INDIAN PHARMACOPOEIA 2010

Volume III

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Government of India
Ministry of Health & Family Welfare

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VOLUME 3

GENERAL NOTICES

General Notices

General Statements

The General Notices provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the Indian Pharmacopoeia (IP), as well as to the statements made in the monographs and other texts of the Pharmacopoeia.

A monograph is to be constructed in accordance with any general monograph or notice or any appendix, note or other explanatory material that is contained in this Pharmacopoeia and that is applicable to that monograph. All statements contained in the monograph, except where a specific general notice indicates otherwise and with the exceptions given hereafter, constitute standards for the official articles. An article is not of pharmacopoeial quality unless it complies with all of the requirements stated.

Exceptions to the General Notices do exist, and where they do, the wording in the individual monograph or an appendix takes precedence and specifically indicates directions or the intent. Thus, the specific wording of standards, tests, assays and other specifications is binding wherever deviations from the General Notices exist. Likewise, where there is no specific mention to the contrary, the General Notices apply.

Name. The full name or title of this book, including addenda thereto, is Indian Pharmacopoeia 2010, abbreviated to IP 2010. In the texts, the term "Pharmacopoeia" or "IP" without qualification means the Indian Pharmacopoeia 2010 and any addenda thereto.

Official and Official Articles. The word 'official' wherever used in this Pharmacopoeia or with reference thereto, is synonymous with 'pharmacopoeial', with 'IP' and with 'compendial'. The designation IP in conjunction with the official title on the label of an article is an indication that the article purports to comply with IP standards.

The following terms are used where the articles for which monographs are provided are to be distinguished.

An official substance is a single drug or a drug entity or a pharmaceutical aid for which the monograph title includes no indication of the nature of a dosage form.

An official preparation is a drug product (dosage form) and is the finished or partially finished preparation or product of one or more official substances formulated for use on the patient.

An article is an item for which a monograph is provided, whether an official substance or an official preparation.

Official Standards. The requirements stated in the monographs apply to articles that are intended for medicinal use but not necessarily to articles that may be sold under the same name for other purposes.

The active pharmaceutical ingredients (drug substances), excipients (pharmaceutical aids), pharmaceutical preparations (dosage forms) and other articles described in the monographs are intended for human and veterinary use (unless explicitly restricted to one of these uses).

The requirements given in the monographs are not framed to provide against all possible impurities, contaminants or adulterants; they provide appropriate limitation of potential impurities only.

A preparation must comply throughout the shelf-life assigned to it by the manufacturer; for opened or broached containers the maximum period of validity for use may sometimes be stated in the individual monograph. Nevertheless, the responsibility for assigning the period of validity shall be with the manufacturer.

Added Substances. An official substance, as distinguished from an official preparation, contains no added substances except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances may be added to an official preparation to enhance its stability, usefulness or elegance, or to facilitate its preparation. Such auxiliary substances shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability or safety of the preparation and shall not interfere with the tests and assays prescribed for determining compliance with the official standards. Particular care should be taken to ensure that such substances are free from harmful organisms. The freedom to the manufacturers to add auxiliary substances imposes on them the responsibility of satisfying the licensing authorities on the purpose of the addition and the innocuity of such substances.

Alternative Methods. The tests and assays described are the official methods upon which the standards of the Pharmacopoeia are based. Alternative methods of analysis may be used for control purposes, provided that the methods used are shown to give results of equivalent accuracy and enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official methods were used. Automated procedures utilising the same basic chemistry as the test procedures given in the monograph may also be used to determine compliance. Such alternative or automated procedures must be validated.

In the event of doubt or dispute, the methods of analysis of the Pharmacopoeia are alone authoritative and only the result obtained by the procedure given in this Pharmacopoeia is conclusive.

Meanings of Terms

Alcohol. The term "alcohol" without qualification means ethanol (95 per cent). Other dilutions of ethanol are indicated by the term "ethanol" or "alcohol" followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Desiccator. A tightly-closed container of suitable size and design that maintains an atmosphere of low moisture content by means of silica gel or phosphorus pentoxide or other suitable desiccant.

Drying and ignition to constant weight. Two consecutive weighings after the drying or igniting operations do not differ by more than 0.5 mg, the second weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Ethanol. The term "ethanol" without qualification means anhydrous ethanol or absolute alcohol.

Filtration. Unless otherwise stated, filtration is the passing of a liquid through a suitable filter paper or equivalent device until the filtrate is clear.

Freshly prepared. Made not more than 24 hours before it is issued for use.

Label. Any printed packing material, including package inserts that provide information on the article.

Negligible. A quantity not exceeding 0.50 mg.

Solution. Where the name of the solvent is not stated, "solution" implies a solution in water. The water used complies with the requirements of the monograph on Purified Water. The term 'distilled water' indicates Purified Water prepared by distillation.

Temperature. The symbol ° used without qualification indicates the use of the Celsius thermometric scale.

Water. If the term is used without qualification it means Purified Water of the Pharmacopoeia. The term 'distilled water' indicates Purified Water prepared by distillation.

Water-bath. A bath of boiling water unless water at another temperature is indicated. Other methods of heating may be used provided the required temperature is approximately maintained but not exceeded.

Provisions Applicable to Monographs and Test Methods

Expression of Contents. Where the content of a substance is defined, the expression "per cent" is used according to circumstances with one of two meanings:

— per cent w/w (percentage, weight in weight) expressing the number of grams of substance in 100 grams of final product,

— per cent v/v (percentage, volume in volume) expressing the number of millilitres of substance in 100 millilitres of final product.

The expression "parts per million" refers to the weight in weight, unless otherwise stated.

Where the content of a substance is expressed in terms of the chemical formula for that substance an upper limit exceeding 100 per cent may be stated. Such an upper limit applies to the result of the assay calculated in terms of the equivalent content of the specified chemical formula. For example, the statement 'contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_6O_2$ implies that the result of the assay is not less than 99.0 per cent and not more than 101.0 per cent, calculated in terms of the equivalent content of $C_7H_6O_2$.

Where the result of an assay or test is required to be calculated with reference to the dried, anhydrous, ignited substance, or the substance free from solvent, the determination of loss on drying, water content, loss on ignition, content of the specified solvent, respectively is carried out by the method prescribed in the relevant test in the monograph.

Expression of Concentrations. The following expressions in addition to the ones given under Expression of Content are also used:

— per cent w/v (percentage, weight in volume) expressing the number of grams of substance in 100 millilitres of product

— per cent v/w (percentage, volume in weight) expressing the number of millilitres of substance in 100 grams of product.

Usually, the strength of solutions of solids in liquids is expressed as percentage weight in volume, of liquids in liquids as percentage volume in volume, of solids in semi-solid bases (e.g. creams) and of gases in liquids as percentage weight in weight.

When the concentration of a solution is expressed as parts of dissolved substance in parts of solution, it means parts by weight (g) of a solid in parts by volume (ml) of the final solution; as parts by weight (g) of a gas in parts by weight (g) of the final solution.

When the concentration of a solution is expressed in molarity designated by the symbol M preceded by a number, it denotes the number of moles of the stated solute contained in sufficient Purified Water (unless otherwise stated) to produce 1 litre of solution.

Abbreviated Statements. Incomplete sentences are employed in parts of the monographs for directness and brevity (for example, Iodine Value. Not more than .....; Relative Density. .....to.....) Where the tests are abbreviated, it is to be understood that the test method referred to in brackets

provides the method to be followed and that the values specified are the applicable limits.

Weights and Measures. The metric system of weights and measures is employed in the Pharmacopoeia. All measures are required to be graduated at 25° and all measurements in tests and assays, unless otherwise stated, are to be made at that temperature. Graduated glass apparatus used in analytical operations shall comply with the requirements stated in Chapter 2.1.6

Monographs

General Monographs

General monographs on dosage forms include requirements of general application and apply to all preparations within the scope of the Introduction section of the general monograph, except where a preamble limits the application. The requirements are not necessarily comprehensive for a given specific preparation; additional requirements may sometimes be given in the individual monograph for it.

Production. Statements given under the heading Production relate to particular aspects of the manufacturing process and are not necessarily comprehensive. However, they are mandatory instructions to manufacturers. They may relate, for example, to source materials, to the manufacturing process and its validation and control, to any in-process testing that is to be carried out by the manufacturer on the final product either on selected batches or on each batch prior to release. All this cannot be verified on a sample of the final product by an independent analyst. It is for the licensing authority to verify that the instructions have been followed.

The absence of a section on Production does not imply that attention to features such as those given above is not required. An article described in a monograph of the Pharmacopoeia is to be manufactured in accordance with the principles of good manufacturing practice and in accordance with the requirements of the Drugs and Cosmetics Rules, 1945. The general principles applicable to the manufacture and quality assurance of drugs and preparations meant for human use apply equally to veterinary products as well.

Manufacture of Drug Products. The opening definitive statement in certain monographs for drug products is given in terms of the active ingredient(s) only. Any ingredient(s) other than those included in the statement, must comply with the general notice on Excipients and the product must conform to the Pharmacopoeial requirements.

Official preparations are prepared only from ingredients that comply with the requirements of the pharmacopoeial monographs for those individual ingredients for which monographs are provided.

Excipients. Any substance added in preparing an official preparation shall be innocuous, shall have no adverse influence in the therapeutic efficacy of the active ingredients and shall not interfere with the tests and assays of the Pharmacopoeia. Care should be taken to ensure that such substances are free from harmful organisms.

Individual Monographs

Drug products that are the subject of an individual monograph are also required to comply with the tests given in the general monographs.

Titles. The main title for a drug substance is the International Non-proprietary Name (INN) approved by the World Health Organization. Subsidiary names and synonyms have also been given in some cases; where included, they have the same significance as the main title.

The main titles of drug products are the ones commonly recognised in practice. Synonyms drawn from the full non-proprietary name of the active ingredient or ingredients have also been given. Where, however, a product contains one or the other of different salts of an active molecule, the main title is based on the full name of the active ingredient. For example, Chloroquine Phosphate Tablets and Chloroquine SulphateTablets.

Chemical Formulae. When the chemical structure of an official substance is known or generally accepted, the graphic and molecular formulae are normally given at the beginning of the monograph for information. This information refers to the chemically pure substance and is not to be regarded as an indication of the purity of the official material. Elsewhere, in statement of purity and strength and in descriptions of processes of assay, it will be evident from the context that the formulae denote the chemically pure substances.

Where the absolute stereochemical configuration is specified, the International Union of Pure and Applied Chemistry (IUPAC) *R/S* and *E/Z* systems of designation have been used. If the substance is an enantiomer of unknown absolute stereochemistry, the sign of the optical rotation, as determined in the solvent and under the conditions specified in the monograph, has been attached to the systematic name. An indication of sign of rotation has also been given where this is incorporated in a trivial name that appears on an IUPAC preferred list.

Atomic and Molecular Weights. The atomic weight or molecular weight is shown , as and when appropriate at the top right hand corner of the monograph. The atomic and molecular weights and graphic formulae do not constitute analytical standards for the substances described.

Definition. The opening statement of a monograph is one that constitutes an official definition of the substance, preparation or other article that is the subject of the

monograph. In certain monographs for pharmaceutical preparations the statement is given in terms of the principal ingredient(s).

In monographs on vegetable drugs, the definition indicates whether the subject of the monograph is, for example, the whole drug or the drug in powdered form.

Certain pharmaceutical substances and other articles are defined by reference to a particular method of manufacture. A statement that a substance or article is prepared or obtained by a certain method constitutes part of the official definition and implies that other methods are not permitted. A statement that a substance may be prepared or obtained by a certain method, however, indicates that this is one possible method and does not imply that other methods are not permissible.

Statement of content. The limits of content stated are those determined by the method described under Assay.

Category. The statement of category is provided for information and is indicative of the medical or pharmaceutical basis for recognition in the Pharmacopoeia. It generally represents an application of the best known pharmacological action of the article or of its active ingredient. In the case of pharmaceutical aids it may indicate the more common usage of the article. The statement is not intended to limit in any way the choice or use of the article nor to indicate that it has no other activity or use.

Dose. Doses mentioned in the Pharmacopoeia are intended merely for general guidance and represent, unless otherwise stated, the average range of quantities which are generally regarded as suitable for adults when administered by mouth. They are not to be regarded as binding upon the prescribers. The medical practitioner will exercise his own judgment and act on his own responsibility in respect of the amount of any therapeutic agent he may prescribe or administer or the frequency of its administration. If it is usual to administer a drug by a method other than by mouth, the single dose suitable for that method of administration is mentioned. In the case of some preparations notes have been given below the statement of doses to show the approximate quantities of active ingredients contained in the maximal doses as information for the prescriber.

Usual Strength. The statement on the usual strength(s) of a preparation given in the individual monograph indicates the strength(s) usually marketed for information of the pharmacist and the medical practitioner. It does not imply that a strength other than the one(s) mentioned in the individual monograph meeting all the prescribed requirements cannot be manufactured and marketed with the approval of the appropriate authority.

Description. The statements under the heading Description are not to be interpreted in a strict sense and are not to be regarded as official requirements.

Solubility. Statements on solubility are given in Chapter 2.4.26 and are intended as information on the approximate solubility at a temperature between 15° and 30°, unless otherwise stated, and are not to be considered as official requirements. However, a test for solubility stated in a monograph constitutes part of the standards for the substance that is the subject of that monograph.

Test Methods

References to general methods of testing are indicated by test method numbers in brackets immediately after the heading of the test or at the end of the text.

Identification. The tests given under the heading Identification are not necessarily sufficient to establish absolute proof of identity. They provide a means of verifying that the identity of the material under examination is in accordance with the label on the container.

In certain monographs alternative series of identification tests are given; compliance with either one or the other set of tests is adequate to verify the identity of the article.

When tests for infrared absorption are applied to material extracted from formulated preparations, strict concordance with the specified reference spectrum may not always be possible, but nevertheless a close resemblance between the spectrum of the extracted material and the specified reference spectrum should be achieved.

Tests and Assays

The tests and assays are the official methods upon which the standards of the Pharmacopoeia depend. The requirements are not framed to take into account all possible impurities. It is not to be presumed, for example, that an impurity that is not detectable by means of the prescribed tests is tolerated. Material found to contain such an impurity is not of pharmacopoeial quality if the nature or amount of the impurity found is incompatible with good pharmaceutical practice.

Pharmacopoeial methods and limits should be used merely as compliance requirements and not as requirements to guarantee total quality assurance. Tests and assays are prescribed for the minimum sample available on which the attributes of the article should be measured. Assurance of quality must be ensured by the manufacturer by the use of statistically valid sampling and testing programmes.

Tests. Unless otherwise stated, the assays and tests are carried out at a temperature between 20° and 30°.

Where it is directed that an analytical operation is to be carried out 'in subdued light', precautions should be taken to avoid exposure to direct sunlight or other strong light. Where a procedure is directed to be performed 'protected from light' precautions should be taken to exclude actinic light by the

use of low-actinic glassware, working in a dark room or similar procedures.

For preparations other than those of fixed strength, the quantity to be taken for a test or an assay is usually expressed in terms of the active ingredient. This means that the quantity of the active ingredient expected to be present and the quantity of the preparation to be taken are calculated from the strength stated on the label.

Other Tests. In the monographs on dosage forms and certain preparations, under the sub-heading 'Other tests' it is stated that the article complies with the tests stated under the general monograph of the relevant dosage form or preparation. Details of such tests are provided in the general monographs.

Limits. The limits given are based on data obtained in normal analytical practice. They take into account normal analytical errors, of acceptable variations in manufacture and of deterioration to an extent that is acceptable. No further tolerances are to be applied to the limits for determining whether or not the article under examination complies with the requirements of the monograph.

Quantities. Unless otherwise stated, the quantities to be taken for assays, limit tests and other tests are of the substance under examination.

In tests with numerical limits and assays, the quantity stated to be taken for testing is approximate. The amount actually used, which may deviate by not more than 10 per cent from that stated, is accurately weighed or measured and the result of analysis is calculated from this exact quantity. In tests where the limit is not numerical but usually depends upon comparison with the behaviour of a reference in the same conditions, the stated quantity is taken for testing. Reagents are used in the prescribed amounts.

Quantities are weighed or measured with an accuracy commensurate with the indicated degree of precision. For weighings, the precision is plus or minus 5 units after the last figure stated. For example, 0.25 g is to be interpreted as 0.245 g to 0.255 g. For the measurement of volumes, if the figure after the decimal point is a zero or ends in a zero, e.g. 10.0 ml or 0.50 ml, the volume is measured using a pipette, a volumetric flask or a burette, as appropriate; in other cases, a graduated measuring cylinder or a graduated pipette may be used. Volumes stated in microlitres are measured using a micropipette or microsyringe.

The term 'transfer' is used generally to indicate a quantitative operation.

Apparatus. Measuring and weighing devices and other apparatus are described in the chapter entitled 'Apparatus for Tests and Assays'. A specification for a definite size or type of container or apparatus in a test or assay is given merely as a recommendation.

Unless otherwise stated, comparative tests are carried out using identical tubes of colourless, transparent, neutral glass with a flat base, commonly known as Nessler cylinders.

Reagents and Solutions. The reagents required for the tests and assays of the Pharmacopoeia are defined in the various chapters showing their nature, degree of purity and the strengths of the solutions to be made from them. The requirements set out are not intended to imply that the materials are suitable for use in medicine; reagents not covered by monographs in the pharmacopoeia shall not be claimed to be of IP quality.

The term 'analytical reagent grade of commerce' implies that the chemical is of a high degree of purity wherein the limits of various impurities are known. Where it is directed to use a 'general laboratory reagent grade of commerce' it is intended that a chemically pure grade material, not necessarily required to be tested for limiting or absence of certain impurities, is to be used.

Indicators. Where the use of an indicator solution is mentioned in an assay or test, approximately 0.1 ml of the solution shall be added, unless otherwise directed.

Reference Substances. Certain monographs require the use of a chemical reference substance or a biological reference preparation or a reference spectrum These are authentic specimens chosen and verified on the basis of their suitability for intended use as prescribed in the Pharmacopoeia and are not necessarily suitable in other circumstances.

IP Reference Substances, abbreviated to IPRS (and referred to as RS in the individual monographs) are issued by the Indian Pharmacopoeia Commission (IPC). They are the official standards to be used in cases of arbitration. Secondary Standards (Working Standards) may be used for routine analysis, provided they are standardized at regular intervals against the Reference Substances

Biological Reference Substances, also abbreviated to IPRS and Standard Preparations of antibiotics are issued by agencies authorised by the IPC. They are standardized against the International Standards and Reference Preparations established by the World Health Organization (WHO). The potency of these preparations is expressed in International Units.

Reference spectra are published by the IPC and they are accompanied by information concerning the conditions used for sample preparation and recording of the spectra.

Test Animals. Unless otherwise directed, animals used in a test or an assay shall be healthy and are drawn from a uniform stock, and have not previously been treated with any material that will interfere with the test or the assay.

Calculation of Results. In determining compliance with a numerical limit in assay or test, the result should be calculated

to one decimal place more than the significant figures stated and then rounded up or down as follows: if the last figure calculated is 5 to 9, the preceding figure is increased by 1; if it is 4 or less, the preceding figure is left unchanged.

Storage. Statements under the side-heading Storage constitute non-mandatory advice. The articles of the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monograph.

Specific directions are given in some monographs with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that usage at a lower or higher temperature may produce undesirable results. The storage conditions are defined by the following terms:

— Store in a dry, well ventilated place at a temperature not exceeding 30°

— Store in a refrigerator (2° to 8°). Do not freeze

— Store in a freezer (-2° to -18°)

— Store in a deep freezer (Below -18°)

Storage conditions not related to temperature are indicated in the following terms:

— Store protected from light

— Store protected from light and moisture

Where no specific storage directions or limitations are given in the monograph or by the manufacturer, it is to be understood that the storage conditions include protection from moisture, freezing and excessive heat (any temperature above 40°).

Storage Containers. The requirements, guidance and information on containers for pharmaceutical use are given in the chapter entitled Containers (6.1)

In general, an article should be packed in a well-closed container i.e. one that protects the contents from contamination by extraneous solids, liquids or vapours and from loss of the article under normal conditions of handling and storage.

Where, additionally, loss or deterioration of the article from effervescence, deliquescence or evaporation under normal conditions of storage is likely, the container must be capable of being tightly closed, and re-closed after use.

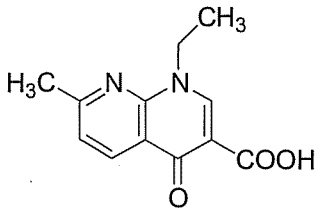
In certain cases, special requirements of pack have been indicated in some monographs under Storage, using expressions that have been defined in chapter 6.1.

Labelling. The labelling of drugs and pharmaceuticals is governed by the Drugs and Cosmetics Rules, 1945. The statements that are given in the monographs under the side-heading 'Labelling' are not comprehensive. Only those that are necessary to demonstrate compliance or otherwise with the monograph have been given and they are mandatory. For example, in the monograph on Betamethasone Sodium Tablets the labelling statement is "The label states the strength in terms of the equivalent amount of betamethasone". Any other statements are included as recommendations.

DRUG SUBSTANCES, DOSAGE FORMS AND PHARMACEUTICAL AIDS

N

Nalidixic Acid



$C_{12}H_{12}N_2O_3$ Mol. Wt. 232.2

Nalidixic Acid is 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid.

Nalidixic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Category. Antibacterial.

Dose. 2 to 4 g daily, in divided doses.

Description. A white to slightly yellow, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nalidixic acid RS* or with the reference spectrum of nalidixic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 258 nm and 334 nm; ratio of the absorbance at about 258 nm to that at about 334 nm, 2.2 to 2.4.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Dissolve 0.1 g in 2 ml of *hydrochloric acid* and add 0.5 ml of a 10 per cent w/v solution of *2-naphthol* in *ethanol (95 per cent)*; an orange-red colour develops.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 70 volumes of *ethanol (95 per cent)*, 20 volumes of *dichloromethane* and 10 volumes of *5 M ammonia*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of *dichloromethane*.

Test solution (b). A 0.1 per cent w/v solution of the substance under examination in *dichloromethane*.

Reference solution (a). A 0.002 per cent w/v solution of the substance under examination in *dichloromethane*.

Reference solution (b). A 0.0008 per cent w/v solution of the substance under examination in *dichloromethane*.

Reference solution (c). A 0.1 per cent w/v solution of *nalidixic acid RS* in *dichloromethane*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18) Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 10 ml of *dichloromethane*, add 30 ml of *2-propanol* and 10 ml of *carbon dioxide-free water* and titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25) and using a glass electrode as the indicator electrode and a silver-silver chloride reference electrode with a sleeve diaphragm or a capillary tip filled with a saturated solution of *lithium chloride* in *ethanol*. Throughout the titration keep the temperature of the solution at 15° to 20° and pass a current of nitrogen through the solution.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02322 g of $C_{12}H_{12}N_2O_3$.

Storage. Store protected from light and moisture.

Nalidixic Acid Tablets

Nalidixic Acid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of nalidixic acid, $C_{12}H_{12}N_2O_3$.

Usual strengths. 250 mg; 500 mg.

Identification

To a quantity of the powdered tablets containing 1 g of Nalidixic Acid add 50 ml of *chloroform*, shake for 15 minutes, filter and evaporate the filtrate to dryness. The residue, after drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nalidixic acid RS* or with the reference spectrum of nalidixic acid.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 258 nm and 334 nm; ratio of the absorbance at about 258 nm to that at about 334 nm, 2.2 to 2.4.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 70 volumes of *ethanol (95 per cent)*, 20 volumes of *dichloromethane* and 10 volumes of *5 M ammonia*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Nalidixic Acid with 50 ml of *chloroform* for 15 minutes, filter, evaporate the filtrate to dryness and dissolve the residue in 5 ml of *chloroform*.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *chloroform*.

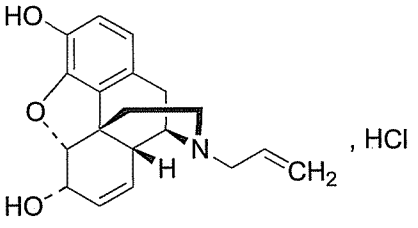
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Nalidixic Acid, add 150 ml of *0.1 M sodium hydroxide*, shake for 3 minutes, dilute to 200.0 ml with *0.1 M sodium hydroxide*, mix and allow to stand for 15 minutes. Dilute 2.0 ml of the solution to 100.0 ml with water and measure the absorbance of the resulting solution at the maximum at about 334 nm (2.4.7), using *0.1 M sodium hydroxide* as the blank. Calculate the content of $C_{12}H_{12}N_2O_3$ taking 494 as the specific absorbance at 334 nm.

Storage. Store protected from light and moisture.

Nalorphine Hydrochloride



$C_{19}H_{21}NO_3,HCl$ Mol. Wt. 347.8

Nalorphine Hydrochloride is 17-allyl-7,8-didehydro-4,5α-epoxymorphinan-3,6α-diol hydrochloride.

Nalorphine Hydrochloride contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{19}H_{21}NO_3,HCl$, calculated on the dried basis.

Category. Narcotic antagonist.

Dose. By intravenous injection, 5 mg, repeated twice at three minute intervals, if necessary.

Description. A white or almost white, crystalline powder; odourless. It slowly darkens on exposure to air and light.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests C and D may be omitted if tests A, B and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nalorphine hydrochloride RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum only at about 298 nm; absorbance at about 298 nm, about 0.6.

C. To 10 ml of a 2 per cent w/v solution add 0.05 ml of *dilute ammonia solution*; a white precipitate soluble in sodium hydroxide solution is produced.

D. Dissolve 2 mg in 2 ml of *water*, add 0.15 ml of *potassium ferricyanide solution* containing, in each ml, 0.05 ml of *ferric chloride solution*; a deep bluish green colour is produced immediately.

E. Gives reaction A of chlorides (2.3.1).

Tests

Melting range (2.4.21). 260° to 263°.

Acidity. Dissolve 0.2 g in 10 ml of freshly boiled and cooled *water* and titrate with *0.02 M sodium hydroxide* using *methyl red solution* as indicator; not more than 0.2 ml of *0.02 M sodium hydroxide* is required to change the colour of the solution.

Specific optical rotation (2.4.22). –122° to –125°, determined in a 2.0 per cent w/v solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. Weigh accurately about 25 mg and dissolve in sufficient *water* to produce 250 ml. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{19}H_{21}NO_3,HCl$ from the absorbance

obtained by repeating the operation with *nalorphine hydrochloride RS* in place of the substance under examination.

Storage. Store protected from light and moisture.

Nalorphine Injection

Nalorphine Hydrochloride Injection

Nalorphine Injection is a sterile solution of Nalorphine Hydrochloride in Water for Injections containing suitable buffering agents.

Nalorphine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nalorphine hydrochloride, $C_{19}H_{21}NO_3,HCl$.

Usual strength. 10 mg per ml.

Identification

A. To a volume containing 50 mg of Nalorphine Hydrochloride add *dilute ammonia solution* until the solution is alkaline and extract with 25 ml of a mixture of 1 volume of *ethanol (95 per cent)* and 3 volumes of *chloroform* and evaporate the extract to dryness. Dry the residue at a pressure not exceeding 2 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nalorphine hydrochloride RS*.

B. To a volume containing 0.1 g of Nalorphine Hydrochloride add 0.05 ml of *dilute ammonia solution*; a white precipitate soluble in *sodium hydroxide solution* is produced.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 6.0 to 7.5.

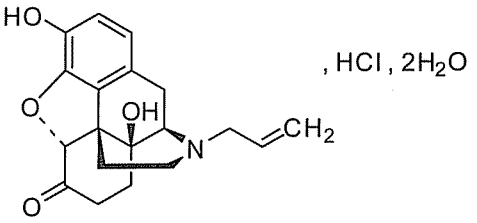
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer an accurately measured volume containing about 10 mg of Nalorphine Hydrochloride to a separating funnel, add 1 ml of *dilute hydrochloric acid* and dilute to 10 ml with *water*. Extract with five successive quantities, each of 5 ml, of *chloroform*, allowing the layers to separate before drawing off each chloroform extract and discard the chloroform extracts. Transfer the aqueous layer to a 100-ml volumetric flask with the aid of small quantities of *water* and dilute to volume with *water*. Measure the absorbance of the resulting solution at the maximum at about 285 nm (2.4.7). Calculate the content of $C_{19}H_{21}NO_3,HCl$ from the absorbance obtained by repeating the operation with *nalorphine hydrochloride RS*.

Storage. Store protected from light.

Naloxone Hydrochloride

Naloxone Hydrochloride Dihydrate



$C_{19}H_{21}NO_4,HCl,2H_2O$ Mol. Wt. 399.9

Naloxone Hydrochloride is 17-allyl-4,5-α-epoxy-3,14-dihydroxymorphinan-3-one hydrochloride dihydrate.

Naloxone Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{22}ClNO_4$, calculated on the anhydrous basis.

Category. Antidote for opioids poisoning.

Description. A white to almost white, hygroscopic, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *naloxone hydrochloride dihydrate RS* or with the reference spectrum of naloxone hydrochloride.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. Mix 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of *ammonia* and 100 ml of *butan-1-ol*.

Test solution. Dissolve 8 mg of the substance under examination in 0.5 ml of *water* and dilute to 1 ml with *methanol*.

Reference solution. Dissolve 8 mg of *naloxone hydrochloride dihydrate RS* in 0.5 ml of *water* and dilute to 1 ml with *methanol*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with a freshly prepared 0.5 per cent w/v solution of *potassium ferricyanide* in *ferric chloride solution* and examine in daylight. The

principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* (Solution A) is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.05 ml of *methyl red solution.* Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (2.4.22). −170° to −181°, determined in solution A.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.125 g of the substance under examination in 25 ml of *0.1 M hydrochloric acid.*

Reference solution (a). Dissolve 5 mg of *naloxone for peak identification RS* (containing naloxone impurity A (4,5α-epoxy-3,14-dihydroxymorphinan-6-one) (noroxymorphone), naloxone impurity B (4,5α-epoxy-14-hydroxy-17-(prop-2-enyl)-3-(prop-2-enyloxy)morphinan-6-one) (3-*O*-allylnaloxone), naloxone impurity C (4,5α-epoxy-3,10á,14-trihydroxy-17-(prop-2-enyl)morphinan-6-one) (10α-hydroxynaloxone), naloxone impurity D (7,8-didehydro-4,5α-epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one) (7,8-didehydronaloxone), naloxone impurity E (4,5 α:4',5' α-diepoxy-3,3',14,14'-tetrahydroxy-17,17'-bis(prop-2-enyl)-2,2'-bimorphinanyl-6,6'-dione) (2,2'-bisnaloxone) and naloxone impurity F (4,5α-epoxy-3,10β,14-trihydroxy-17-(prop-2-enyl)morphinan-6-one) (10α-hydroxynaloxone) in 1 ml of *0.1 M hydrochloric acid.*

Reference solution (b). Dilute 1.0 ml of the test solution to 20 ml with *0.1 M hydrochloric acid.* Dilute 1.0 ml of this solution to 25.0 ml with *0.1 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of 20 volumes of *acetonitrile*, 40 volumes of *tetrahydrofuran* and 940 volumes of the solution prepared by dissolving 1.1 g of *sodium octanesulphonate* in 1000 ml of *water.* Adjust to pH 2.0 with a 50 per cent v/v solution of *orthophosphoric acid,*

  B. a mixture of 40 volumes of *tetrahydrofuran* , 170 volumes of *acetonitrile* and 790 volumes of the solution prepared by dissolving 1.1 g of *sodium octanesulphonate* in 1000 ml of *water.* Adjust to pH 2.0 with a 50 per cent v/v solution of *orthophosphoric acid,*
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 → 40	100 → 0	0 → 100
40 → 50	0	100

Inject reference solution (a). Adjust the sensitivity of the system so that the peak-to-valley ratio is minimum 2.0, where H_p is height above the baseline of the peak due to impurity D and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to naloxone.

The relative retention time with reference to naloxone, for impurity C, impurity A, impurity F, impurity D, impurity E and impurity B is about 0.6 minute, 0.8 minute, 0.9 minute, 1.1 minutes, 3.0 minutes and 3.2 minutes respectively.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of each secondary peak corresponding to naloxone impurities A, B, C, E, F is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of secondary peak corresponding to naloxone impurity D is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The area of any other impurities is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all other secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.05 per cent).

Water (2.3.43). 7.5 per cent to 11.0 per cent, determined on 0.2 g.

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *ethanol (95 per cent)* and add 5.0 ml of *0.01 M hydrochloric acid.* Titrate with *0.1 M ethanolic sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.03638 g of $C_{19}H_{22}ClNO_4$.

Storage. Store protected from light.

Naloxone Injection

Naloxone Injection is a sterile solution of Naloxone Hydrochloride in Water for Injections.

Naloxone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of naloxone hydrochloride, $C_{19}H_{21}NO_4,HCl$.

Usual strength. 400 µg per ml.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of *M ammonia* and 100 ml of *butan-1-ol.*

Test solution. Add 1 ml of *ammonia buffer pH 10.0* to a volume of the injection containing the equivalent of 2 mg of anhydrous naloxone hydrochloride, extract with three 20 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol.* Dilute 1 ml of this solution to 20 ml with *methanol.*

Reference solution. A 0.01 per cent w/v solution of *naloxone hydrochloride RS* in *methanol.*

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm, protecting the plate from light. After development, dry the plate in a current of air, spray with a freshly prepared 0.5 per cent w/v solution of *potassium hexacyanoferrate (III)* in *iron(III) chloride solution* and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 4.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 5 volumes of *methanol* and 95 volumes of the upper layer from a mixture of 60 ml of *2 M ammonia* and 100 ml of *butan-1-ol.*

Test solution. Transfer a volume of the injection containing about 2 mg of Naloxone Hydrochloride in 1 ml of *ammonia buffer pH 10.0*, extract with three 20 ml quantities of a mixture of 1 volume of *propan-2-ol* and 3 volumes of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 1 ml of *methanol.*

Reference solution. Dilute 1 ml of the test solution to 200 ml with *methanol.*

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm, protecting the plate from light. After development, dry the plate in a current of air, spray with a freshly prepared 0.5 per cent w/v solution of *potassium hexacyanoferrate(III)* in *iron(III) chloride solution* and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent). Ignore any spot remaining on the line of application.

Bacterial endotoxins (2.2.3). Not more than 70 IU per ml of the injection, diluted if necessary, with *water BET* to give a solution containing 0.04 per cent w/v of anhydrous Naloxone Hydrochloride.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 0.1 volume of *orthophosphoric acid*, 45 volumes of *methanol* and 55 volumes of *water.*

Test solution. Dilute the injection equivalent to 0.001 per cent w/v of Naloxone Hydrochloride with the solvent mixture.

Reference solution (a). A 0.001 per cent w/v solution of *naloxone hydrochloride RS* in the solvent mixture.

Reference solution (b). A 0.001 per cent w/v solution of *naloxone hydrochloride RS* and 0.0005 per cent w/v solution of *noroxymorphone* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 to 10 µm) (such as Zorbax C18, 7 to 8 µm),
- mobile phase: a solution containing 0.068 per cent w/v of *sodium octanesulphonate* and 0.1 per cent w/v of *sodium chloride* in the solvent mixture,
- flow rate. 1 ml per minute,
- spectrophotometer set at 229 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naloxone and noroxymorphone in the chromatogram obtained with reference solution (b) is not less than 1.3.

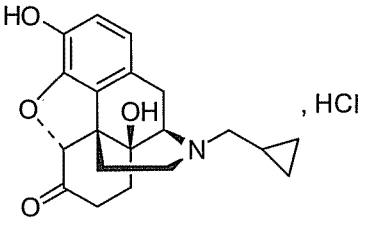
Injection reference solution (a) and the test solution.

Calculate the content of $C_{19}H_{21}NO_4,HCl$ in the injection.

Storage. Store protected from light.

Labelling. The label states the quantity of active ingredient in terms of the equivalent amount of anhydrous naloxone hydrochloride. When naloxone is prescribed for neonatal use, Neonatal Naloxone Injection (containing the equivalent of 20 micrograms per ml of anhydrous naloxone hydrochloride) shall be dispensed.

Naltrexone Hydrochloride



$C_{20}H_{24}ClNO_4$ Mol. Wt. 377.9

Naltrexone Hydrochloride is 17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one hydrochloride.

Naltrexone Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{24}ClNO_4$, calculated on the anhydrous and ethanol free basis.

Category. Antidote for opioids poisoning.

Description. A white or almost white powder, very hygroscopic.

Identification

A. Dissolve 20 mg of the substance under examination in 5 ml of *water* and make alkaline with *dilute ammonia*. Shake with 10 ml of *dichloromethane*, separate the organic layer and evaporate the solvent. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *naltrexone hydrochloride RS* or with the reference spectrum of naltrexone hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon-dioxide free water* is clear (2.4.1) and not more intensely coloured than reference solution YS5 or BS5 (2.4.1).

Acidity or alkalinity. To 10 ml of 2.0 per cent w/v solution in *carbon-dioxide free water*, add 0.05 ml of *methyl red solution*. Not more than 0.2 ml of *0.02 M sodium hydroxide* or *0.02 M hydrochloric acid* is required to change the colour of the indicator.

Specific optical rotation (2.4.22). - 187° to - 195°, determined in a 2.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of *0.1 M hydrochloric acid*.

Reference solution (a). Dissolve 5 mg of *17-but-3-enyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one RS (naltrexone impurity C RS)* in 2.5 ml of *0.1 M hydrochloric acid*.

Reference solution (b). Dilute 1.0 ml of the test solution and 1.0 ml of reference solution (a) to 100 ml with *0.1 M hydrochloric acid*. Dilute 1.0 ml of this solution to 10 ml with *0.1 M hydrochloric acid*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a 0.11 per cent w/v solution of *sodium octanesulphonate*, adjust the pH to 2.3 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-45	90→55	10→45
45-47	55→90	45→10
47-55	90	10

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naltrexone and naltrexone impurity C is not less than 2.0. The relative retention time with reference to naltrexone for 17-formyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one (naltrexone impurity A) is about 0.4, for 4,5α-epoxy-3,14-dihydroxymorphinan-6-one (noroxymorphone) (naltrexone impurity B) is about 0.7, For 17-(cyclopropylmethyl)-4,5α-epoxy-3,10α,14-trihydroxymorphinan-6-one (naltrexone impurity F) is about 0.8, for 17-(cyclopropylmethyl)-4,5α-epoxy-3,10β ,14-trihydroxymorphinan-6-one (naltrexone impurity G) is about 0.9, for 17-but-3-enyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one (naltrexone impurity C) is about 1.05, for 17-butyl-4,5α-epoxy-3,14-dihydroxymorphinan-6-one (naltrexone impurity H) is about 1.1, for 17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6,10-dione (naltrexone impurity I) is about 1.2, for 7-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one (naltrexone impurity J) is about 1.3, for 17,17′-bis(cyclopropylmethyl)-4,5α:4′,5′-α-diepoxy-

3,3′,14,14′-tetrahydroxy-2,2′-bimorphinanyl-6,6′-dione (pseudonaltrexone) (naltrexone impurity D) is about 1.4, for 3-(cyclopropylmethoxy)-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxymorphinan-6-one (naltrexone impurity E) is about 1.7.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area any peak corresponding to naltrexone impurity C, D, E, F and G is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent); the area of any peak corresponding to naltrexone impurity A, B, H, I and J is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of the areas of all secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Ethanol (2.3.45). Not more than 3.0 per cent v/v, determined by Method I using the following solutions.

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of *water.*

Reference solution. Dilute 0.75 g of *anhydrous ethanol* to 1000 ml with *water.*

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 10.0 per cent, determined on 0.2 g.

Assay. Weigh accurately about 0.2 g of the substance under examination, dissolve in 60 ml of *ethanol (95 per cent)* and add 1.0 ml of *0.1 M hydrochloric acid.* Titrate with *0.1 M sodium hydroxide,* determining the end-point potentiometrically (2.4.25). The curve shows 3 points of inflexion. Read the volume added between the first 2 points of inflexion.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03779 g of $C_{20}H_{24}ClNO_4$.

Storage. Store protected from light and moisture.

Naltrexone Tablets

Naltrexone Hydrochloride Tablets

Naltrexone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of naltrexone hydrochloride, $C_{20}H_{23}NO_4.HCl$.

Usual strength. 50 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A solution of *naltrexone hydrochloride RS* in *water* to obtain the same concentration as given in the test solution.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 600 volumes of 0.05 M buffer solution prepared by dissolving 7 g of *monobasic sodium phosphate* in 1000 ml of *water*, add 1.1 g of *sodium 1-octane sulphonate monohydrate* and 400 ml of *methanol*, adjust the pH to 6.7 with *dilute sodium hydroxide*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 100 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{23}NO_4.HCl$ in the tablets.

D. Not less than 80 per cent of the stated amount of $C_{20}H_{23}NO_4.HCl$.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay, using the following solution as the test solution.

Test solution. Disperse 1 tablet in 100 ml of *0.1 M orthophosphoric acid.*

Calculate the content of $C_{20}H_{23}NO_4.HCl$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Shake a quantity of powdered tablets containing about 250 mg of Naltrexone with 80 ml of *0.1 M orthophosphoric acid*, sonicate for 30 minutes and dilute to 100 ml with the same solvent, filter.

Reference solution (a). Dissolve 22.5 mg of *naltrexone RS* in 1.5 ml of *methanol* and 0.6 ml of *0.1 M hydrochloric acid.* Dilute to 10 ml with *0.1 M orthophosphoric acid.*

Reference solution (b). Dissolve about 3 mg of *N-(3-butenyl)-noroxymorphone hydrochloride RS (naltrexone impurity A RS)* in 3.0 ml of *methanol* and dilute to 10 ml with *0.1 M orthophosphoric acid.* To 0.5 ml of this solution, add 5.0 ml of reference solution (a) and dilute to 10 ml with *0.1 M orthophosphoric acid.*

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: A. dissolve about 1.08 g of *sodium 1-octanesulphonate* and 23.8 g of *sodium acetate* in 800 ml of *water*. Add 1.0 ml of *triethylamine* and 200 ml of *methanol*, adjust the pH to 6.5 with *glacial acetic acid*, B. dissolve about 1.08 g *sodium 1-octanesulphonate* and 23.8 g *sodium acetate* in 400 ml of *water*. Add 1.0 ml of *triethylamine* and 600 ml of *methanol*, adjust the pH to 6.5 with *glacial acetic acid*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

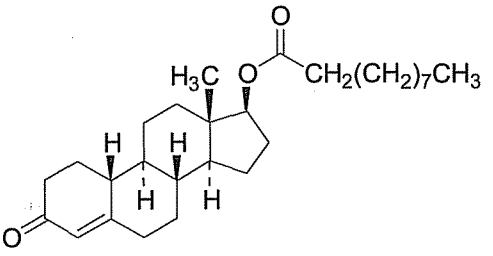
Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
1-35	100→0	0→100
35-36	0→100	100→0

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naltrexone and naltrexone impurity A is not less than 2.0, the tailing factor is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to naltrexone for noroxymorphone is about 0.55, for 10-hydroxynaltrexone is about 0.7, for naltrexone impurity A is about 1.26, for 2,2α-bisnaltrexone is about 1.80 and for 10-ketonaltrexone is about 1.99.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{20}H_{23}NO_4.HCl$ in the Tablets.

Nandrolone Decanoate



$C_{28}H_{44}O_3$ Mol. Wt. 428.7

Nandrolone Decanoate is 3-oxo-4-estren-17β-yl decanoate.

Nandrolone Decanoate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{28}H_{44}O_3$, calculated on the dried basis.

Category. Anabolic steroid.

Dose. By intramuscular injection, 25 to 50 mg, every 3 weeks.

Description. A white to creamy-off white, crystalline powder; odour, faint and characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nandrolone decanoate RS* or with the reference spectrum of nandrolone decanoate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 239 nm; absorbance at about 239 nm, about 0.41.

C. Dissolve 25 mg in 1 ml of *methanol*, add 2 ml of *semicarbazide acetate solution*, heat under a reflux condenser for 30 minutes and cool; the precipitate, after recrystallisation from *ethanol (95 per cent)*, melts at about 175° (2.4.21).

Tests

Specific optical rotation (2.4.22). +32.0° to +36.0°, determined in a 2.0 per cent w/v solution in *dioxan*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *heptane* and 30 volumes of *acetone.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform.*

Reference solution (a). A 0.005 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (b). A 0.01 per cent w/v solution of *nandrolone RS* in *chloroform.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to nandrolone is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 10 mg and dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml. Dilute 5.0 ml to 50.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 239 nm (2.4.7). Calculate the content of $C_{28}H_{44}O_3$ taking 407 as the specific absorbance at 239 nm.

Storage. Store protected from light and moisture.

Nandrolone Decanoate Injection

Nandrolone Decanoate Injection is a sterile solution of Nandrolone Decanoate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these.

Nandrolone Decanoate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nandrolone decanoate, $C_{28}H_{44}O_3$.

Usual strength. 25 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *heptane* and 30 volumes of *acetone*.

Test solution. Dilute a suitable volume of the injection with *carbon tetrachloride* to give a solution containing 0.5 per cent w/v solution of Nandrolone Decanoate.

Reference solution. A 0.5 per cent w/v solution of *nandrolone decanoate RS* in *carbon tetrachloride*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol (95 per cent)*, heat at 105° for 30 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Ignore any subsidiary spots due to the vehicle.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

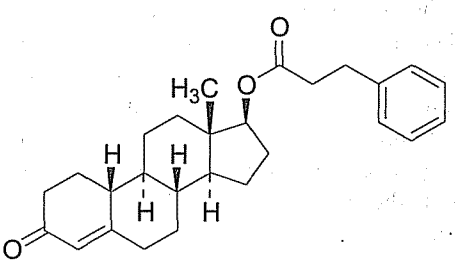
Assay. To an accurately measured volume containing about 0.1 g of Nandrolone Decanoate add sufficient *chloroform* to produce 100.0 ml. Dilute 3.0 ml of the solution to 50.0 ml with *chloroform*. To 5.0 ml of this solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{28}H_{44}O_3$ from the absorbance obtained by repeating the operation using a suitable quantity of *nandrolone RS*.

1 mg of $C_{18}H_{26}O_2$ is equivalent to 1.562 mg of $C_{28}H_{44}O_3$.

Storage. Store protected from light.

Nandrolone Phenylpropionate

Nandrolone Phenpropionate



$C_{27}H_{34}O_3$ Mol.Wt. 406.6

Nandrolone Phenylpropionate is 3-oxo-4-estren-17β-yl 3-phenylpropionate.

Nandrolone Phenylpropionate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{27}H_{34}O_3$, calculated on the dried basis.

Category. Anabolic steroid.

Dose. By deep intramuscular injection, 25 to 50 mg weekly.

Description. A white to creamy-white, crystalline powder; odour, characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nandrolone phenylpropionate RS* or with the reference spectrum of nandrolone phenylpropionate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an

absorption maximum only at about 240 nm; absorbance at about 240 nm, about 0.43.

C. Dissolve 25 mg in 1 ml of *methanol*, add 2 ml of *semicarbazide acetate solution*, heat under a reflux condenser for 30 minutes and cool; the precipitate, after recrystallisation from *ethanol (95 per cent)* melts at about 182° (2.4.21).

Tests

Specific optical rotation (2.4.22). +48.0° to +51.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *heptane* and 30 volumes of *acetone*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

Reference solution (a). A 0.005 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.01 per cent w/v solution of *nandrolone RS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to nandrolone is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 10 mg, dissolve in sufficient *ethanol* to produce 100.0 ml, dilute 5.0 ml to 50.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{27}H_{34}O_3$ taking 430 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Nandrolone Phenylpropionate Injection

Nandrolone Phenylpropionate Injection is a sterile solution of Nandrolone Phenylpropionate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in a mixture of these.

Nandrolone Phenylpropionate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of nandrolone phenylpropionate, $C_{27}H_{34}O_3$.

Usual strengths. 25 mg per ml; 50 mg per ml.

Identification

Dissolve a volume of the injection containing 50 mg of Nandrolone Phenylpropionate in 8 ml of *light petroleum (40° to 60°)* and extract with three 8-ml quantities of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand for 2 hours in ice and filter. The precipitate, after washing with *water* and drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, complies with the following test.

Determine by thin-layer chromatography (2.4.17), using a *silica gel GF254* precoated plate the surface of which has been modified by chemically-bonded octadecylsilyl groups.

Mobile phase. A mixture of 20 volumes of *water*, 40 volumes of *acetonitrile* and 60 volumes of *propan-2-ol*.

Test solution. A 0.5 per cent w/v solution of the dried precipitate in *chloroform*.

Reference solution (a). A 0.5 per cent w/v solution of *nandrolone phenylpropionate RS* in *chloroform*.

Reference solution (b). A mixture of equal volumes of the test solution and the reference solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the solvent has evaporated and heat it at 100° for 10 minutes. Allow to cool and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single spot.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

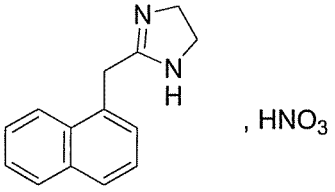
Assay. To an accurately measured volume containing about 0.1 g of Nandrolone Phenylpropionate add sufficient *chloroform* to produce 100.0 ml. Dilute 3.0 ml of this solution to 50.0 ml with *chloroform*. To 5.0 ml of the resulting solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the solution at the maximum at about 380 nm (2.4.7), using as blank 5 ml of *chloroform* treated in the same manner. Calculate the content of $C_{27}H_{34}O_3$ from the

absorbance obtained from a 0.006 per cent w/v solution of *nandrolone phenylpropionate RS* treated in the same manner.

Storage. Store protected from light.

Labelling. The label states that the preparation is for intramuscular injection only.

Naphazoline Nitrate



$C_{14}H_{14}N_2,HNO_3$ Mol. Wt. 273.3

Naphazoline Nitrate is 2-(1-napthylmethyl)-2-imidazoline nitrate.

Naphazoline Nitrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_4H_{14}N_2,HNO_3$ calculated on the dried basis.

Category. Sympathomimetic.

Description. A white or almost white crystalline powder.

Identification

Test A *may be omitted if tests B, C and D are carried out. Tests B and* C *may be omitted if tests* A *and D are carried out.*

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *naphazoline nitrate RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 270 nm, 280 nm, 287 nm and 291 nm; absorbances at these maxima are about 0.43, 0.50, 0.35 and 0.34 respectively.

C. Dissolve about 0.5 mg in 1 ml of *methanol,* add 0.5 ml of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside* and 0.5 ml of a 2 per cent w/v solution of *sodium hydroxide*, allow to stand for 10 minutes and add 1 ml of a 8 per cent w/v solution of *sodium bicarbonate;* a violet colour is produced.

D. Dissolve about 10 mg in 5 ml of *water,* add 0.2 g of *magnesium oxide,* shake mechanically for 30 minutes, add 10 ml of *chloroform* and shake vigorously. Allow to stand, separate the chloroform layer, filter and evaporate the aqueous layer to dryness. The residue gives reaction A for nitrates (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and colourless (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg of *1-naphthylacetic acid* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *naphazoline impurity A RS* in 100 ml of the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (c). Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: dissolve 1.1 g of *sodium octanesulphonate* in a mixture of 5 volumes of *glacial acetic acid,* 300 volumes of *acetonitrile* and 700 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to naphazoline and naphazoline impurity B is not less than 5.0. The relative retention time with reference to naphazoline for naphthylacetylethylenediamine (naphazoline impurity A) is about 0.76, for 1-naphthylacetic acid (naphazoline impurity B) is about 1.27, for 1-naphthylacetonitrile (naphazoline impurity C) is about 2.8, for ß-naphazoline (naphazoline impurity D) is about 1.28.

Inject the test solution, reference solution (b) and (c). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to naphazoline impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principle peak in the chromatogram obtained with reference solution (c) (1.0 per cent), Ignore any peak with an area less than 0.25 times the

area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Naphthylacetylethylenediamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *methanol* and 1.5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Reference solution. A solution containing 2 per cent w/v of *naphazoline nitrate RS* and 0.01 per cent w/v of *naphthylacetylethylenediamine hydrochloride RS*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 5 minutes, spray with a 0.5 per cent w/v solution of *ninhydrin* in *methanol* and heat at 105° for 10 minutes. Any spot corresponding to naphthylacetylethylenediamine hydrochloride in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram obtained with the reference solution. The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Chlorides (2.3.12). 15.0 ml of 1.0 per cent w/v solution in *carbon dioxide-free water* complies with the limit test for chlorides (375 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

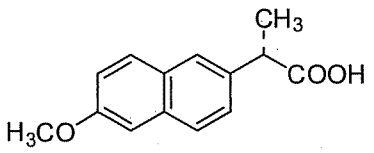
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.2 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02733 g of $C_4H_{14}N_2,HNO_3$.

Storage. Store protected from light.

Naproxen



$C_{14}H_{14}O_3$ Mol. Wt. 230.3

Naproxen is (2*S*)-2-(6-methoxynaphthalen-2-yl)propionic acid.

Naproxen contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{14}O_3$, calculated on the dried basis.

Category. Nonsteroidal antiinflammatory.

Description. A white or almost white crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *naproxen RS* or with the reference spectrum of naproxen.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.04 per cent w/v solution in *methanol* shows absorption maxima at about 262 nm, 271 nm, 316 nm and 331 nm. The absorbance at the maxima are 216 to 238, 219 to 241, 61 to 69 and 79 to 87 respectively.

C. Melting range (2.4.21). 154° to 158°.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Specific optical rotation (2.4.22). + 59° to + 62°, determined in a 2.0 per cent w/v solution in *ethanol (95 per cent)*.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

NOTE—Protect the solutions from light.

Test solution. Dissolve 25 mg of the substance under examination in 50 ml of *tetrahydrofuran*. Dilute 2.0 ml of this solution to 20 ml with the mobile phase.

Reference solution (a). Dilute 2.5 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *racemic naproxen RS* in 10 ml of *tetrahydrofuran*, dilute to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica gel π-acceptor/π-donor for chiral separations (5 µm),
- column temperature. 25°,
- mobile phase: a mixture of 5 volumes of *glacial acetic acid*, 50 volumes of *acetonitrile*, 100 volumes of *2-propanol* and 845 volumes of *hexane*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 263 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to (2*R*)-2-(6-methoxynaphthalen-2-yl) propanoic acid (naproxen impurity G) ((*R*)-enantiomer) and naproxen is not less than 3.0.

Inject reference solution (a) and the test solution. Run the chromatogram 1.5 times the retention time of the principal peak. The area of the peak due to naproxen impurity G is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Protect the solutions from light.

Test solution. Dissolve 12 mg of the substance under examination in 20 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 50 ml with the mobile phase. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (b). Dissolve 6 mg of *2-bromo-6-methoxynaphthalene RS (Naproxen impurity N RS),* 6 mg of *1-(6-methoxynaphthalen-2-yl)ethanone RS(Naproxen impurity L RS)* and 6 mg of *(1RS)-1-(6- methoxynaphthalen-2-yl)ethanol RS (Naproxen impurity K RS)* in 10 ml of *acetonitrile.* To 1.0 ml of this solution, add 1.0 ml of the test solution and dilute to 50 ml with the mobile phase. Dilute 1.0 ml of this solution to 20 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 50°,
- mobile phase: a mixture of 42 volumes of *acetonitrile* and 58 volumes of a 0.136 per cent w/v solution of *potassium dihydrogen phosphate* adjust the pH to 2.0 with *orthophosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naproxen impurity K and naproxen is not less than 2.2. The relative retention time with reference to naproxen for naproxen impurity K is about 0.9, for naproxen impurity L is about 1.4 and naproxen impurity N is about 5.3.

Inject reference solution (a), (b) and the test solution . Run the chromatogram 1.5 times the retention time of naproxen impurity N. The area of the peak due to naproxen impurity L is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (b) (0.1 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of all impurities is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.2 g and dissolve in a mixture of 25 ml of *water* and 75 ml of *methanol.* Titrate with *0.1 M sodium hydroxide* using 1 ml of *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02303 g of $C_{14}H_{14}O_3$.

Storage. Store protected from light.

Naproxen Oral Suspension

Naproxen Oral Suspension is an aqueous suspension of Naproxen in a suitable flavoured vehicle.

Naproxen Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of naproxen, $C_{14}H_{14}O_3$.

Usual strength. 25 mg per ml.

Identification

Evaporate 50 ml of solution A obtained in the Assay, to dryness using a rotary evaporator. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *naproxen RS* or with the reference spectrum of naproxen.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *methanol* exhibits maxima at 262 nm, 271 nm, 316 nm and 331 nm.

Tests

pH (2.4.24). 2.1 to 4.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 3 volumes of *glacial acetic acid,* 9 volumes of *tetrahydrofuran* and 90 volumes of *toluene.*

Test solution. Evaporate solution A obtained in the Assay to dryness on a rotary evaporator and dissolve the residue in sufficient *methanol* to produce a solution containing 5.0 per cent w/v of naproxen.

Reference solution. Dilute 1 ml of the test solution to 200 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Complies with the tests stated under Oral Liquids.

Assay. To a quantity of the oral suspension containing 0.5 g of Naproxen add 20 ml of *3.5 M hydrochloric acid*, mix, extract with three 50 ml quantities of *chloroform*, filter each extract through *anhydrous sodium sulphate*, combine the filtrates and add sufficient *chloroform* to produce 200 ml (solution A). To 5 ml of solution A add sufficient *methanol* to produce 250 ml and measure the absorbance of the resulting solution at the maximum at about 331 nm (2.4.7).

Calculate the content of $C_{14}H_{14}O_3$ taking 81 as the specific absorbance at 331 nm.

Naproxen Suppositories

Naproxen Suppositories contain Naproxen in a suitable suppository base.

Naproxen Suppositories contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of naproxen, $C_{14}H_{14}O_3$.

Usual strength. 500 mg.

Identification

Dissolve a quantity of the suppositories containing 0.5 g of Naproxen in 50 ml of *2,2,4-trimethylpentane* and extract with four 25 ml quantities of *methanol (80 per cent)*. To the combined extracts add 100 ml of a 2 per cent w/v solution of *sodium chloride*, extract with four 25 ml quantities of *chloroform*, dry the combined extracts over *anhydrous sodium sulphate*, filter and add sufficient *chloroform* to produce 200 ml (solution A). Evaporate 100 ml of solution A to dryness using a rotary evaporator. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *naproxen RS* or with the reference spectrum of naproxen.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.004 per cent w/v solution in *methanol* exhibits maxima at 262 nm, 271 nm, 316 nm and 331 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 3 volumes of *glacial acetic acid*, 9 volumes of *tetrahydrofuran* and 90 volumes of *toluene.*

Test solution. Weigh 20 suppositories and cut into small pieces. Dissolve a quantity of the suppositories containing 0.5 g of Naproxen in 50 ml of *2,2,4-trimethylpentane* and extract with four 25-ml quantities of *methanol (80 per cent).* Combine the extracts, add 100 ml of a 2 per cent w/v solution of *sodium chloride*, extract with four 25-ml quantities of *chloroform* filtering each extract through a layer of *anhydrous sodium sulphate* on an absorbent cotton plug moistened with *chloroform.* Evaporate the combined filtrates to dryness using a rotary evaporator with the aid of gentle heat. Shake the residue with 10 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution. Dilute1 ml of the test solution to 200 ml with *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Suppositories.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse ten suppositories in 500 ml of *methanol* on a water-bath for 40 minutes with the aid of ultrasound and by swirling the flask. Cool at 5° for 1 hour, centrifuge and use the clear, supernatant liquid; if the solution is still cloudy filter through glass-fibre paper (such as Whatman GF/C). To 5 ml of the filtrate add sufficient of the mobile phase to produce a solution containing 0.01 per cent w/v of naproxen.

Reference solution (a). A 0.01 per cent w/v solution of *naproxen RS* in the mobile phase.

Reference solution (b). A 0.01 per cent w/v each of *naproxen RS* and *2-naphthylacetic acid* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm × 4 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- mobile phase: a mixture of 1 volume of a 0.52 per cent w/v solution of *sodium acetate*, adjusted to pH 5.8 using *glacial acetic acid* and 1 volume of *methanol,*

- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to naproxen and 2-naphthylacetic acid in the chromatogram obtained with the reference solution (b) is more than 3.0.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{14}O_3$ in the suppositories.

Storage. Store protected from light.

Naproxen Sustained-release Tablets

Naproxen Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of naproxen, $C_{14}H_{14}O_3$.

Usual strength. 375 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7), the solution used in test B of dissolution, exhibits an absorption maximum at about 332 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (b).

Tests

Dissolution (2.5.2).

A. Apparatus No. 1,
Medium. 1000 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 2 hours.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 332 nm (2.4.7). Calculate the content of $C_{14}H_{14}O_3$ in the medium from the absorbance obtained from a solution of known concentration of *naproxen RS* in the same medium.

D. Not more than 10 per cent of the stated amount of $C_{14}H_{14}O_3$.

B. Apparatus No. 1,

Medium. 1000 ml of *0.2 M phosphate buffer pH 6.8,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 332 nm (2.4.7). Calculate the content of $C_{14}H_{14}O_3$ in the medium from the absorbance obtained from a solution of known concentration of *naproxen RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_{14}H_{14}O_3$.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Test solution. Disperse 1 tablet in about 140 ml of solvent mixture B. Shake for 15 minutes, sonicate for 15 minutes, dilute to 200 ml with solvent mixture B, filter. Dilute 2.0 ml of the filtrate to 50 ml with the mobile phase .

Reference solution. A 0.025 per cent w/v solution of *naproxen RS* in solvent mixture A. Dilute 10 ml of this solution to 25 ml with solvent mixture B.

Calculate the content of $C_{14}H_{14}O_3$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture A. 90 volumes of *acetonitrile* and 10 volumes of *water.*

Solvent mixture B. 50 volumes of *acetonitrile* and 50 volumes of *water.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 250 mg of Naproxen in 70 ml of solvent mixture B, sonicate for 15 minutes and dilute to 100.0 ml with solvent mixture B and filter. Dilute 2.0 ml of the filtrate to 50 ml with the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *naproxen RS* in solvent mixture A.

Reference solution (b). Dilute 10 ml of reference solution (a) to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of 1.0 per cent v/v solution of *acetic acid* and 45 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

Inject reference solution (b). The test is not valid unless the tailing factor of the principal peak is not more than 1.5 per cent and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b).

Calculate the content of $C_{14}H_{14}O_3$ in the tablet.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Naproxen Tablets

Naproxen Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of naproxen, $C_{14}H_{14}O_3$.

Usual strengths. 250 mg; 500 mg.

Identification

A. Extract a quantity of the powdered tablets containing 20 mg of Naproxen with 100 ml of *methanol* and filter. Evaporate and dry the residue at 105°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *naproxen RS* or with the reference spectrum of naproxen.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* exhibits maxima at 262 nm, 271 nm, 316 nm and 331 nm.

Tests

Dissolution (2.5.2).

Apparatus No.1,
Medium. 900 ml of a phosphate buffer prepared by dissolving 2.62 g of *sodium dihydrogen orthophospate monohydrate* and 11.5 g of *anhydrous disodium hydrogen orthophosphate* in sufficient *water* to produce 1000 ml, adjusted to pH 7.4 with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at about 332 nm (2.4.7). Calculate the content of $C_{14}H_{14}O_3$, in the medium from the absorbance obtained from a solution of known concentration of *naproxen RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{14}H_{14}O_3$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *glacial acetic acid*, 9 volumes of *tetrahydrofuran* and 90 volumes of *toluene*.

Test solution. Shake a quantity of the powdered tablets containing about 0.5 g of Naproxen with 10 ml of *methanol* for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution. Dilute 1 ml of the test solution to 200 ml with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

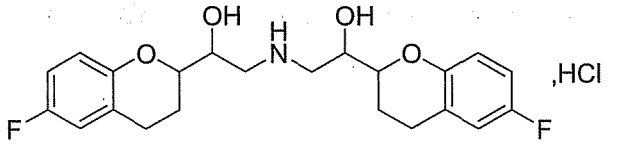
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powder containing about 50 mg of Naproxen with 70 ml of *methanol* for 30 minutes, add sufficient *methanol* to produce 100 ml and filter. Dilute 10 ml of the filtrate to 50 ml with *methanol* and measure the absorbance at the maximum at about 331 nm (2.4.7) using a 0.01 per cent w/v solution of *naproxen RS* in *methanol*.

Calculate the content of $C_{14}H_{14}O_3$ in the tablets.

Storage. Store protected from light.

Nebivolol Hydrochloride



$C_{22}H_{25}F_2NO_4,HCl$ Mol. Wt. 441.9

Nebivolol Hydrochloride is (1*RS*,1'*RS*)-1, 1'-[(2*RS*,2'*SR*)-bis (6-flurochroman-2-yl)]-2,2'-iminodiethanol hydrochloride.

Nebivolol Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{25}F_2NO_4,HCl$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nebivolol hydrochloride RS* or with the reference spectrum of nebivolol hydrochloride.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 30 mg of the substance under examination in 5 ml of *acetonitrile* and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 30 mg of *nebivolol hydrochloride RS* in 5 ml of *acetonitrile* and dilute to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica (5 µm) with chemically bonded phenyl groups,

- mobile phase: a mixture of 28 volumes of *acetonitrile*, 72 volumes of a buffer solution prepared by dissolving 3.4 g of *tetrabutyl ammonium hydrogen sulphate* in 1000 ml *water*, and 0.3 volume of *diethylamine*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency in not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 35 mg of the substance under examination in 5 ml of *acetonitrile* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution. Dissolve 35 mg of *nebivolol hydrochloride RS* in 5 ml of *acetonitrile* and dilute to 50.0 ml with the mobile phase. Dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Use the chromatographic system described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{25}F_2NO_4$, HCl.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Nebivolol Tablets

Nebivolol Hydrochloride Tablets

Nebivolol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nebivolol, $C_{22}H_{25}F_2NO_4$.

Usual strength. 5 mg

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 500 ml *0.1 M hydrochloric acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions and a flow rate of 1.5 ml per minute.

Test solution. The filtrate obtained as given above.

Reference solution. A solution containing *nebivolol hydrochloride RS* equivalent to 0.001 per cent w/v of nebivolol, in the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{22}H_{25}F_2NO_4$.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and the reference solution described in the Assay.

Test solution. Disperse one tablet in the mobile phase, dilute to obtain a solution containing 0.005 per cent w/v of nebivolol in the mobile phase and filter.

Calculate the content of $C_{22}H_{25}F_2NO_4$ in the tablets.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of nebivolol, disperse in 100.0 ml of the mobile phase and filter.

Reference solution. A solution containing *nebivolol hydrochloride RS* equivalent to 0.005 per cent w/v of nebivolol, in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adding 2 ml of *triethylamine* and adjusting the pH to 3.0 with 10 per cent v/v *orthophosphoric acid*, 25 volumes of *acetonitrile* and 30 volumes of *methanol*,

- flow rate. 1 ml per minute,
- spectrophotometer set at 281 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

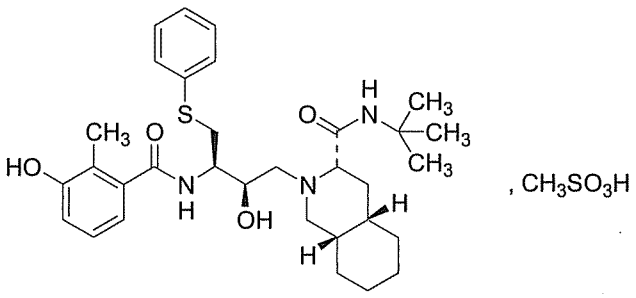
Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{25}F_2NO_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of nebivolol.

Nelfinavir Mesylate



$C_{32}H_{45}N_3O_4S,CH_4O_3S$ Mol. Wt. 663.9

Nelfinavir Mesylate is (3*S*,4a*S*,8a*S*)-*N*-(*tert*-butyldecahydro-2-[(2*R*,3*R*)-3-(3-hydroxy-*o*-toluamido)-hydroxy-4-(phenylthio)butyl]isoquinoline-3-carboxamide methyl sulphonate.

Nelfinavir Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{32}H_{45}N_3O_4S,CH_4O_3S$, calculated on the anhydrous basis.

Category. Antiretroviral.

Dose. 1.25 g twice daily.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nelfinavir mesylate RS* or with the reference spectrum of nelfinavir mesylate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –105° to –120°, determined in a 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14), using the chromatographic system described in the Assay.

Test solution. A 0.1 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution (b). A 0.01 per cent w/v solution of *methanesulphonic acid* in the mobile phase.

Inject reference solution (a). The test is not valid unless the column efficiency determined from the nelfinavir peak is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Separately inject reference solution (b) and record the chromatograms. Separately inject the test solution and continue the chromatography for at least three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than half of the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak due to methanesulphonic acid corresponding to the retention time of the principal peak in the chromatogram obtained with reference solution (b).

Methanesulphonic acid. 13.5 per cent to 15.5 per cent w/w, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.6 g, dissolve in 50 ml of *dimethylformamide* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00961 g of CH_3SO_3H.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.01 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *nelfinavir mesylate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile*, 20 volumes of *methanol* and 35 volumes of a buffer prepared by dissolving 4.0 g of *sodium dihydrogen phosphate* in 1000 ml of *water*, to which 1 ml of *dimethylamine* solution and 1 g of *sodium octanesulphonate* are added and mixed to dissolve,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nelfinavir peak is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the responses for the principal peak. Calculate the content of $C_{32}H_{45}N_3O_4S,CH_4O_3S$.

Storage. Store protected from light.

Nelfinavir Mesylate Oral Powder

Nelfinavir Mesylate Oral Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nelfinavir, $C_{32}H_{45}N_3O_4S$.

Usual strength. 50 mg per g.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 1

Medium. 900 ml of *0.1 M hydrochloric acid.*

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate and, if necessary, dilute with the dissolution medium.

Reference solution. A 0.065 per cent w/v solution of *nelfinavir mesylate RS* in *methanol.* Dilute 10 ml of the solution to 100 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the test solution and the reference solution.

D. Not less than 75 per cent of the stated amount of $C_{32}H_{45}N_3O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the oral powder containing 50 mg of Nelfinavir Mesylate, disperse in 10 ml of *methanol*, dilute to 50 ml with the mobile phase and filter.

Reference solution (a). Dissolve 10 mg of *nelfinavir mesylate RS* in 2 ml of *methanol* and dilute to 10 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 28 volumes of a buffer solution prepared by dissolving 4.88 g of *anhydrous sodium dihydrogen phosphate* in 1000 ml of *water*, adjusting the pH to 3.4 with *phosphoric acid* and filtering, 27 volumes of *acetonitrile*, 20 volumes of *methanol* and 25 volumes of *water*. Adjust the pH to 4.8 with *0.1 M sodium hydroxide* or *orthophosphoric acid.*
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 4000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Water (2.3.43). Not more than 12.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol.*

Test solution. Weigh accurately a quantity of the powder containing 50 mg of Nelfinavir Mesylate, disperse in 50 ml of *0.1 M hydrochloric acid,* dilute to 250.0 ml with the solvent mixture and filter.

Reference solution. Dissolve 10 mg of *nelfinavir mesylate RS* in 10 ml of *0.1 M hydrochloric acid* and dilute to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 4 g of *sodium dihydrogen phosphate dihydrate* and 1g of *1-octane sulphonic acid sodium salt* into 1000 ml of *water*, adding 1ml of *dimethylamine* and filtering, 45 volumes *acetonitrile* and 20 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{32}H_{45}N_3O_4S$ in the oral powder.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of nelfinavir.

Nelfinavir Tablets

Nelfinavir Mesylate Tablets

Nelfinavir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nelfinavir mesylate, $C_{32}H_{45}N_3O_4S,CH_4O_3S$.

Usual strengths. 250 mg; 625 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 0.1 g of Nelfinavir Mesylate with 80 ml of *methanol* for 10 minutes, add sufficient *methanol* to produce 100 ml, mix and filter. Dilute 5 ml of the filtrate to 100 ml with *methanol*.

When examined in the range 200 nm to 300 nm the resulting solution shows an absorption maximum only at about 254 nm (2.4.7).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.01 M hydrochloric acid*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{32}H_{45}N_3O_4S,CH_4O_3S$ from the absorbance of a solution of known concentration of *nelfinavir mesylate RS*.

D. Not less than 75 per cent of the stated amount of $C_{32}H_{45}N_3O_4S, CH_4O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 100 mg of Nelfinavir Mesylate, add about 20 ml of *methanol*, mix with the aid of ultrasound for 10 minutes and dilute to 100 ml with the mobile phase.

Reference solution. Weigh accurately about 10 mg of *nelfinavir mesylate RS*, add about 10 ml of *methanol*, shake for 10 minutes and dilute to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica particles or ceramic microparticles (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile*, 20 volumes of *methanol* and 35 volumes of a buffer prepared by dissolving 4.0 g of *sodium dihydrogen phosphate* in 1000 ml of *water*, to which are added 1 ml of *dimethylamine solution* and 1 g of *sodium octanesulphonate* and mixing to dissolve,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nelfinavir mesylate peak is not less than 4000 theoretical plates and the tailing factor is not more than 2.0.

Inject separately the diluent (10 ml of *methanol* diluted to 50 ml with the mobile phase) and the test solution and continue the chromatography for 4 times the retention time of the principal peak. Examine the diluent chromatogram for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent

and the sum of the areas of all the secondary peaks should not be more than 2.0 per cent when calculated by percentage area normalisation. Inhibit integration of peak due to methanesulphonic acid.

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 200 mg of Nelfinavir Mesylate, add about 20 ml of *methanol*, mix with the aid of ultrasound for 10 minutes and dilute to 100.0 ml with the mobile phase. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Further dilute 5.0 ml of the filtrate to 100.0 ml with the mobile phase.

Reference solution. Weigh accurately about 50 mg of *nelfinavir mesylate RS*, add about 10 ml of *methanol*, mix with the aid of ultrasound to dissolve and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Use the chromatographic system described in the test for Related substances.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nelfinavir mesylate peak is not less than 5000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak. Calculate the content of $C_{32}H_{45}N_3O_4S,CH_4O_3S$ in the tablets.

Storage. Store protected from light.

Neomycin Sulphate

Neomycin Sulphate is a mixture of the sulphates of substances obtained by the growth of certain selected strains of *Streptomyces fradiae*.

Neomycin Sulphate has a potency of not less than 600 Units per mg, calculated on the dried basis.

Category. Antibacterial (topical and systemic).

Dose. 1 g every 4 hours.

Description. A white or yellowish-white powder; odourless or almost odourless; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A freshly prepared 3.85 per cent w/v solution of *ammonium acetate.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *water.*

Reference solution. A 2.0 per cent w/v solution of *neomycin sulphate RS* in *water.*

Apply to the plate 1 µl of each solution. After development, dry the plate in air for 10 minutes, heat at 100° for 1 hour and spray with a 0.1 per cent w/v solution of *ninhydrin* in *1-butanol* saturated with *water*. Heat again at 100° for 5 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve about 10 mg in 5 ml of *water*, add 0.1 ml of *pyridine* and 2 ml of a 0.1 per cent w/v solution of *ninhydrin* and heat on a water-bath at a temperature of about 70° for 10 minutes; a deep violet colour is produced.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22).+53.5° to +59.0°, determined in a 10.0 per cent w/v solution.

Neamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 30 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *dichloromethane.*

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of *water.*

Reference solution. A 0.05 per cent w/v solution of *neamine RS* in *water.*

Apply to the plate as 5-mm bands 5 µl of each solution. Dry the bands; allow the mobile phase to rise at least 8 cm. Dry the plate in a current of warm air, heat at 110° for 10 minutes, spray the plate with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any band corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Neomycin C. Determine by thin-layer chromatography (2.4.17), coating the plate with silica gel of a suitable grade.

Mobile phase. A mixture of 80 volumes of a 20 per cent w/v solution of *sodium chloride* and 20 volumes of *methanol.*

Test solution. Dissolve 40 mg of the substance under examination in water and dilute to 5 ml with the same solvent.

Reference solution (a). Dissolve 30 mg of *framycetin sulphate RS* in *water* and dilute to 25 ml with the same solvent.

Reference solution (b). Dilute 5 ml of reference solution (a) to 25 ml with *water.*

Reference solution (c). Dissolve 40 mg of *neomycin sulphate RS* in *water* and dilute to 5 ml with the same solvent.

Apply to the plate as 5 mm bands 5 µl of each solution. Dry the bands; allow the mobile phase to rise at least 12 cm. Dry the plate at 100° to 105° for 10 minutes. Spray the plate with *ethanolic ninhydrin solution* and heat at 100° to 105° for 10 minutes. In the chromatogram obtained with the test solution the principal band corresponds to the principal band in the chromatogram obtained with reference solution (c) and the band due to neomycin C with R_f value slightly less than that of the principal band is not more intense than the band obtained with reference solution (a) (15 per cent) but is more intense than the band in the chromatogram obtained with reference solution (b) (3 per cent). The test is not valid unless in the chromatogram obtained with reference solution (c) a band appears with R_f value slightly less than that of the principal band.

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 0.5 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10).

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of Units of neomycin per mg.

Neomycin Eye Drops

Neomycin Sulphate Eye Drops

Neomycin Sulphate Eye Drops are a sterile solution of Neomycin Sulphate in Purified Water.

Neomycin Sulphate Eye Drops contain not less than 90.0 per cent and not more than 115.0 per cent w/v of the stated amount of neomycin sulphate.

Usual strength. 0.5 per cent w/v (3500 Units per ml).

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel.*

Mobile phase. A mixture of 60 volumes of *methanol,* 40 volumes of *strong ammonia solution* and 20 volumes of *chloroform.*

Test solution. Dilute if necessary a volume of the eye drops to produce a solution containing 0.5 per cent w/v of Neomycin Sulphate in *water.*

Reference solution (a). A 0.5 per cent w/v solution of *neomycin sulphate RS* in *water.*

Reference solution (b). A mixture of equal volumes of the eye drops and reference solution (a).

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with a 1 per cent w/v solution of *ninhydrin* in *1-butanol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and the principal red spot in the chromatogram obtained with reference solution (b) appears as a single spot.

Tests

Neamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 30 volumes of *methanol,* 20 volumes of *strong ammonia solution* and 10 volumes of *dichloromethane.*

Test solution. A volume of the eye drops containing 5 µg (3.5 Units).

Reference solution. The same volume of water containing 0.1 µg of *neamine RS.*

Apply to the plate each solution. After development, dry the plate in a current of warm air, heat at 110° for 10 minutes, spray the plate with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any spot corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Neomycin C. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops with *0.02 M borax* to contain 1 mg (700 Units) per ml. To 0.5 ml of the diluted solution add 1.5 ml of a freshly prepared 2 per cent w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol,* dilute to 25 ml with the mobile phase, allow to stand and use the clear lower layer.

Reference solution. Add 1.5 ml of the 1-fluoro-2,4-dinitrobenzene solution to 0.5 ml of a 0.1 per cent w/v solution of *neomycin sulphate RS* in *0.02 M borax,* heat in a water-bath at 60° for 1 hour and cool; dilute the solution to 25 ml with the mobile phase, allow to stand and use the clear lower layer.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with porous silica particles (5 µm) (such as Nucleosil 100-5),
- mobile phase: a mixture of 97 ml of *tetrahydrofuran*, 1.0 ml of *water* and 0.5 ml of *glacial acetic acid* diluted with sufficient of a 2.0 per cent v/v solution of *ethanol* in *ethanol-free chloroform* to produce 250 ml,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 10 µl.

If necessary the tetrahydrofuran and water content of the mobile phase may be adjusted so that the chromatogram obtained with the reference solution shows resolution similar to that in the specimen chromatogram supplied with *framycetin sulphate RS*. The mobile phase should be passed through the column for several hours before the solutions are injected. Continue the chromatography for 1.4 times the retention time of the peak due to neomycin B.

The column efficiency, determined using the peak due to Neomycin B in the chromatogram obtained with the test solution, should be not less than 13,000 theoretical plates.

In the chromatogram obtained with the test solution the area of the peak corresponding to neomycin C is not less than 3.0 per cent and not more than 15.0 per cent of sum of the areas of the peaks corresponding to Neomycin B and Neomycin C.

Other tests. Complies with the tests stated under Eye Drops.

Assay. Measure accurately a quantity containing 5 mg of Neomycin Sulphate and dilute to 50.0 ml with sterile *phosphate buffer pH 8.0* and mix. Dilute 10.0 ml of the resulting solution to 100.0 ml with the same solvent.

Determine by the microbiological assay of antibiotics, Method A (2.2.10)

The upper fiducial limit of error is not less than 90.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units per ml.

Storage. Store protected from light.

Labelling. The strength is stated in terms of percentage w/v as well as the number of Units per ml.

Neomycin Eye Ointment

Neomycin Sulphate Eye Ointment

Neomycin Sulphate Eye Ointment is a sterile preparation containing Neomycin Sulphate in a suitable basis.

Neomycin Sulphate Eye Ointment contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of neomycin sulphate.

Usual strength. 0.5 per cent w/w (3500 Units per g).

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 60 volumes of *methanol*, 40 volumes of *strong ammonia solution* and 20 volumes of *chloroform*.

Test solution. Disperse a quantity of the eye ointment containing 20 mg of Neomycin Sulphate in 20 ml of *chloroform*, extract with 5 ml of *water* and use the aqueous extract.

Reference solution (a). A 0.4 per cent w/v solution of *neomycin sulphate RS* in *water*.

Reference solution (b). A mixture of equal volumes of test solution and reference solution (a).

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with a 1 per cent w/v solution of *ninhydrin* in *1-butanol* and heat at 105° for 2 minutes. The principal red spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and the principal red spot in the chromatogram obtained with reference solution (b) appears as a single spot.

Tests

Neamine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 30 volumes of *methanol*, 20 volumes of *strong ammonia solution* and 10 volumes of *dichloromethane*.

Test solution. Disperse a quantity of the eye ointment containing 20 mg of Neomycin Sulphate in 20 ml of *chloroform*, shake gently with 8 ml of *water*, allow the layers to separate and use the aqueous layer.

Reference solution. A 0.005 per cent w/v solution of *neamine RS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in a current of warm air, heat at 110° for 10 minutes, spray with *ninhydrin and stannous chloride reagent* and heat at 110° for 15 minutes. Spray the plate again with the same reagent and heat at 110° for 15 minutes. Any spot corresponding to neamine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Neomycin C. Determine by liquid chromatography (2.4.17)

Test solution. Disperse a quantity of the eye ointment containing 5 mg of Neomycin Sulphate in 20 ml of *light petroleum (120° to 160°)*, add 5 ml of *0.02 M borax*, shake, separate the aqueous layer and centrifuge. To 0.5 ml of the separated aqueous layer add 1.5 ml of a freshly prepared 2 per

cent w/v solution of *1-fluoro-2,4-dinitrobenzene* in *methanol*, heat on a water-bath at 60° for 1 hour and cool. Dilute the resulting solution to 25 ml with the mobile phase, allow to stand and use the clear lower layer.

Reference solution. Add 1.5 ml of the *1-fluoro-2,4-dinitrobenzene solution* to 0.5 ml of a 0.1 per cent w/v solution of *neomycin sulphate RS* in *0.02 M borax* and proceed as for the test solution.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with porous silica particles (5 µm),
- mobile phase: 97 ml of *tetrahydrofuran*, 1.0 ml of *water* and 0.5 ml of *glacial acetic acid* with sufficient of a 2.0 per cent v/v solution of *ethanol* in *ethanol-free chloroform* to produce 250 ml,
- flow rate. 1.6 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 10 µl.

If necessary the tetrahydrofuran and water content of the mobile phase may be adjusted so that the chromatogram obtained with reference solution shows resolution similar to that in the specimen chromatogram supplied with *framycetin sulphate RS*. The mobile phase should be passed through the column for several hours before the solutions are injected. Continue the chromatography for 1.4 times the retention time of the peak due to neomycin B.

The column efficiency, determined using the peak due to Neomycin B in the chromatogram obtained with the test solution, should be not less than 13,000 theoretical plates.

In the chromatogram obtained with the test solution the area of the peak corresponding to neomycin C is not less than 3.0 per cent and not more than 15.0 per cent of the sum of the areas of the peaks corresponding to Neomycin B and Neomycin C.

Other tests. Complies with the tests stated under Eye Ointments.

Assay. Weigh accurately a quantity containing 5 mg of Neomycin Sulphate, dissolve in 25 ml of *chloroform*, extract with four quantities, each of 20 ml, of sterile *phosphate buffer pH 8.0*, combine the extracts and add sufficient of the buffer solution to produce 100.0 ml.

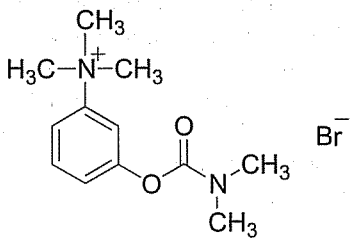
Carry out the microbiological assay of antibiotics, Method A (2.2.10).

The upper fiducial limit of error is not less than 90.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units per g.

Storage. Store protected from light.

Labelling. The strength is stated in terms of percentage w/v as well as the number of Units per ml.

Neostigmine Bromide



$C_{12}H_{19}BrN_2O_2$ Mol. Wt. 303.2

Neostigmine Bromide is 3-(dimethylcarbamoyloxy) trimethylanilinium bromide.

Neostigmine Bromide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{12}H_{19}BrN_2O_2$, calculated on the dried basis.

Category. Anticholinesterase.

Dose. 15 to 30 mg, repeated at suitable intervals; total daily dose, 75 to 300 mg.

Description. Colourless crystals or a white, crystalline powder; odourless; hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *neostigmine bromide RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.02 per cent w/v solution in *0.5 M sulphuric acid* shows absorption maxima at about 260 nm and 266 nm.

C. Warm about 50 mg with 1 ml of *dilute sodium hydroxide solution*; an odour of dimethylamine develops slowly.

D. Warm about 50 mg with 0.4 g of *potassium hydroxide* and 2 ml of *ethanol (95 per cent)* on a water-bath for 3 minutes, replacing the evaporated *ethanol*. Cool, add 2 ml of *dilute diazobenzenesulphonic acid solution*; an orange-red colour is produced.

E. Gives the reactions of bromides (2.3.1).

Tests

Appearance of solution. A 0.5 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

Acidity. Dissolve 0.2 g in 20 ml of *carbon dioxide-free water* and titrate to pH 7.0 with *0.02 M sodium hydroxide* (carbonate-free); not more than 0.1 ml is required.

3-Hydroxytrimethylanilinium bromide. Dissolve 50 mg in a mixture of 1 ml of *sodium carbonate solution* and 9 ml of *water*. Absorbance of the resulting solution at about 294 nm, measured immediately after preparation, not more than 0.25 (2.4.7).

Sulphates (2.3.17). 0.75 g complies with the limit test for sulphates (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 20 ml of *anhydrous glacial acetic acid*, add 5 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03032 g of $C_{12}H_{19}BrN_2O_2$.

Storage. Store protected from light and moisture.

Neostigmine Tablets

Neostigmine Bromide Tablets

Neostigmine Bromide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of neostigmine bromide, $C_{12}H_{19}BrN_2O_2$.

Usual strength. 15 mg.

Identification

Triturate a quantity of the powdered tablets containing about 0.3 g of Neostigmine Bromide with three quantities, each of 5 ml of *ether* and discard the ether. Macerate the residue with several quantities, each of 10 ml of *ethanol (95 per cent)*, filtering after each maceration. Evaporate the combined filtrates on a water-bath and dry the residue at 105° for 1 hour. The residue melts at about 167°, with decomposition. The residue complies with the following tests.

A. Warm about 50 mg with 0.4 g of *potassium hydroxide* and 2 ml of *ethanol (95 per cent)* on a water-bath for 3 minutes, replacing the evaporated *ethanol*. Cool, add 2 ml of *dilute diazobenzenesulphonic acid solution*; an orange-red colour is produced.

B. Gives the reactions of bromides (2.3.1).

Tests

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Neostigmine Bromide, transfer to a semi-micro ammonia-distillation apparatus, add 20 ml of a 50 per cent w/v solution of *sodium hydroxide* and 0.5 ml of a 2 per cent w/v solution of *2-octanol* in *liquid paraffin*. Pass a current of steam through the mixture, collect the distillate in 50 ml of *0.01 M sulphuric acid* until the volume is about 200 ml and titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sulphuric acid required to neutralise the dimethylamine produced.

1 ml of *0.01 M sulphuric acid* is equivalent to 0.006064 g of $C_{12}H_{19}BrN_2O_2$.

Storage. Store protected from light and moisture.

Neostigmine Methylsulphate

$C_{13}H_{22}N_2O_6S$ Mol. Wt. 334.4

Neostigmine Methylsulphate is 3-(dimethylcarbamoyloxy)-trimethylanilinium methyl sulphate.

Neostigmine Methylsulphate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{22}N_2O_6S$, calculated on the dried basis.

Category. Anticholinesterase.

Dose. By subcutaneous or intramuscular injection, 1 to 2.5 mg, repeated at suitable intervals; total daily dose, 5 to 20 mg.

Description. Colourless crystals or a white, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *neostigmine methylsulphate RS* or with the reference spectrum of neostigmine methylsulphate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.5 M sulphuric acid* shows absorption maxima, at about 261 nm and 267 nm. The ratio of the absorbance at the maximum at about 267 nm to that at the maximum at 261 nm is 0.84 to 0.87.

C. Dissolve 0.1 g in 5 ml of *distilled water* and add 1 ml of a 6 per cent w/v solution of *barium chloride*; no precipitate is produced. Add 2 ml of *hydrochloric acid* and heat in a water-bath for 10 minutes; a white precipitate is produced.

D. Warm about 50 mg with 0.4 g of *potassium hydroxide* and 2 ml of *ethanol (95 per cent)* on a water-bath for 3 minutes,

replacing the evaporated *ethanol*. Cool, add 2 ml of *dilute diazobenzenesulphonic acid solution*; an orange-red colour is produced.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *distilled water* is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 4.0 ml of a 5.0 per cent w/v solution in *distilled water* add 6.0 ml of *water* and 0.1 ml of *phenolphthalein solution*; the solution is colourless. Add 0.3 ml of *0.01 M sodium hydroxide*; the solution becomes red. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution becomes colourless. Add 0.1 ml of *methyl red solution*; the solution becomes red or yellowish-red.

3-Hydroxytrimethylanilinium methyl sulphate. Dissolve 50 mg in a mixture of 1 ml of *sodium carbonate solution* and 9 ml of *water*. Absorbance of the resulting solution at about 294 nm, measured immediately after preparation, not more than 0.20 (2.4.7).

Chlorides (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 0.75 g complies with the limit test for sulphates (200 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.3 g and dissolve in 150 ml of *water*. Add 100 ml of *2 M sodium hydroxide*, distill and collect the distillate in 50 ml of a 4 per cent w/v solution of *boric acid* until a total volume of 250 ml is reached. Titrate the distillate with *0.1 M hydrochloric acid* using 0.25 ml of *methyl red-methylene blue solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of hydrochloric acid required.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.03344 g of $C_{13}H_{22}N_2O_6S$.

Storage. Store protected from light and moisture.

Neostigmine Injection

Neostigmine Methylsulphate Injection

Neostigmine Injection is a sterile solution of Neostigmine Methylsulphate in Water for Injections.

Neostigmine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of neostigmine methylsulphate, $C_{13}H_{22}N_2O_6S$.

Usual strengths. 0.5 mg per ml; 2.5 mg per ml.

Identification

A. Dilute, if necessary, a volume of the injection containing 2.5 mg of Neostigmine Methylsulphate to 5 ml with *water*, shake with three quantities, each of 10 ml, of *ether* and discard the ether extracts.

When examined in the range 230 nm to 360 nm (2.4.7), a 2 cm layer of the resulting solution shows absorption maxima at about 260 nm and 267 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 35 volumes of *methanol*, 10 volumes of *formic acid* and 5 volumes of *water*.

Test solution. Dilute the injection under examination, if necessary, with *water* to produce a solution containing 0.05 per cent w/v of Neostigmine Methylsulphate.

Reference solution (a). A 0.05 per cent w/v solution of *neostigmine methylsulphate RS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. To 1 ml add 0.5 ml of *sodium hydroxide solution* and evaporate to dryness on a water-bath. Heat quickly in an oil-bath to about 250° and maintain at this temperature for about 30 seconds. Cool, dissolve the residue in 1 ml of *water*, cool in ice water and add 1 ml of *diazobenzenesulphonic acid solution*; an orange-red colour is produced.

Tests

pH. (2.4.24) 4.5 to 6.5.

3-Hydroxy trimethylanilinium methyl sulphate. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the injection if necessary, with *water* to contain a 0.05 per cent w/v solution of Neostigmine Methylsulphate.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *water*.

Reference solution (b). Add 0.05 ml of *5 M sodium hydroxide* to 1 ml of the test solution and allow to stand for 5 minutes. Add 0.1 ml of *5 M hydrochloric acid* and use immediately.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane chemically bonded to porous silica particles (5 µm) (such as Lichrosphere 60 RP-select B),
- mobile phase: *0.0015 M* solution of *sodium heptanesulphonate* in a mixture of 15 volumes of *acetonitrile* and 85 volumes of *0.05 M potassium dihydrogen orthophosphate* adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. of 1.1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

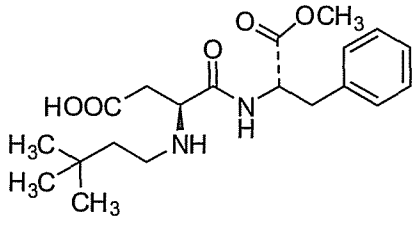
In the chromatogram obtained with reference solution (b) the principal peak has a retention time of about 6.8 minutes (neostigmine methylsulphate) and there is a peak with a relative retention time of about 0.5 (3-hydroxy) trimethylanilinium methylsulphate. In the chromatogram obtained with the test solution, the area of any secondary peak with a retention time corresponding to that of the peak due to (3-hydroxy)trimethylanilinium methylsulphate in the chromatogram obtained with reference solution (b) is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume containing about 25 mg of Neostigmine Methylsulphate to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 260 nm (2.4.7). Calculate the content of $C_{13}H_{22}N_2O_6S$ taking 14.35 as the specific absorbance at 260 nm.

Storage. Store protected from light.

Neotame



$C_{20}H_{30}N_2O_5$ Mol.Wt. 378.4

Neotame is *N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine 2-methyl ester.

Neotame contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{20}H_{30}N_2O_5$, calculated on the dried basis.

Category. Non-nutritive sweetener.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *neotame RS* or with the reference spectrum of neotame.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). –40° to –43.4°, determined in a 0.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.002 per cent w/v solution of *neotame RS* in the mobile phase.

Reference solution (b). A 0.003 per cent w/v solution of *neotame impurity A RS* in the mobile phase.

Use the chromatographic system as described under Assay.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of peak corresponding to neotame impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent) and the sum of the areas of all other secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent).

Loss on ignition (2.4.20). Not more than 0.2 per cent.

Water (2.3.43). Not more than 5.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of substance under examination in 25.0 ml of mobile phase. Dilute 5.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *neotame RS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),

- column temperature. 45°,
- mobile phase: dissolve 3 gm of *sodium 1-heptanesulphonate* and 3.8 ml of *triethylamine* in 750 ml of *water*, adjust the pH to 3.5 with *orthophosphoric acid*. Add 250 ml of *acetonitrile*, adjust the pH to 3.7 with *orthophosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

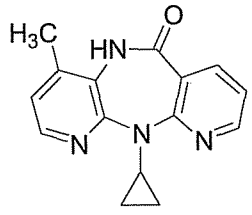
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{20}H_{30}N_2O_5$.

Storage. Store protected from moisture.

Nevirapine



$C_{15}H_{14}N_4O$ Mol. Wt. 266.3

Nevirapine is 11-cyclopropyl-5,11-dihydro-4-methy-6*H*-dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one.

Nevirapine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{14}N_4O$, calculated on the dried basis.

Category. Antiretroviral.

Dose. 200 mg once or twice daily.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nevirapine RS* or with the reference spectrum of nevirapine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol.*

Reference solution. Dilute 1 ml of the test solution to 100 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a filtered and degassed mixture of 20 volumes of *methanol*, 20 volumes of *acetonitrile* and 60 volumes of a buffer prepared by dissolving 12.0 g of *sodium dihydrogen phosphate* in about 800 ml of *water*, adjusting the pH to 3.0 with *phosphoric acid* and diluting to 1000.0 ml with *water,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nevirapine peak is not less than 5000 theoretical plates and the tailing factor is not more than 2.0.

Separately inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than half of the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such peaks is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 105° for 3 hours.

Assay: Determine by liquid chromatography (2.4.14).

Test solution. A 0.005 per cent w/v solution of the substance under examination in *methanol.*

Reference solution. A 0.005 per cent w/v solution of *nevirapine RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a filtered and degassed mixture of 20 volumes of *methanol*, 20 volumes of *acetonitrile* and 60 volumes of a buffer prepared by dissolving 12.0 g of *sodium dihydrogen phosphate* in about 800 ml of *water*, adjusting the pH to 3.0 with *orthophosphoric acid* and diluting to 1000.0 ml with *water,*

- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nevirapine peak is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the responses for the principal peak. Calculate the content of $C_{15}H_{14}N_4O$.

Storage. Store protected from moisture.

Nevirapine Oral Suspension

Nevirapine Oral Suspension is a suspension of Nevirapine in a suitable flavoured vehicle.

Nevirapine Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nevirapine, $C_{15}H_{14}N_4O$.

Usual strength. 50 mg in 5 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *1-butanol*, 30 volumes of *heptane*, 30 volumes of *acetone* and 10 volumes of *strong ammonia solution*.

Test solution. Dilute the preparation under examination with *methanol* to obtain a solution containing 1 mg of Nevirapine per ml.

Reference solution. A 0.1 per cent w/v solution of *nevirapine RS* in a mixture of 75 volumes of *methanol* and 25 volumes of *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.0 to 7.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To an accurately measured volume of the preparation under examination containing about 25 mg of Nevirapine add about 10 ml of *methanol*, mix with the aid of ultrasound for 10 minutes, dilute to 50 ml with *water*, mix and filter.

Reference solution. Weigh accurately about 25 mg of *nevirapine RS*, add about 10 ml of *methanol*, mix with the aid of ultrasound to dissolve and dilute to 50 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilyl silica gel for chromatography (5 µm) (Such as Hypersil C8),
- mobile phase: A. *0.1 M ammonium acetate*, B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
25	20	80
30	20	80
31	95	5
40	95	5

Inject the reference solution. The test is not valid unless the column efficiency determined from the nevirapine peak is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject separately the diluent (dilute 10 ml of *methanol* to 50 ml with *water*) and the test solution. Examine the diluent chromatogram for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution. Ignore any peaks due to preservatives also.

Any secondary peak observed in the chromatogram obtained with the test solution should not be more than 1.0 per cent and the sum of the areas of all the secondary peaks should not be more than 2.0 per cent when calculated by percentage area normalisation.

Other tests. Comply with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the preparation under examination containing 25 mg of Nevirapine, add about 10 ml of *methanol*, mix with the aid of ultrasound for 10 minutes, dilute to 50.0 ml with *water*, mix and filter. Further dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

Reference solution. Weigh accurately about 50 mg of *nevirapine RS,* add about 20 ml of *methanol,* mix with the aid of ultrasound to dissolve and dilute to 100.0 ml with *water.* Dilute 10.0 ml of this solution to 25.0 ml with *water.*

Use the chromatographic system described under the test for Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the principal peak.

Determine the weight per ml of the oral suspension (2.4.29) and calculate the content of $C_{15}H_{14}N_4O$ weight in volume.

Storage. Store protected from light.

Nevirapine Tablets

Nevirapine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nevirapine, $C_{15}H_{14}N_4O$.

Usual strength. 200 mg.

Identification

A. When examined in the range 200 nm to 400 nm (2.4.7) a 0.001 per cent w/v solution in the mobile phase described under Assay, shows an absorption maximum at about 230 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).
Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute suitably, if necessary. Measure the absorbance of the resulting solution, at the maximum at about 313 nm (2.4.7).

Calculate the content of $C_{15}H_{14}N_4O$ from the absorbance obtained from a solution of known concentration of *nevirapine RS* in *0.1 M hydrochloric acid.*

D. Not less than 70 per cent of the stated amount of $C_{15}H_{14}N_4O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets with a suitable quantity of the mobile phase to obtain a mixture containing 0.05 per cent w/v of Nevirapine and filter through a membrane filter disc with an average diameter not exceeding 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A 0.05 per cent w/v solution of *nevirapine RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a filtered and degassed mixture of 20 volumes of *methanol,* 20 volumes of *acetonitrile* and 60 volumes of a buffer prepared by dissolving 12.0 g of *sodium dihydrogen phosphate* in about 800 ml of *water,* adjusting the pH to 3.0 with *orthophosphoric acid* and diluting to 1000.0 ml with *water,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the nevirapine peak is not less than 7500 theoretical plates and the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2 per cent.

Inject the test solution and continue the chromatography for at least five times the retention time of the principal peak. Determine the amount of related substances by the area normalisation method. Any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Shake a quantity of powder containing about 100 mg of Nevirapine with sufficient of the mobile phase to obtain a mixture containing 0.05 per cent w/v of Nevirapine. Mix and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution. A 0.05 per cent w/v solution of *nevirapine RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a filtered and degassed mixture of 20 volumes of *methanol,* 20 volumes of *acetonitrile* and 60 volumes of a buffer prepared by dissolving 12.0 g of *sodium dihydrogen phosphate* in about 800 ml of *water,* adjusting the pH to 3.0 with *phosphoric acid* and diluting to 1000.0 ml with *water,*

- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

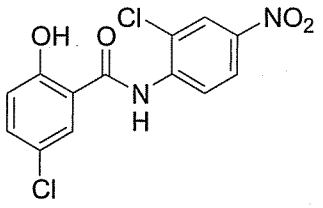
Inject the reference solution. The test is not valid unless the column efficiency determined from the nevirapine peak is not less than 7500 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Separately inject the test solution and the reference solution and measure the responses for the principal peak. Calculate the content of $C_{15}H_{14}N_4O$ in the tablets.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Niclosamide

Anhydrous Niclosamide



$C_{13}H_8Cl_2N_2O_4$ Mol. Wt. 327.1

Niclosamide is 2′ ,5-dichloro-4′ -nitrosalicylanilide.

Niclosamide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_8Cl_2N_2O_4$, calculated on the dried basis.

Category. Anthelmintic.

Dose. 2 g as a single dose after a light breakfast followed by a purgative 2 hours later.

Description. A yellowish white to yellowish, fine crystals or powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *niclosamide RS* or with the reference spectrum of niclosamide.

B. Heat 50 mg with 5 ml of *1 M hydrochloric acid* and 0.1 g of *zinc powder* in a water-bath for 10 minutes, cool and filter. To the filtrate add 1 ml of a 0.5 per cent w/v solution of *sodium nitrite* and allow to stand for 3 minutes. Add 2 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 3 minutes and add 2 ml of a 0.5 per cent w/v solution of N-*(1-naphthyl) ethylenediamine dihydrochloride*; a violet colour is produced.

C. Heat the substance under examination on a copper wire in a non-luminous flame; a green colour is imparted to the flame.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile*. Dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile*.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, 50 volumes of a solution containing 0.2 per cent w/v of *potassium dihydrogen orthophosphate*, 0.1 per cent w/v of *disodium hydrogen phosphate* and 0.2 per cent w/v of *tetrabutylammonium hydrogen sulphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. Adjust the sensitivity so that the height of the principal peak is not less than 20 per cent of full scale of the recorder.

Inject the test solution and the reference solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks is not more than 4 times the area of the principle peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than 10 per cent of the area of the principal peak in the chromatogram obtained with the reference solution.

Chlorides (2.3.12). To 2.0 g add a mixture of 40 ml of *water* and 1.2 ml of *5 M acetic acid*, boil for 2 minutes, cool and filter; 10 ml of the filtrate diluted to 15 ml with *water* complies with the limit test for chlorides (500 ppm).

2-Chloro-4-nitroaniline. Not more than 100 ppm, determined by the following method. Boil 0.25 g with 5 ml of *methanol*, cool, add 45 ml of *1 M hydrochloric acid*, heat to boiling, cool, filter and dilute the filtrate to 50.0 ml with *1 M hydrochloric acid*. To 10.0 ml of this solution add 0.5 ml of a 0.5 per cent w/v solution of *sodium nitrite* and allow to stand for 3 minutes. Add 1.0 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 3 minutes

and add 1.0 ml of a 0.5 per cent w/v solution of N- *(1-naphthyl) ethylenediamine dihydrochloride*. Any pinkish violet colour produced is not more intense than that obtained in a solution prepared at the same time and in the same manner using 10.0 ml of a solution prepared by diluting 2.0 ml of a 0.00050 per cent w/v solution of *2-chloro-4-nitroaniline* in *methanol* to 20 ml with *1 M hydrochloric acid* and beginning at the words "add 0.5 ml of a 0.5 per cent w/v solution of *sodium nitrite*.....".

5-Chlorosalicylic acid. Not more than 60 ppm, determined by the following method. Boil 1.0 g with 15 ml of *water* for 2 minutes, cool, filter through a membrane filter (pore size 0.45 µm), wash the filter and dilute the combined filtrate and washings to 20 ml with *water* (solution A). Dissolve 30 mg of *5-chlorosalicylic acid* in 20 ml of *methanol* and add sufficient *water* to produce 100.0 ml. Dilute 1.0 ml of this solution to 100.0 ml with *water* (solution B). To 10.0 ml of each of solutions A and B add separately 0.1 ml of *ferric chloride solution*; any violet colour produced in solution A is not more intense than that obtained in solution B.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.3 g, dissolve in 80 ml of a mixture of equal volumes of *acetone* and *methanol*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03271 g of $C_{13}H_8Cl_2N_2O_4$.

Storage. Store protected from light and moisture.

Niclosamide Tablets

Niclosamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of niclosamide, $C_{13}H_8Cl_2N_2O_4$. The tablets may contain sweetening and flavouring agents.

Usual strength. 500 mg.

Identification

Heat a quantity of the powdered tablets containing 0.5 g of Niclosamide with 25 ml of hot *ethanol (95 per cent)*, filter while hot and evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *niclosamide RS* or with the reference spectrum of niclosamide.

B. Heat 50 mg with 5 ml of *1 M hydrochloric acid* and 0.1 g of *zinc powder* in a water-bath for 10 minutes, cool and filter. To the filtrate add 1 ml of a 0.5 per cent w/v solution of *sodium nitrite* and allow to stand for 3 minutes. Add 2 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 3 minutes and add 2 ml of a 0.5 per cent w/v solution of N- *(1-naphthyl) ethylenediamine dihydrochloride*; a violet colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing about 100 mg of anhydrous nicolsamide with 80 ml of *methanol* for 15 minutes and dilute to 100.0 ml with the same solvent.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with *acetonitrile* and further dilute 1.0 ml of this solution to 20.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of a solution containing about 0.2 per cent w/v of *potassium dihydrogen orthophosphate*, 0.2 per cent w/v of *tetrabutylammonium hydrogen sulphate* and 0.1 per cent w/v of *disodium hydrogen orthophosphate*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. Adjust the sensitivity so that the height of the principal peak is not less than 20 per cent of full scale of the recorder.

Inject the test solution and the reference solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks is not more than 4 times the area of the principle peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than 10 per cent of the area of the principal peak in the chromatogram obtained with the reference solution.

2-Chloro-4-nitroaniline. Not more than 100 ppm, Boil a quantity of the powdered tablets containing 0.1 g of Niclosamide with 20 ml of *methanol* and 20 ml of a solution in *methanol* containing 10 µg of *2-chloro-4-nitroaniline*, cool, add 45 ml of *1 M hydrochloric acid*, heat to boiling, cool, filter and dilute the filtrate to 50.0 ml with *1 M hydrochloric acid*. To 10.0 ml of this solution add 0.5 ml of a 0.5 per cent w/v solution of *sodium nitrite* and allow to stand for 3 minutes.

Add 1.0 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 3 minutes and add 1.0 ml of a 0.5 per cent w/v solution of N-*(1-naphthyl) ethylenediamine dihydrochloride*. Any pinkish violet colour produced is not more intense than that obtained in a solution prepared at the same time and in the same manner using 10.0 ml of a solution prepared by diluting 2.0 ml of a 0.0005 per cent w/v solution of *2-chloro-4-nitroaniline* in *methanol* to 20.0 ml with *1 M hydrochloric acid* and beginning at the words "add 0.5 ml of a 0.5 per cent w/v solution of *sodium nitrite*.....".

5-Chlorosalicylic acid. Boil a quantity of the powdered tablets containing 0.5 g of Niclosamide with 10 ml of *water* for 2 minutes, cool, filter and to the filtrate add 0.2 ml of *ferric chloride solution*; no red or violet colour is produced.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 0.3 g of Niclosamide dissolved in 60 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

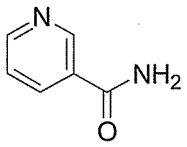
1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03271 g of $C_{13}H_8Cl_2N_2O_4$.

Storage. Store protected from light and moisture.

Labelling. The label states that the tablets should be chewed thoroughly before swallowing.

Nicotinamide

Niâcinamide



$C_6H_6N_2O$ Mol. Wt. 122.1

Nicotinamide is pyridine-3-carboxamide.

Nicotinamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_6N_2O$, calculated on the dried basis.

Category. B-group vitamin.

Dose. Orally, prophylactic, 15 to 30 mg daily; therapeutic, 50 to 250 mg daily. By intravenous injection, 50 to 250 mg daily.

Description. Colourless crystals or a white, crystalline powder; odour, faint and characteristic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nicotinamide RS* or with the reference spectrum of nicotinamide.

B. Heat about 5 mg in a dry tube; pyridine is evolved.

C. Boil 0.1 g with 1 ml of *dilute sodium hydroxide solution*; ammonia is evolved.

D. To 2 ml of a 0.1 per cent w/v solution add 6 ml of *cyanogen bromide solution* and 1 ml of a 2.5 per cent w/v solution of *aniline*; a golden yellow colour develops.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 6.0 to 7.5, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 48 volumes of *chloroform*, 45 volumes of *ethanol* and 4 volumes of *water*.

Test solution. Dissolve 0.8 g of the substance under examination in 10 ml of *ethanol (50 per cent)*.

Reference solution. A 0.02 per cent w/v solution of the substance under examination in *ethanol (50 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). Dissolve 0.67 g in 10 ml of *water*, 7.5 ml of *1 M hydrochloric acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (30 ppm).

Chlorides (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 1.2 g complies with the limit test for sulphates (125 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.7 kPa for 18 hours.

Assay. Weigh accurately about 0.25 g, dissolve in 20 ml of *anhydrous glacial acetic acid*, heating slightly if necessary. Add 5 ml of *acetic anhydride*. Titrate with *0.1 M perchloric*

acid, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01221 g of $C_6H_6N_2O$.

Storage. Store protected from moisture.

Nicotinamide Tablets

Niacinamide Tablets

Nicotinamide Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of nicotinamide, $C_6H_6N_2O$.

Usual strength. 50 mg.

Identification

Shake a quantity of the powdered tablets containing 0.2 g of Nicotinamide with 50 ml of *ethanol* for 15 minutes, filter and evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nicotinamide RS* or with the reference spectrum of nicotinamide.

B. Boil 0.1 g with 1 ml of *dilute sodium hydroxide solution*; ammonia is evolved.

C. To 2 ml of a 0.1 per cent w/v solution add 6 ml of *cyanogen bromide solution* and 1 ml of a 2.5 per cent w/v solution of *aniline*; a golden yellow colour develops.

D. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 262 nm and two shoulders at about 258 nm and 269 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 48 volumes of *chloroform*, 45 volumes of *ethanol* and 4 volumes of *water*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinamide with 15 ml of *ethanol* for 15 minutes, filter, evaporate to dryness on a water-bath and dissolve the residue as completely as possible in 1 ml of *ethanol*.

Reference solution. Dilute 1 volume of the test solution to 400 volumes with *ethanol*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

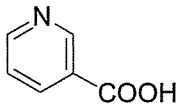
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Nicotinamide, shake with 50 ml of *ethanol (95 per cent)* for 15 minutes and dilute to 100.0 ml with *ethanol (95 per cent)*. Mix, filter, dilute 5.0 ml of the filtrate to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 262 nm (2.4.7). Calculate the content of $C_6H_6N_2O$ taking 241 as the specific absorbance at 262 nm.

Storage. Store protected from light and moisture.

Nicotinic Acid

Niacin



$C_6H_5NO_2$ Mol. Wt. 123.1

Nicotinic Acid is pyridine-3-carboxylic acid.

Nicotinic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_6H_5NO_2$, calculated on the dried basis.

Category. B-group vitamin.

Dose. Prophylactic, 15 to 30 mg daily; therapeutic, 50 to 250 mg daily.

Description. A white or creamy-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nicotinic acid RS* or with the reference spectrum of nicotinic acid.

B. Heat a small quantity with twice its weight of *soda lime*; pyridine is evolved.

C. Dissolve about 50 mg in 20 ml of *water*, neutralise to *litmus paper* with *0.1 M sodium hydroxide*, add 3 ml of *copper sulphate solution*; a blue precipitate is gradually produced.

D. To 2 ml of a 0.1 per cent w/v solution add 6 ml of *cyanogen bromide solution* and 1 ml of a 2.5 per cent w/v solution of *aniline*; a golden yellow colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 85 volumes of *1-propanol*, 10 volumes of *anhydrous formic acid* and 5 volumes of *water.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *water.* Warm slightly, if necessary.

Reference solution. A 0.01 per cent w/v solution of the substance under examination in *water.*

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° for 10 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). Mix 1.0 g with 1.5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml, heat gently and cool to room temperature. The resulting solution complies with the limit test for heavy metals, Method B (20 ppm).

Chlorides (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *carbon dioxide-free water* and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01231 g of $C_6H_5NO_2$.

Storage. Store protected from light and moisture.

Nicotinic Acid Tablets

Niacin Tablets

Nicotinic Acid Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of nicotinic acid, $C_6H_5NO_2$.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 48 volumes of *chloroform*, 45 volumes of *ethanol (95 per cent)* and 8 volumes of *water.*

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 50 ml of hot *ethanol (95 per cent)*, filter and allow the filtrate to cool.

Reference solution. A 0.1 per cent w/v solution of *nicotinic acid RS* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Triturate a quantity of the powdered tablets containing 50 mg of Nicotinic Acid with 10 ml of *water* and filter. To 2 ml of the filtrate add 6 ml of *cyanogen bromide solution* and 1 ml of a 2.5 per cent w/v solution of *aniline*; a golden yellow precipitate is produced.

C. Shake a quantity of the powdered tablets containing 0.1 g of Nicotinic Acid with *ethanol (95 per cent)*, filter and evaporate the filtrate to dryness. Add to the residue 10 mg of *citric acid* and 0.15 ml of *acetic anhydride* and heat on a water-bath; a reddish-violet colour is produced.

Tests

Other tests. Comply with the tests stated under Tablets.

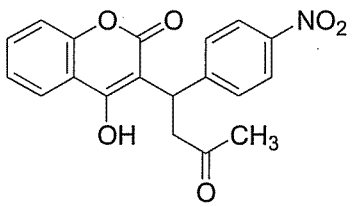
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Nicotinic Acid, add 40 ml of hot *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution*, and shake. Allow to stand for 15 minutes, swirling occasionally, and then shake for 10 minutes. Filter through a plug of cotton and wash the filter with *ethanol (95 per cent).* Add 50 ml of *carbon dioxide-free water* and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01231 g of $C_6H_5NO_2$.

Storage. Store protected from light and moisture.

Nicoumalone

Acenocoumarol



$C_{19}H_{15}NO_6$ Mol. Wt. 353.3

Nicoumalone is (*RS*)-4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]coumarin.

Nicoumalone contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{19}H_{15}NO_6$, calculated on the dried basis.

Category. Anticoagulant.

Dose. Initial dose, first day, 8 to 12 mg; second day, 4 to 8 mg; maintenance dose, 1 to 8 mg daily.

Description. A white to brownish-white powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nicoumalone RS* or with the reference spectrum of nicoumalone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in a mixture of 9 volumes of *methanol* and 1 volume of *1 M hydrochloric acid* shows absorption maxima at about 283 nm and 306 nm; absorbances at the maxima, about 0.65 and about 0.52, respectively.

C. Heat 50 mg with 2.5 ml of *glacial acetic acid*, 0.5 ml of *hydrochloric acid* and 0.2 g of *zinc powder* on a water-bath for 5 minutes, cool and filter. To the filtrate add 0.05 ml of *sodium nitrite solution* and add the mixture to 10 ml of a 1 per cent w/v solution of *2-naphthol* containing 3 ml of *5 M sodium hydroxide*; a bright red precipitate is produced.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *acetone* is clear (2.4.1).

B. A 2.0 per cent w/v solution in *0.1 M sodium hydroxide* is clear (2.4.1), and yellow.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 50 volumes of *cyclohexane* and 20 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *acetone*.

Reference solution. A 0.002 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and immediately examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.75 g, dissolve in 50 ml of *acetone* and titrate with *0.1 M sodium hydroxide* using *bromothymol blue solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03533 g of $C_{19}H_{15}NO_6$.

Storage. Store protected from light.

Nicoumalone Tablets

Acenocoumarol Tablets

Nicoumalone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nicoumalone, $C_{19}H_{15}NO_6$.

Usual strengths. 1 mg; 2 mg; 3 mg; 4 mg.

Identification

A. Heat a quantity of the powdered tablets containing 50 mg of Nicoumalone with 30 ml of *acetone* under a reflux condenser for 5 minutes, filter and wash the residue with two quantities, each of 10 ml, of *acetone*. Evaporate the combined filtrate and washings to 5 ml, add *water* dropwise until the solution becomes turbid, heat on a water-bath until the solution is clear and allow to stand. Filter, wash the crystals with a mixture of equal volumes of *acetone* and *water* and dry at 100° at a pressure of 2 kPa for 30 minutes.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nicoumalone RS* or with the reference spectrum of nicoumalone.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 283 nm and 306 nm.

C. Heat 50 mg of the residue obtained in test A, with 2.5 ml of *glacial acetic acid*, 0.5 ml of *hydrochloric acid* and 0.2 g of *zinc powder* on a water-bath for 5 minutes, cool and filter. To the filtrate add 0.05 ml of *sodium nitrite solution* and add the mixture to 10 ml of a 1 per cent w/v solution of *2-naphthol* containing 3 ml of *5 M sodium hydroxide*; a bright red precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 50 volumes of *cyclohexane* and 20 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 20 mg of Nicoumalone with 5 ml of *acetone*, centrifuge and use the supernatant liquid.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *acetone*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and immediately examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content. Comply with the test stated under Tablets.

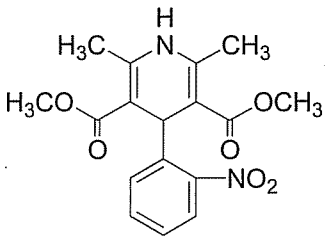
Finely crush one tablet, add 30 ml of *methanol*, stir the mixture for 30 minutes and filter through sintered glass, washing the residue with three quantities, each of 15 ml, of *methanol*. To the combined filtrate and washings add 10 ml of *1 M hydrochloric acid* and sufficient *methanol* to produce 100.0 ml. If necessary, dilute further with a solvent prepared by diluting 1 volume of *1 M hydrochloric acid* to 10 volumes with *methanol* to produce a solution containing about 0.001 per cent w/v solution of Nicoumalone. Measure the absorbance of the resulting solution at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the specific absorbance at 306 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Nicoumalone, add 30 ml of *methanol*, stir the mixture for 30 minutes and filter through sintered-glass, washing the residue with three quantities, each of 15 ml, of *methanol*. To the combined filtrate and washings add 10 ml of *1 M hydrochloric acid* and sufficient *methanol* to produce 100.0 ml. Dilute 5.0 ml of this solution to 50.0 ml with a solvent prepared by diluting 1 volume of *1 M hydrochloric acid* to 10 volumes with *methanol* and measure the absorbance of the resulting solution at the maximum at about 306 nm (2.4.7). Calculate the content of $C_{19}H_{15}NO_6$ taking 521 as the specific absorbance at 306 nm.

Storage. Store protected from light and moisture.

Nifedipine



$C_{17}H_{18}N_2O_6$ Mol. Wt. 346.3

Nifedipine is dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate.

Nifedipine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{18}N_2O_6$, calculated on the dried basis.

Category. Antianginal (calcium channel blocker).

Dose. Initial dose, 5-20 mg daily, in divided doses; subsequent doses, in accordance with the needs of the patient but total daily dose not to exceed 100 mg.

Description. A yellow, crystalline powder; readily affected by exposure to light.

NOTE— Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenyl derivative. Exposure to ultraviolet light leads to the formation of a nitrophenyl derivative. Perform the tests and assay in the dark or under long-wavelength light (greater than 420 nm). Use low-actinic glassware.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nifedipine RS* or with the reference spectrum of nifedipine.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to nifedipine in the chromatogram obtained with the reference solution (a).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *cyclohexane* and 40 volumes of *ethyl acetate*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution. A 0.1 per cent w/v solution of *nifedipine RS* in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 25 mg add 10 ml of a mixture of 5 volumes of *ethanol (95 per cent)*, 3.5 volumes of *water* and 1.5 volumes of *hydrochloric acid* and dissolve with gentle heating. Add 0.5 g of granulated zinc and allow to stand for 5 minutes, swirling occasionally. Filter, add 5 ml of a 1 per cent w/v solution of *sodium nitrite* to the filtrate and allow to stand for 2 minutes. Add 2 ml of a 5 per cent w/v solution of *ammonium sulphamate*, shake vigorously with care and add 2 ml of a 0.5 per cent w/v solution of N-*(1- naphthyl) ethylenediamine dihydrochloride*; an intense red colour develops which persists for more than 5 minutes.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 20 ml of *methanol* and dilute to 50 ml with the mobile phase.

Reference solution (a). Dissolve an accurately weighed quantity of *nifedipine RS* in sufficient *methanol* to produce a 1.0 per cent w/v solution and dilute quantitatively with the mobile phase to obtain a 0.4 per cent w/v solution.

Reference solution (b). A 0.04 per cent w/v solution of *dimethyl-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicaboxylate RS* (nitrophenylpyridine analogue) in *methanol.*

Reference solution (c). A 0.04 per cent w/v solution of *dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)pyridine-3,5-dicarboxylate RS* (nitroso- phenylpyridine analogue) in *methanol.*

Reference solution (d). Mix 1 volume of each of reference solutions (b) and (c) and 0.1 volume of the test solution, dilute to 10 volumes with the mobile phase and then dilute 2 volumes of the resulting solution to 10 volumes with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 55 volumes of *water*, 36 volumes of *methanol* and 9 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (d). The peaks appear in the order nitrophenylpyridine analogue, nitrosophenylpyridine analogue and nifedipine (retention time about 15.5 minutes). The test is not valid unless, in the chromatogram obtained with reference solution (d), (a) the resolution factor between the peaks due to the nitrophenylpyridine analogue and the nitrosophenylpyridine analogue is greater than 1.5, (b) the resolution between the peaks due to the nitrosophenylpyridine analogue and nifedipine is greater than 1.5, and (c) the height of the peak due to the nitrophenyl-pyridine analogue is at least 20 per cent of the full-scale deflection.

Inject the test solution and reference solutions (a) and (d) and record the chromatograms for twice the retention time of nifedipine. In the chromatogram obtained with the test solution no secondary peak other than any peaks corresponding to the nitrophenylpyridine analogue and the nitrosophenylpyridine analogue has an area greater than that of the peak due to nifedipine in the chromatogram obtained with reference solution (d) and the areas of any peaks corresponding to the nitrophenylpyridine analogue and the nitrosophenylpyridine analogue are not greater than the areas of the corresponding peaks in the chromatogram obtained with reference solution (d). The total amount of related substances is not greater than 0.3 per cent. Ignore any peak with an area less than 10 per cent of the area of the peak due to nifedipine in the chromatogram obtained with reference solution (d).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 0.13 g, dissolve in a mixture of 25 ml of *2-methyl-2-propanol* and 25 ml of *1 M perchloric acid* and titrate with *0.1 M ceric ammonium sulphate*, using 0.1 ml of *ferroin solution* as indicator until the pink colour is discharged, titrating slowly towards the end-point. Carry out a blank titration.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.01732 g of $C_{17}H_{18}N_2O_6$.

Storage. Store protected from light.

Nifedipine Capsules

Nifedipine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nifedipine, $C_{17}H_{18}N_2O_6$.

Usual strengths. 5 mg; 10 mg.

NOTE — Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a

nitrosophenyl derivative. Exposure to ultraviolet light leads to the formation of a nitrophenyl derivative. Perform the tests and assay in the dark or under long-wavelength light (greater than 420 nm). Use low-actinic glassware.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of equal volumes of *ethyl acetate* and *cyclohexane.*

Test solution. Transfer a quantity of the contents of the capsules containing 30 mg of Nifedipine into a centrifuge tube containing *0.1 M sodium hydroxide*, add 25 ml of *dichloromethane*, stopper the tube and shake gently for 1 hour. Centrifuge for 10 minutes at 2000 to 2500 rpm. Remove the supernatant aqueous layer by aspiration with a syringe and transfer 5 ml of the clarified lower layer to a suitable vial.

Reference solution (a). A 0.12 per cent w/v solution of *nifedipine RS* in *dichloromethane.*

Reference solution (b). A mixture of equal volumes of test solution and reference solution (a).

Apply to the plate 500 µl of each solution as bands 20 mm by 3 mm. After development, dry the plate in air until the solvent is not detectable and immediately examine in ultraviolet light at 254 nm. The principal band, appearing as a dark blue band, in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a). Spray with a solution prepared in the following manner. Dissolve 3 g of *bismuth subnitrate* and 30 g of *potassium iodide* in 10 ml of *3 M hydrochloric acid* and dilute with *water* to 100 ml; dilute 10 ml to 100 ml with *0.3 M hydrochloric acid.* In the chromatogram obtained with test solution the principal band, appearing as a compact light orange band against a yellow background, corresponds to that in the chromatogram obtained with reference solution (a). The band obtained with reference solution (b) appears as a single band under both visualisation procedures.

Tests

Uniformity of content. Comply with the test stated under Capsules.

Transfer the contents of a capsule quantitatively to a 200-ml volumetric flask with the aid of *methanol*, dilute to volume with *methanol* and mix. Complete the Assay beginning at the words "Measure the absorbance...." and calculate the content of $C_{17}H_{18}N_2O_6$ in the capsule.

Other tests. Comply with the tests stated under Capsules.

Assay. Transfer the contents of 5 capsules containing about 50 mg of Nifedipine quantitatively to a 200-ml volumetric flask with the aid of small quantities of *methanol.* Dilute to volume with *methanol* and mix. To 20.0 ml add sufficient *methanol* to produce 100.0 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{17}H_{18}N_2O_6$ in the capsules from the absorbance obtained by repeating the operation with a 0.005 per cent w/v solution of *nifedipine RS* in *methanol.*

Storage. Store protected from light.

Nifedipine Sustained-release Tablets

Nifedipine Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nifedipine, $C_{17}H_{18}N_2O_6$.

NOTE — Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenyl derivative. Exposure to ultraviolet light leads to the formation of a nitrophenyl derivative. Perform the tests and the assay in the dark or under long-wavelength light (greater than 420 nm). Use low-actinic glassware.

Usual strengths. 10 mg; 20 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of equal volumes of *ethyl acetate* and *cyclohexane.*

Test solution. Transfer a quantity of the powdered tablets containing 30 mg of Nifedipine into a centrifuge tube containing *0.1 M sodium hydroxide*, add 25 ml of *dichloromethane*, stopper the tube and shake gently for 1 hour. Centrifuge for 10 minutes at 2000 rpm to 2500 rpm. Remove the supernatant aqueous layer by aspiration with a syringe and use 5 ml of the clarified lower layer.

Reference solution. A 0.12 per cent w/v solution of *nifedipine RS* in *dichloromethane.*

Apply to the plate 500 µl of each solution as bands 20 mm by 3 mm. After development, dry the plate in air until the odour of the solvent is not detectable and immediately examine in ultraviolet light at 254 nm. The principal band, appearing as a dark blue band, in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Spray with a solution prepared in the following manner. Dissolve 3 g of *bismuth subnitrate* and 30 g of *potassium iodide* in 10 ml of *3 M hydrochloric acid* and dilute to 100 ml with *water*; dilute 10 ml of this solution to 100 ml with *0.3 M hydrochloric acid.* In the chromatogram obtained with the test solution the principal band, appearing

as a compact light orange band against a yellow background, corresponds to that in the chromatogram obtained with reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 150 rpm and 120 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium, if necessary, at the maximum at about 340 nm (2.4.7).

Calculate the content of $C_{17}H_{18}N_2O_6$ in the medium from the absorbance obtained from a solution of known concentration of *nifedipine RS* prepared by dissolving in minimum volume of *methanol* and then diluting with the dissolution medium.

D. Not less than 25 per cent and not more than 45 per cent of the stated amount of $C_{17}H_{18}N_2O_6$.

B. Apparatus No. 1,

Medium. 900 ml of phosphate buffer pH 6.8 prepared by dissolving 30 g of *sodium lauryl sulphate,* 24.8 g of *disodium hydrogen phosphate anhydrous,* 2.85 g of *citric acid monohydrate* and 0.75 ml of *orthophosphoric acid* in sufficient *water* to produce 6000 ml. Adjust the pH to 6.8, if necessary,

Replace *0.1 M hydrochloric acid* with phosphate buffer pH 6.8 and run the apparatus at 150 rpm for 6 hours. Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium, if necessary, at the maximum at about 340 nm (2.4.7).

Calculate the content of $C_{17}H_{18}N_2O_6$ in the medium from the absorbance obtained from a solution of known concentration of *nifedipine RS* prepared by dissolving in minimum volume of *methanol* and then diluting with the dissolution medium.

D. Not less than 60 per cent of the stated amount of $C_{17}H_{18}N_2O_6$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Nifedipine, disperse in *methanol*, shake and dilute to 100.0 ml with *methanol*, filter. Dilute 20.0 ml of the filtrate to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{17}H_{18}N_2O_6$ from the absorbance obtained with a 0.005 per cent w/v solution of *nifedipine RS* in *methanol*.

Storage. Store protected from light and moisture.

Nifedipine Tablets

Nifedipine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nifedipine, $C_{17}H_{18}N_2O_6$. The tablets may be coated.

Usual strengths. 5 mg; 10 mg.

NOTE — Nifedipine, when exposed to daylight and certain wavelengths of artificial light, readily converts to a nitrosophenyl derivative. Exposure to ultraviolet light leads to the formation of a nitrophenyl derivative. Perform the tests and assay in the dark or under long-wavelength light (greater than 420 nm). Use low-actinic glassware.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of equal volumes of *ethyl acetate* and *cyclohexane.*

Test solution. Transfer a quantity of the powdered tablets containing 30 mg of Nifedipine to a centrifuge tube containing 20 ml of *0.1 M sodium hydroxide,* add 25 ml of *dichloromethane,* stopper the tube and shake gently for 1 hour. Centrifuge for 10 minutes at 2000 to 2500 rpm. Remove the supernatant aqueous layer by aspiration with a syringe and transfer 5.0 ml of the clarified lower layer to a suitable vial.

Reference solution (a). A 0.12 per cent w/v solution of *nifedipine RS* in *dichloromethane.*

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 500 µl of each solution as bands 20 mm by 3 mm. After development, dry the plate in air until the solvent is not detectable and immediately examine in ultraviolet light at 254 nm. The principal band, appearing as a dark blue band, in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). Spray with a solution prepared in the following manner. Dissolve 3 g of *bismuth subnitrate* and 30 g of *potassium iodide* in 10 ml of *3 M hydrochloric acid* and dilute with *water* to 100 ml; dilute 10 ml to 100 ml with *0.3 M hydrochloric acid.* In the chromatogram obtained with the test solution the principal band, appearing as a compact light orange band against a yellow background, corresponds to that in the chromatogram obtained with reference solution (a). The band obtained with reference solution (b) appears as a single band under both visualisation procedures.

Tests

Uniformity of content. Comply with the test stated under Tablets

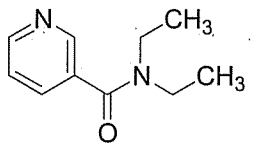
Shake one tablet with *methanol* in a 200-ml volumetric flask, dilute to volume with *methanol*, mix and filter. Complete the Assay beginning at the words "Measure the absorbance...." and calculate the content of $C_{17}H_{18}N_2O_6$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of Nifedipine into a 200 ml volumetric flask. Dissolve with the aid of 50 ml of *methanol*. Dilute to volume with *methanol*, mix and filter. Dilute 20 ml of the filtrate to 100 ml with *methanol* and mix. Measure the absorbance of the resulting solution at the maximum at about 350 nm (2.4.7). Calculate the content of $C_{17}H_{18}N_2O_6$ from the absorbance obtained by repeating the operation with a 0.005 per cent w/v solution of *nifedipine RS* in *methanol*.

Storage. Store protected from light and moisture.

Nikethamide



$C_{10}H_{14}N_2O$ Mol. Wt. 178.2

Nikethamide is *N,N*-diethylpyridine-3-carboxamide.

Nikethamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{14}N_2O$, calculated on the anhydrous basis.

Category. CNS and Respiratory stimulant.

Dose. By slow intravenous injection, 500 mg to 2 g repeated at intervals of 15 to 30 minutes as necessary.

Description. A colourless or slightly yellowish, oily liquid or crystalline mass; odour, slight and characteristic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nikethamide RS* or with the reference spectrum of nikethamide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution shows an absorption maximum only at about 263 nm; absorbance at about 263 nm, about 0.57.

C. Heat 0.1 g with 1 ml of *2 M sodium hydroxide*; diethylamine, recognisable by its odour, is evolved progressively; the fumes turn red litmus paper blue.

D. To 2 ml of a 0.1 per cent w/v solution add 2 ml of *cyanogen bromide solution* and 3 ml of a 2.5 per cent w/v solution of *aniline* and mix; a yellow colour is produced.

Tests

Appearance of solution. The substance, in liquid form or liquefied by gentle heating, is clear (2.4.1), and not more intensely coloured than reference solution YS5 (2.4.1).

pH (2.4.24). 6.0 to 7.8, determined in a 25.0 per cent w/v solution.

Congealing temperature (2.4.10). 23° to 24.5°.

Refractive index (2.4.27). 1.522 to1.526.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *chloroform* and 25 volumes of *1-propanol*.

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.04 per cent w/v solution of *ethylnicotinamide RS* in *methanol*.

Reference solution (b). A 0.004 per cent w/v solution of *ethylnicotinamide RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to ethylnicotinamide in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.3 per cent, determined on 2.0 g.

Assay. Weigh accurately about 0.15 g, dissolve in 20 ml of *anhydrous glacial acetic acid* and 5 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01782 g of $C_{10}H_{14}N_2O$.

Storge. Store protected from light.

Nikethamide Injection

Nikethamide Injection is a sterile solution containing 25 per cent w/v solution of Nikethamide in Water for Injections.

Nikethamide Injection contains not less than 24.0 per cent and not more than 26.0 per cent w/v solution of nikethamide, $C_{10}H_{14}N_2O$.

Identification

A. Make 1 ml alkaline with *5 M sodium hydroxide*, extract with 5 ml of *dichloromethane* and evaporate the solvent.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nikethamide RS* or with the reference spectrum of nikethamide.

B. Gives a voluminous precipitate with *alkaline potassium mercuri-iodide solution* and a greyish-brown flocculent precipitate with *tannic acid solution*. Gives no precipitate with *iodine solution* or with *potassium mercuri-iodide solution*.

C. Heat 1 ml with 0.2 g of *sodium hydroxide*; diethylamine, recognisable by its odour, is evolved.

Tests

pH (2.4.24). 6.0 to 8.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *chloroform* and 25 volumes of *1-propanol*.

Test solution. Dilute 1 ml of the injection to 5 ml with *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *ethylnicotinamide RS* in *methanol*.

Reference solution (b). A 0.005 per cent w/v solution of *ethylnicotinamide RS* in *methanol*.

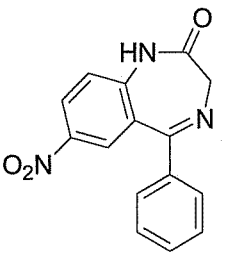
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to ethylnicotinamide in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute 5.0 ml to 500.0 ml with *water*. To 5.0 ml of the solution add 5 ml of *1 M hydrochloric acid* and sufficient *water* to produce 500.0 ml. Measure the absorbance of the resulting solution at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{10}H_{14}N_2O$ taking 282 as the specific absorbance at 263 nm.

Storage. Store protected from light, in single dose containers.

Nitrazepam



$C_{15}H_{11}N_3O_3$ Mol. Wt. 281.3

Nitrazepam is 1,3-dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one.

Nitrazepam contains not less than 99.0 per cent and not more than 101.0 per cent $C_{15}H_{11}N_3O_3$, calculated on the dried basis.

Category. Hypnotic; sedative.

Dose. 5 to 10 mg daily, at bed time.

Description. A yellow, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nitrazepam RS* or with the reference spectrum of nitrazepam.

B. *Carry out the following procedure in subdued light.*

When examined in the range 230 nm to 360 nm (2.4.7), a freshly prepared 0.0005 per cent w/v solution in a 0.5 per cent w/v solution of *sulphuric acid* in *methanol* shows an absorption maximum only at about 280 nm; absorbance at about 280 nm, about 0.45.

C. Dissolve 10 mg in 1 ml of *methanol*, warming if necessary, and add 0.05 ml of *2 M sodium hydroxide*; an intense yellow colour is produced.

D. Dissolve 20 mg in a mixture of 5 ml of *hydrochloric acid* and 10 ml of *water*, boil for 5 minutes, cool and add 2 ml of a 0.1 per cent w/v solution of *sodium nitrite*. Allow to stand for 1 minute, add 1 ml of a 0.5 per cent w/v solution of *sulphamic*

acid, mix, allow to stand for 1 minute, add 1 ml of a 0.1 per cent w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*; a red colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 85 volumes of *nitromethane* and 15 volumes of *ethyl acetate*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *acetone*.

Reference solution. A 0.002 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02813 g of $C_{15}H_{11}N_3O_3$.

Storage. Store protected from light and moisture.

Nitrazepam Tablets

Nitrazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nitrazepam, $C_{15}H_{11}N_3O_3$.

Usual strengths. 5 mg; 10 mg.

Identification

Carry out the following procedure in subdued light.

A. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 280 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution. Shake a quantity of the powdered tablets with sufficient *methanol* to produce a solution containing 5 mg of Nitrazepam per ml, allow to settle and decant the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *nitrazepam RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray it with *ethanolic sulphuric acid (10 per cent v/v)*, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To a quantity of the powdered tablets containing 5 mg of Nitrazepam add 5 ml of *hydrochloric acid* and 10 ml of *water*, heat on a water-bath for 15 minutes, filter and cool. To the clear filtrate add 1 ml of a 0.1 per cent w/v solution of *sodium nitrite*, allow to stand for 3 minutes and add 1 ml of a 0.5 per cent w/v solution of *sulphamic acid*. Allow to stand for 3 minutes and add 1 ml of a 0.1 per cent w/v solution of N-*(1-naphthyl) ethylenediamine dihydrochloride*; a red colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{15}H_{11}N_3O_3$, in the medium from the absorbance obtained from a solution of known concentration of *nitrazapam RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{15}H_{11}N_3O_3$.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *nitromethane*, 40 volumes of *toluene* and 20 volumes of *chloroform*.

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Nitrazepam with 25 ml of *chloroform*, filter, carefully evaporate the filtrate to dryness and dissolve the residue in 2 ml of *chloroform*.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *chloroform*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content. Comply with the test stated under Tablets.

NOTE — Carry out the following procedure in subdued light.

Powder 1 tablet, add 5 ml of *water*, mix and allow to stand for 15 minutes protected from light. Add 70 ml of a 0.5 per cent v/v solution of *hydrochloric acid* in *methanol*, shake for 15 minutes protected from light, add sufficient of the *hydrochloric acid solution* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate with sufficient of the *hydrochloric acid solution* to produce a solution containing 0.0005 per cent w/v solution of Nitrazepam. Measure the absorbance of the resulting solution immediately at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{15}H_{11}N_3O_3$ in the tablet taking 910 as the specific absorbance at 280 nm.

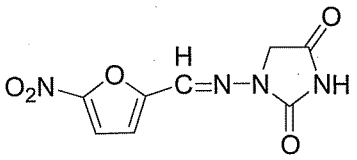
Other tests. Comply with the tests stated under Tablets.

Assay. *Carry out the following procedure in subdued light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Nitrazepam, add 5 ml of *water*, mix and allow to stand for 15 minutes protected from light. Add 70 ml of a 0.5 per cent v/v solution of *hydrochloric acid* in *methanol*, shake for 15 minutes protected from light, add sufficient of the *hydrochloric acid solution* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with the same solvent and measure the absorbance of the resulting solution immediately at the maximum at about 280 nm (2.4.7). Calculate the content of $C_{15}H_{11}N_3O_3$ taking 910 as the specific absorbance at 280 nm.

Storage. Store protected from light and moisture.

Nitrofurantoin



$C_8H_6N_4O_5$ Mol. Wt. 238.2 (anhydrous)

$C_8H_6N_4O_5,H_2O$ Mol. Wt. 256.2 (hydrous)

Nitrofurantoin is 1-(5-nitrofurfurylideneamino)imidazolidine-2,4-dione. It is anhydrous or contains one molecule of water of hydration.

Nitrofurantoin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_6N_4O_5$, calculated on the dried basis.

Category. Antibacterial.

Dose. 50 to 100 mg four times daily.

Description. Lemon yellow crystals or a crystalline powder; odourless or almost odourless.

Identification

Carry out the following test in subdued light.

A. When examined in the range 230 nm to 400 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 266 nm and 367 nm; the ratio of the absorbance at the maximum at about 367 nm to that at the maximum at about 266 nm is 1.36 to 1.42.

B. To 1 ml of a 0.1 per cent w/v solution in *dimethylformamide* add 0.1 ml of *0.5 M ethanolic potassium hydroxide*; a brown colour develops.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 90 volumes of *nitromethane* and 10 volumes of *methanol*.

Test solution. Dissolve 0.25 g of the substance under examination in minimum volume of *dimethylformamide* and dilute to 10 ml with *acetone*.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and examine in ultraviolet light at 254 nm. Spray with *phenylhydrazine hydrochloride solution* and heat the plate at 105° for further 10 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution by both methods of visualisation.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 5.0 to 7.1 per cent (hydrous form) and not more than 1.0 per cent (anhydrous form), determined on 1.0 g by drying in an oven at 105°.

Assay. *Carry out the following procedure in subdued light.*

Weigh accurately about 75 mg and dissolve in 25.0 ml of *dimethylformamide*, add sufficient *water* to produce 500.0 ml and mix. Dilute 5.0 ml to 100.0 ml with a solution containing 1.8 per cent w/v solution of *sodium acetate* and 0.14 per cent v/v of *glacial acetic acid*. Measure the absorbance of the

resulting solution at the maximum at about 367 nm (2.4.7), using as the blank the sodium acetate-acetic acid solution. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the specific absorbance at 367 nm.

Storage. Store protected from light and moisture.

Labelling. The label states whether the material is anhydrous or hydrous.

Nitrofurantoin Tablets

Nitrofurantoin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nitrofurantoin, $C_8H_6N_4O_5$.

Usual strengths. 50 mg; 100 mg.

Identification

Carry out the following procedure in subdued light.

A. When examined in the range 230 nm to 400 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 266 nm and 367 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 90 volumes of *nitromethane* and 10 volumes of *methanol*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Nitrofurantoin with 10 ml of a mixture of 9 volumes of *acetone* and 1 volume of *dimethylformamide* and filter.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, heat at 105° for 5 minutes and examine in ultraviolet light at 254 nm. Spray with *phenylhydrazine hydrochloride solution* and heat the plate at 105° for further 10 minutes. Any secondary spot in the chromatogram obtained with the test solution, is not more intense than the spot in the chromatogram obtained with the reference solution by both methods of visualisation.

Other tests. Comply with the tests stated under Tablets.

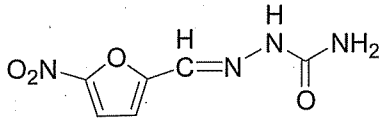
Assay. *Carry out the following procedure in subdued light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Nitrofurantoin, add 50.0 ml of *dimethylformamide*, shake for 5 minutes, add sufficient *water* to produce 1000.0 ml and mix. Dilute 5.0 ml to 100.0 ml with a solution containing 1.8 per cent w/v solution of *sodium acetate* and 0.14 per cent v/v of *glacial acetic acid*. Measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7), using as the blank the sodium acetate-acetic acid solution. Calculate the content of $C_8H_6N_4O_5$ taking 765 as the specific absorbance at 367 nm.

Storage. Store protected from light and moisture.

Nitrofurazone

Nitofural



$C_6H_6N_4O_4$ Mol. Wt. 198.1

Nitrofurazone is 5-nitro-2-furaldehyde semicarbazone.

Nitrofurazone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_6H_6N_4O_4$, calculated on the dried basis.

Category. Antibacterial.

Description. A yellow to brownish-yellow, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nitrofurazone RS* or with the reference spectrum of nitrofurazone.

B. Dissolve 1 mg in 1 ml of *dimethylformamide* and add 0.05 ml of *1 M ethanolic potassium hydroxide*; a ruby red colour is produced.

Tests

pH (2.4.24). 5.0 to 7.0, determined in the filtrate obtained by shaking 1.0 g with 100 ml of *carbon dioxide-free water* and filtering.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.0005 per cent w/v solution of *(5-nitro-2-furyl)methylene diacetate* (Nitrofurazone impurity B) in the mobile phase.

Reference solution (b). Dissolve 10 mg each of *nitrofural RS* and *nitrofurantoin* in 100 ml of the mobile phase. Dilute 5 ml of this solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 310 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to nitrofural and nitrofurantoin is not less than 2.0.

Inject the test solution and reference solution (a). Run the chromatogram 10 times the retention time of nitrofural. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peaks with the area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.025 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. *Carry out the following procedure in subdued light.*

Weigh accurately about 60 mg, add 20.0 ml of *dimethylformamide*, swirl to dissolve and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml of the solution to 100.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 375 nm (2.4.7). Calculate the content of $C_6H_6N_4O_4$ taking 822 as the specific absorbance at 375 nm.

Storage. Store protected from light and moisture.

Nitrous Oxide

N_2O Mol. Wt. 44.0

Nitrous Oxide contains not less than 98.0 per cent v/v of N_2O.

NOTE — Carry out the following tests on a full cylinder from which no gas has been withdrawn. The cylinder from which the gas is taken should be kept at room temperature for not less than 6 hours before carrying out the tests. Keep the cylinder in the vertical position with the outlet valve uppermost and deliver the gas at a rate of 4 litres per hour, unless otherwise directed. The test for Carbon monoxide should be carried out on the first portion of gas drawn from the cylinder and that for Nitric oxide and nitrogen dioxide immediately thereafter.

Category. General anaesthetic.

Dose. By inhalation, 60 to 80 per cent, with oxygen 20 to 40 per cent, as required.

Description. A colourless gas; odourless.

Identification

A. A glowing splinter of wood bursts into flame on contact with the gas.

B. Shake with *alkaline pyrogallol solution*; the gas being examined is not absorbed and the solution does not become brown.

Tests

Acidity or alkalinity. Use hermetically-closed, flat-bottomed, glass cylinders with dimensions such that 50 ml of liquid reaches a height of 12 to 14 cm, fitted with an outlet tube and with an inlet tube with an orifice of 1 mm in internal diameter reaching to within 2 mm of the bottom of the cylinder. For solution (1) pass 2.0 litres of the gas under examination through a mixture of 0.1 ml of *0.01 M hydrochloric acid* and 50 ml of *carbon dioxide-free water*. For solution (2) use 50 ml of *carbon dioxide-free water*. For solution (3) add 0.2 ml of *0.01 M hydrochloric acid* to 50 ml of *carbon dioxide-free water*. To each solution add 0.1 ml of a 0.02 per cent w/v solution of *methyl red* in *ethanol (70 per cent)*. The intensity of the colour of solution (1) is between those of solutions (2) and (3).

Arsine and phosphine. Through a *mercuric chloride paper* attached to a glass tube as in the limit test for arsenic (2.3.10), pass 2.0 litres of the gas; no visible stain is produced.

Carbon dioxide. Not more than 300 ppm v/v determined by the following method. Use the apparatus described in the test for Acidity or alkalinity. Pass 1.0 litre through 50 ml of clear *barium hydroxide solution*. Any turbidity produced in the resulting solution is not more than that obtained in a reference solution prepared at the same time by adding 1 ml of a 0.11 per cent w/v solution of *sodium bicarbonate* in *carbon dioxide-free water* to 50 ml of *barium hydroxide solution*.

Carbon monoxide. Not more than 10 ppm v/v, determined by the following method. Connect in series a U-tube containing *silica gel* impregnated with *chromium trioxide*, a drechsel bottle containing 100 ml of a 40 per cent w/v solution of *potassium hydroxide*, a U-tube containing pellets of *potassium hydroxide*, a U-tube containing *phosphorus pentoxide* dispersed on previously granulated, fused pumice, a tube containing *iodine pentoxide* in granules, previously dried at 200° and kept at a temperature of 120°, packed in 1-cm columns separated by 1-cm columns of glass wool giving an effective length of 5 cm, and a flask containing 2.0 ml of *1 M potassium iodide* and 0.15 ml of *starch solution*.

Flush the apparatus with 5.0 litres of carbon dioxide-free air and, if necessary, discharge the blue colour in the iodide solution by adding a small quantity of freshly prepared *0.002 M sodium thiosulphate*. Continue flushing until not more than 0.045 ml of *0.002 M sodium thiosulphate* is required after passing 5.0 litres of carbon dioxide-free air. Pass 5.0 litres of the gas under examination through the apparatus and flush the last traces of liberated iodine into the reaction flask by passing through the apparatus 1.0 litre of carbon monoxide-free air. Titrate the liberated iodine with *0.002 M sodium thiosulphate*. Carry out a blank titration under the same conditions, using 5.0 litres of carbon dioxide-free air. The difference between the volumes of *0.002 M sodium thiosulphate* used in the two titrations is not greater than 1.0 ml.

Halogens and hydrogen sulphide. Pass a volume containing 1.0 litre measured at 25° and at 101.3 kPa through a mixture of 100 ml of *water* and 1 ml of *silver nitrate solution*; neither opalescence nor darkening is produced.

Nitric oxide and nitrogen dioxide. Not more than 2 ppm v/v in both the liquid and gaseous phases, determined by the following method. Use two of the cylinders described in the test for Acidity or alkalinity connected in series. Examine separately both the liquid and gaseous phases of the gas under examination. To obtain the liquid phase invert the cylinder. The liquid vaporises on leaving the valve.

For solution A dissolve 1 g of *sulphanilic acid* in a mixture of 10 ml of *glacial acetic acid* and 180 ml of *water*. For solution B dissolve 0.2 g of *N-(1-naphthyl)ethylenediamine dihydrochloride* in 10 ml of a 50 per cent v/v solution of *glacial acetic acid*, heating gently, and dilute to 200 ml with *water*. Mix 9 volumes of solution A with 1 volume of solution B (reagent A).

In the first cylinder place 15 ml of a solution containing 2.5 per cent w/v solution of *potassium permanganate* and 1.2 per cent v/v of *sulphuric acid (96 per cent)*. Place 20 ml of reagent A in the second cylinder and connect the outlet tube of the first cylinder to the inlet tube of the second cylinder. Pass 2.5 litres of the gas under examination through the reagents at a rate of 15 litres per hour. Prepare a reference solution by adding 0.25 ml of a 0.00616 per cent w/v solution of *sodium nitrite* to 20 ml of reagent A. Allow both the sample and reference solutions to stand for 10 minutes. For both liquid and gaseous phases, any red colour in the sample solution is not more intense than that in the reference solution.

Oxidising substances. Pass a volume containing 2.0 litres measured at 25° and at 101.3 kPa through a freshly prepared solution of 0.5 g of *soluble starch* and 0.5 g of *potassium iodide* in 100 ml of *water* containing 0.05 ml of *glacial acetic acid*; the colour of the liquid is not changed.

Water. Pass a measured quantity at a rate of 6 litres per hour through an absorption tube containing *magnesium perchlorate*; the increase in weight of the tube does not exceed 2 mg per litre of gas, the initial and final weighings of the tube being made when the air in it has been displaced by the nitrous oxide.

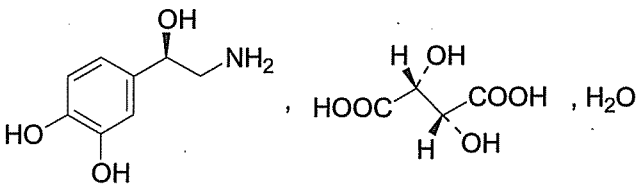
Assay. Carry out the assay of nitrous oxide (2.3.32), using 100 ml of the gas under examination. Use a cylinder of the gas under examination from which at least 1 per cent w/w of the contents have been removed.

Storage. Store under pressure in metal cylinders of the type conforming to the appropriate safety regulations and at a temperature not exceeding 37°.

Labelling. The cylinder is painted blue and carries a label stating "Nitrous Oxide". In addition, "Nitrous Oxide" or the symbol "N_2O" should be stencilled in paint on the shoulder of the cylinder.

Noradrenaline Bitartrate

Noradrenaline Acid Tartrate; Levarterenol Bitartrate; Norepinephrine Bitartrate



$C_8H_{11}NO_3,C_4H_6O_6,H_2O$ Mol. Wt. 337.3

Noradrenaline Bitartrate is (*R*)-2-amino-1-(3,4-dihydroxyphenyl)ethanol tartrate monohydrate.

Noradrenaline Bitartrate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{11}NO_3,C_4H_6O_6$, calculated on the anhydrous basis.

Category. Sympathomimetic.

Dose. By intravenous infusion, 2 to 20 µg per minute, according to the blood pressure of the patient.

Description. A white or almost white, crystalline powder; odourless. It gradually darkens on exposure to air and light.

Identification

Test A may be omitted if tests B, C, D, E and F are carried out. Tests C, D, E may be omitted if tests A, B and F are carried out.

A. Dissolve 0.2 g in 2 ml of *water* containing about 10 mg of *sodium sulphite* and add sufficient *dilute ammonia solution* to give an alkaline reaction. Keep the mixture at about 4° for 1 hour and filter.

On the residue (residue R) determine by infrared absorption spectrophotometry (2,4,6). Compare the spectrum with that obtained with *noradrenaline acid tartrate RS* treated in the same manner.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum at about 279 nm; absorbance at about 279 nm, about 0.40.

C. Wash residue R obtained in test A with three quantities, each of 2 ml, of *water*, followed by 5 ml of *ethanol (95 per cent)* and 5 ml of *ether* and dry the precipitate under pressure of 1.5 to 2.5 kPa for 3 hours. The specific optical rotation (2.4.22), determined in a 2.0 per cent w/v solution of the dried precipitate in *0.5 M hydrochloric acid* is –44° to –48°.

D. To 1 ml of a 1 per cent w/v solution, add 0.05 ml of *ferric chloride solution*; an intense green colour is produced. Add, drop by drop, *sodium bicarbonate solution*; the colour changes to blue and then red.

E. To 1 ml of a 0.1 per cent w/v solution add 10 ml of *phthalate buffer pH 3.6*, add 1 ml of *0.05 M iodine*, set aside for 5 minutes and add 2 ml of *0.1 M sodium thiosulphate*; not more than a faint red colour is produced. Repeat the test using *buffer solution pH 6.6* instead of *phthalate buffer pH 3.6*; a strong reddish violet colour is produced (distinction from adrenaline and isoprenaline).

F. The filtrate obtained in test A gives the reactions of tartrates (2.3.1).

Tests

Appearance of solution. A freshly prepared 2.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BY*S5* (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in a 1.0 per cent w/v solution.

Melting range (2.4.21). 100° to 106°, with decomposition.

Adrenaline. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *acetone*, 50 volumes of *dichloromethane* and 0.5 volume of *anhydrous formic acid*.

Prepare the following solutions immediately before use.

Test solution. Dissolve 0.25g of the substance under examination in 10 ml of *water*.

Reference solution (a). A 0.125 per cent w/v solution of *adrenaline tartrate RS* in *water*.

Reference solution (b). A 0.025 per cent w/v solution of *adrenaline tartrate RS* in *water*.

Reference solution (c). A mixture of equal volumes of the test solution and reference solution (b).

Apply to the plate 6 µl of each of test solution, reference solutions (a) and (b) and 12 µl of reference solution (c) as bands 20 mm by 2 mm. Allow the applied bands to dry in air, spray them with a saturated solution of *sodium bicarbonate*, allow to dry in air and spray the bands twice with *acetic anhydride*, drying between the two sprayings. Heat the plate at 50° for 90 minutes and develop the chromatograms. After removal of the plate, allow it to dry in air and spray with a freshly prepared mixture of 8 volumes of *methanol*, 2 volumes of *ethylenediamine* and 2 volumes of a 0.5 per cent w/v solution of *potassium ferricyanide*. Dry the plate at 60° for 10 minutes and examine in ultraviolet light at 254 and 365 nm. In the chromatogram obtained with the test solution any band with a slightly higher R_f value than the principal band is not more intense than the corresponding band in the chromatogram obtained with reference solution (b). The chromatogram obtained with reference solution (c) shows a clearly separated band corresponding to the most intense band in the chromatogram obtained with reference solution (a) at a higher R_f value than the most intense band.

Noradrenalone. Absorbance of a 0.2 per cent w/v solution in *0.01 M hydrochloric acid* at 310 nm, not more than 0.40 (2.4.7).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.5 to 5.8 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.6 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, warming if necessary.Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator, until a bluish green colour is obtained. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03193 g of $C_8H_{11}NO_3,C_4H_6O_6$.

Storage. Store protected from moisture.

Noradrenaline Bitartrate Injection

Noradrenaline Acid Tartrate Injection; Noradrenaline Injection; Levarterenol Bitartrate Injection; Norepinephrine Bitartrate Injection

Noradrenaline Bitartrate Injection is a sterile solution of Noradrenaline Bitartrate. It is prepared by diluting Sterile Noradrenaline Concentrate to 250 times its volume with Sodium Chloride and Dextrose Injection or with Dextrose Injection (5 per cent w/v) immediately before use.

Noradrenaline Bitartrate Injection contains in 1 ml 8 µg of Noradrenaline Bitartrate equivalent to approximately 4 µg of noradrenaline.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Sterile Noradrenaline Concentrate

Sterile Noradrenaline Concentrate is a sterile, isotonic solution containing 0.2 per cent w/v of Noradrenaline Bitartrate in Water for Injections.

Sterile Noradrenaline Concentrate contains not less than 0.18 per cent and not more than 0.23 per cent w/v of noradrenaline bitartrate, $C_8H_{11}NO_3,C_4H_6O_6,H_2O$.

Identification

Mix 0.5 ml with 10 ml of *phthalate buffer pH 3.6*, add 1 ml of *0.05 M iodine*, allow to stand for 5 minutes and add 2 ml of *0.1 M sodium thiosulphate*; not more than a very faint red colour is produced. Repeat the test using *phosphate buffer pH 6.6* instead of *phthalate buffer pH 3.6*; a strong reddish violet colour is produced.

Tests

pH (2.4.24). 3.0 to 4.6.

Other tests. Complies with the tests stated under Parenteral Preparations (Concentrated Solutions for Injection).

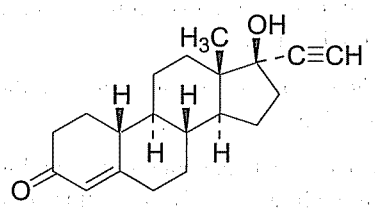
Assay. Dilute 5.0 ml to 200.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 279 nm (2.4.7). Calculate the content of $C_8H_{11}NO_3,C_4H_6O_6,H_2O$ taking 80 as the specific absorbance at 279 nm.

Storage. Store protected from light, in single dose containers.

Labelling. The label states (1) "Sterile Noradrenaline Concentrate"; (2) that 1 volume of the solution diluted to 250 volumes with Sodium Chloride and Dextrose Injection or with Dextrose Injection (5 per cent w/v) produces Noradrenaline Bitartrate Injection, which must be used immediately after preparation; (3) that if the solution is brown it should not be used.

Norethisterone

Norethindrone



$C_{20}H_{26}O_2$ Mol. Wt. 298.4

Norethisterone is 17β-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one.

Norethisterone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{26}O_2$, calculated on the dried basis.

Category. Progestogen.

Dose. 5 to 20 mg daily, in single or divided doses.

Description. A white or yellowish-white, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *norethisterone RS* or with the reference spectrum of norethisterone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 40 volumes of *hexane* and 10 volumes of *dioxan*.

Test solution. Dissolve 10 mg of the substance under examination in 10 ml of *chloroform*.

Reference solution. Dissolve 10 mg of *norethisterone RS* in 10 ml of *chloroform*.

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to and exhibits fluorescence similar to that in the chromatogram obtained with the reference solution.

C. Dissolve about 2 mg in 2 ml of *ethanol (95 per cent)* and add 1 ml of a 1 per cent w/v solution of *butylated hydroxytoluene* in *ethanol (95 per cent)* and 2 ml of *1 M sodium hydroxide*. Heat in a water-bath for 30 minutes and cool; a yellowish pink colour is produced.

Tests

Appearance of solution. Dissolve 0.2 g in sufficient *dioxan* to produce 10 ml (solution A). The solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Specific optical rotation (2.4.22). –33.0° to –37.0°, determined in a solution prepared by diluting 5.0 ml of solution A to 10.0 ml with *dioxan*.

Light absorption. Dissolve 10 mg in sufficient *ethanol (95 per cent)* to produce 100 ml, dilute 10 ml of the solution to 100 ml with *methanol (98 per cent)*. Absorbance of the resulting solution at the maximum at about 240 nm, 0.55 to 0.59 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 40 volumes of *water* and 60 volumes of *acetonitrile*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with end-capped octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water*,
  B. *acetonitrile*,
- a linear gradient programme using the condition given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0-20	63	37
20-25	63-20	37-80
25-35	20	80
35-36	20-63	80-37
36-50	63	37

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (0.3 per cent). Ignore any peaks with the area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.4 g, dissolve in 40 ml of *tetrahydrofuran*, add 10 ml of a 10 per cent w/v solution of *silver nitrate* and titrate with *0.1 M sodium hydroxide* using 2 ml of *bromocresol green solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02984 g of $C_{20}H_{26}O_2$.

Storage. Store protected from light and moisture.

Norethisterone Tablets

Norethindrone Tablets

Norethisterone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of norethisterone, $C_{20}H_{26}O_2$.

Usual strength. 5 mg.

Identification

Place a quantity of the powdered tablets containing 25 mg of Norethisterone on a small filter, wash with three quantities, each of 5 ml, of *light petroleum (60° to 80°)* and discard the washings. Extract the residue with 15 ml of *chloroform*, evaporate the extract to dryness and recrystallise from aqueous *methanol*. The residue complies with the following test.

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

Mobile phase. A mixture of 40 volumes of *cyclohexane* and 10 volumes of *toluene*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *norethisterone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Uniformity of content. (*For tablets containing 10 mg or less*)– Comply with the test stated under Tablets.

Carry out the procedure described under Assay but using the following test solution.

Test solution. Powder one tablet and dissolve as completely as possible in 2 ml of *water* with the aid of ultrasound for 15 minutes and dilute to 5.0 ml with *methanol*. Centrifuge for 15 minutes and use the clear supernatant liquid.

Calculate the content of $C_{20}H_{26}O_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 5 mg of Norethisterone in 10 ml of *water*, sonicate for 15 minutes and dilute to 25.0 ml with *methanol*, filter.

Reference solution. A 0.02 per cent w/v solution of *norethisterone RS* in *methanol.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 28 volumes of *water* and 72 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

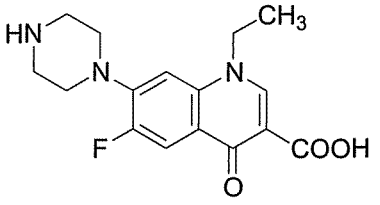
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{20}H_{26}O_2$ in the tablets.

Storage. Store protected from light and moisture.

Norfloxacin



$C_{16}H_{18}FN_3O_3$ Mol. Wt. 319.3

Norfloxacin is 1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

Norfloxacin contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{18}FN_3O_3$, calculated on the dried basis.

Category. Antibacterial.

Dose. 400 mg to 800 g daily, in divided doses.

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *norfloxacin RS* or with the reference spectrum of norfloxacin.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum at about 273 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*, previously washed with *methanol* and dried.

Mobile phase. A mixture of 40 volumes of *dichloromethane*, 40 volumes of *methanol*, 20 volumes of *toluene*, 14 volumes of *diethylamine* and 8 volumes of *water.*

Test solution. Dissolve 0.8 g of the substance under examination in 100 ml of a mixture of equal volumes of *methanol* and *dichloromethane.*

Reference solution. Dissolve 4.0 mg of *norfloxacin RS* in 1 ml of *glacial acetic acid*, add 4 ml of *methanol* and mix; dilute 1 ml of the solution with 9 ml of a mixture of equal volumes of *methanol* and *dichloromethane* (reference solution A). Dilute a portion of reference solution A with an equal volume of the *methanol-dichloromethane* mixture (reference solution B).

Apply separately to the plate spots of the three solutions in quantities indicated below. For spot 1 use 5 µl of the test

solution; for spots 2, 3 and 4 use 1 µl, 1.5 µl and 2 µl respectively of reference solution A; for spot 5 use 5 µl of reference solution B. Place the plate in a paper-lined chamber previously equilibrated with the mobile phase and allow the solvent front to move about nine-tenths of the length of the plate. After development, dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm. Compare the intensities of any secondary spots in the chromatogram obtained with the test solution with those of the principal spots (2), (3), (4) and (5). The sum of the intensities of secondary spots obtained with the test solution is not more than 0.5 per cent of impurities. (The spots (2) (3) (4) and (5) represent 0.2 per cent, 0.3 per cent, 0.4 per cent and 0.5 per cent respectively of impurities).

Heavy metals (2.3.13). 1.33 g complies with the limit test for heavy metals, Method B (15 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 1.0 g in a platinum crucible.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 0.5 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.3 g and dissolve in 100 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25) and using a suitable anhydrous electrode system. (The electrode system may be rendered anhydrous by filling the electrode with *0.1 M lithium perchlorate* in *acetic anhydride* after removing any aqueous solution contained in it). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03193 g of $C_{16}H_{18}FN_3O_3$.

Storage. Store protected from light and moisture.

Norfloxacin Eye Drops

Norfloxacin Eye Drops are a sterile solution of Norfloxacin in Purified water.

Norfloxacin Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of norfloxacin, $C_{16}H_{18}FN_3O_3$.

Usual strength. 0.3 per cent w/v.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.6 to 5.5.

Other tests. Comply with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable volume of the eye drops with a 0.1 per cent v/v solution of *orthophosphoric acid* to produce a solution containing 0.005 per cent w/v of Norfloxacin.

Reference solution. A 0.005 per cent w/v solution of *norfloxacin RS* in 0.1 per cent v/v solution of *orthophosphoric acid*.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (10 µm) (Such as Bondapack C18),
- column temerature. 50°,
- mobile phase: a mixture of 300 volumes of *methanol* and 700 volumes of 0.1 per cent v/v *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Precondition the column using *0.01 M anhydrous sodium dihydrogen orthophosphate*, adjusted to pH 4.0 with *orthophosphoric acid*, at a flow rate of 0.5 ml per minute for 8 hours. Equilibrate the column with the mobile phase for about 30 minutes before starting the chromatography.
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{16}H_{18}FN_3O_3$ in the eye drops.

Storage. Store protected from light.

Norfloxacin Tablets

Norfloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of norfloxacin, $C_{16}H_{18}FN_3O_3$.

Usual strengths. 200 mg; 400 mg; 800 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *chloroform*, 40 volumes of *methanol*, 20 volumes of *toluene*, 14 volumes of *diethylamine* and 8 volumes of *water*.

Test solution. Shake a quantity of the finely powdered tablets containing 75 mg of Norfloxacin with 50 ml of a mixture of equal volumes of acidified *methanol* (containing 0.9 per cent

v/v of *hydrochloric acid*) and *dichloromethane*, centrifuge and use the clear supernatant solution.

Reference solution. A 0.15 per cent w/v solution of *norfloxacin RS* in the same solvent mixture.

Apply to the plate 50 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to norfloxacin in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 750 ml of *acetate buffer pH 4.0*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with *acetate buffer pH 4.0*, if necessary, at the maximum at about 278 nm (2.4.7). Concomitantly measure the absorbance of a solution of known concentration of *norfloxacin RS* in the same medium. Calculate the total content of $C_{16}H_{18}FN_3O_3$ in the medium.

D. Not less than 70 per cent of the stated amount of $C_{16}H_{18}FN_3O_3$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Add 80 ml of the mobile phase to an accurately weighed quantity of the powdered tablets containing about 100 mg of Norfloxacin, mix with the aid of ultrasound for 10 minutes, dilute with a 0.1 per cent v/v solution of *phosphoric acid* to 200.0 ml and mix. Dilute 10.0 ml of this solution to 25.0 ml with the mobile phase, mix and use the resulting solution after filtration through a filter with porosity of not more than 0.1 µm.

Reference solution. A 0.02 per cent w/v solution of *norfloxacin RS* in the mobile phase.

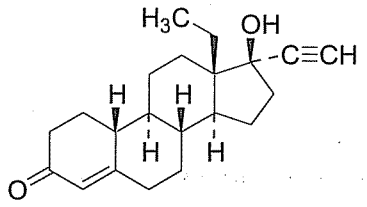
Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 85 volumes of a 0.1 per cent v/v solution of *phosphoric acid* and 15 volumes of *acetonitrile*,
- temperature. column 40° ± 1°, after preconditioning with degassed *0.01 M sodium dihydrogen phosphate* adjusted to pH 4.0 with *phosphoric acid* flowing at a rate of 0.5 ml per minute for 8 hours,
- flow rate. 2 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. The assay is not valid unless the capacity factor is not less than 2, the column efficiency is not less than 1500 theoretical plates, the tailing factor for the norfloxacin peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{16}H_{18}FN_3O_3$ in the tablets.

Storage. Store protected from light and moisture.

Norgestrel



$C_{21}H_{28}O_2$ Mol. Wt. 312.5

Norgestrel is *rac*-13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one.

Category. Progestogen.

Dose. As a contraceptive, 150 to 300 µg in combination with 20 to 50 µg of ethinyloestradiol daily.

Description. A white or almost white, crystalline powder; practically odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *norgestrel RS*.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 240 nm.

C. Melting range (2.4.21). 205° to 212°, but the range between beginning and end of melting does not exceed 4°.

Tests

Specific optical rotation (2.4.22). –0.1° to +0.1°, determined in a 5.0 per cent w/v solution in *chloroform*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *dichloromethane* and 20 volumes of *ethyl acetate*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform.*

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (b). A 0.004 per cent w/v solution of the substance under examination in *chloroform.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *phosphomolybdic acid solution.* Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash. (2.3.18) Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hours.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml, dilute stepwise with *ethanol (95 per cent)* to obtain a solution containing 0.001 per cent w/v of levonorgestrel and measure the absorbance of the resulting solution at the maximum at about 241 nm, (2.4.7). Calculate the content of $C_{21}H_{28}O_2$ from the absorbance obtained with a 0.001 per cent w/v solution of *norgestrel RS* in *ethanol (95 per cent).*

Storage. Store protected from moisture.

Norgestrel and Ethinyloestradiol Tablets

Norgestrel and Ethinyloestradiol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of norgestrel, $C_{21}H_{28}O_2$ and ethinyloestradiol, $C_{20}H_{24}O_2$.

Category. Oral contraceptive.

Dose. One tablet daily for 21 days starting from the fifth day of menstrual cycle.

Usual strength. Norgestrel, 300 µg and Ethinyloestradiol, 30 µg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 96 volumes of *dichloromethane* and 4 volumes of *ethanol (95 per cent).*

Test solution. Powder 20 tablets finely, triturate with 20 ml of *dichloromethane,* allow the solids to sediment and use the clear supernatant liquid.

Reference solution. A solution containing 0.06 per cent w/v solution of *norgestrel RS* and 0.006 per cent w/v solution of *ethinyloestradiol RS.*

Apply to the plate 40 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (80 per cent v/v),* heat at 110° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spots in the chromatogram obtained with the test solution correspond to the spots for norgestrel (red fluorescence) and ethinyloestradiol (orange-yellow fluorescence) in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Carry out the procedure described under Assay but using the following test solution.

Test solution. Add 2.0 ml of *methanol (70 per cent)* and 2.0 ml of a 0.00002 per cent w/v solution of *diphenyl* in *methanol (70 per cent)* (internal standard solution) to one tablet, shake for 20 minutes, centrifuge, filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 mm and use the filtrate.

Calculate the contents of norgestrel $C_{21}H_{28}O_2$, and ethinyloestradiol, $C_{20}H_{24}O_2$, in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) using the chromatographic system described under Uniformity of content.

Test solution. Weigh and powder 20 tablets. To a quantity of the powder equivalent to one tablet add 2.0 ml of *methanol (70 per cent)* and 2.0 ml of a 0.00002 per cent w/v solution of *diphenyl* in *methanol (70 per cent)* (internal standard solution), shake for 20 minutes, centrifuge, filter the supernatant liquid through a membrane filter with a pore size of not more than 0.2 mm and use the filtrate.

Reference solution. A solution in *methanol (70 per cent)* containing 0.15 mg per ml of *norgestrel RS* and 0.015 mg per ml of *ethinyloestradiol RS.* Take 2.0 ml of this solution and add 2.0 ml of a 0.00002 per cent w/v solution of *diphenyl* in *methanol (70 per cent)* and use the resulting solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 to 7 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile,* 15 volumes of *methanol* and 45 volumes of *water,*
- flow rate. 1 to1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

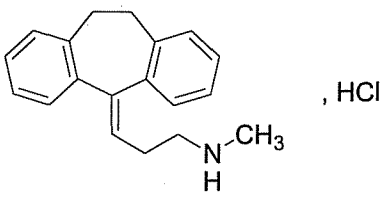
Inject the reference solution. The resolution between the two major peaks is not less 2.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. The relative retention times are about 0.7 for ethinyloestradiol and about 1.0 for norgestrel.

Calculate the contents of norgestrel, $C_{21}H_{28}O_2$, and ethinyloestradiol, $C_{20}H_{24}O_2$ in the tablets.

Storage. Store protected from light.

Nortriptyline Hydrochloride



, HCl

$C_{19}H_{21}N,HCl$ Mol. Wt. 299.8

Nortriptyline Hydrochloride is 3-(10,11-dihydro-5*H*-dibenzo [*a*,*d*]cyclohept-5-ylidene)propyl(methyl)amine hydrochloride.

Nortriptyline Hydrochloride contains not less than 98.0 per cent and not more than 101.5 per cent of $C_{19}H_{21}N,HCl$, calculated on the dried basis.

Category. Antidepressant.

Dose. Initially, 50 to 150 mg daily; maintenance dose, 30 to 75 mg daily.

Description. A white to off-white powder; odour slight and characteristic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and, D are carried out.

A. Dissolve 0.1 g in 10 ml of *water*, make alkaline with *1 M sodium hydroxide*, extract with 5 ml of *chloroform* and evaporate to dryness using a current of nitrogen.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nortriptyline hyrdochloride RS* treated in the same manner or with the reference spectrum of nortriptyline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 239 nm; absorbance at about 239 nm, about 0.48.

C. To about 50 mg dissolved in 3 ml of warm *water*, add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; a red colour is produced after a few minutes (distinction from amitriptyline).

D. Gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 85 volumes of *cyclohexane*, 15 volumes of *ethyl acetate* and 3 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)* prepared in subdued light.

Reference solution. A 0.001 per cent w/v solution of *dibenzosuberone RS* in *ethanol (95 per cent)* prepared in subdued light.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 14 cm in an unsaturated tank protected from light. Dry the plate in air, spray with a freshly prepared solution of *sulphuric acid* containing 4 per cent v/v of *formaldehyde solution* and examine immediately in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.25 g and dissolve in 25 ml of *anhydrous glacial acetic acid*, warm slightly, if necessary, to effect solution. Cool, add 5 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02998 g of $C_{19}H_{21}N,HCl$.

Storage. Store protected from light and moisture.

Nortriptyline Tablets

Nortriptyline Hydrochloride Tablets

Nortriptyline Tablets contain less than 90.0 per cent and not more than 110.0 per cent of the stated amount of nortriptyline, $C_{19}H_{21}N$. The tablets are coated.

Usual strengths. The equivalent of 10 mg and 25 mg of nortriptyline.

Identification

A. Shake a quantity of the powdered tablets containing about 5 mg of nortriptyline with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5 per cent w/v solution of *sodium bicarbonate*, 1 ml of a 2 per cent w/v solution of *sodium periodate* and 1 ml of a 0.3 per cent w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with *1 M sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting trimethylpentane solution shows an absorption maximum only at about 265 nm.

B. Triturate a quantity of the powdered tablets containing 0.1 g of nortriptyline with 10 ml of *chloroform*, filter and evaporate the filtrate to a low volume. Add *ether* until a turbidity is produced and allow to stand. Dissolve 50 mg of the precipitate in 3 ml of warm *water*, cool and add 1 drop of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*; a red colour is produced after a few minutes (distinction from amitriptyline).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 85 volumes of *cyclohexane*, 15 volumes of *ethyl acetate* and 3 volumes of *diethylamine*.

Test solution. Extract a quantity of the powdered tablets containing 20 mg of nortriptyline with 5 ml of a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *2 M hydrochloric acid*, centrifuge and use the supernatant liquid.

Reference solution. A 0.001 per cent w/v solution of *dibenzosuberone RS* in *ethanol (95 per cent)* prepared in subdued light.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 14 cm in an unsaturated tank protected from light. Dry the plate in air, spray with a freshly prepared solution of *sulphuric acid* containing 4 per cent v/v of *formaldehyde solution* and examine immediately in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content *(For tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Powder one tablet, add 2.5 ml of *water*, shake vigorously to completely disperse the tablet, add 5 ml of *methanol* and shake for 30 minutes. Add sufficient *water* to produce 10 ml, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.01 per cent w/v solution of *nortriptyline hydrochloride RS* in *methanol (50 per cent)*.

Follow the procedure given in the Assay. Calculate the content of $C_{20}H_{23}N$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake vigorously 20 tablets with 50 ml of *water* until the tablets disintegrate completely, add 100.0 ml of *methanol* and shake for 30 minutes. Add sufficient *water* to produce 200.0 ml, filter and dilute a volume of the filtrate containing about 25 mg of nortriptyline to 100.0 ml with *methanol (50 per cent)*.

Reference solution. A 0.025 per cent w/v solution of *nortriptyline hydrochloride RS* in *methanol (50 per cent)*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a 0.56 per cent w/v solution of *sodium hexanesulphonate* in a mixture of equal volumes of *water* and *acetonitrile* adjusted to pH 4.5 with *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

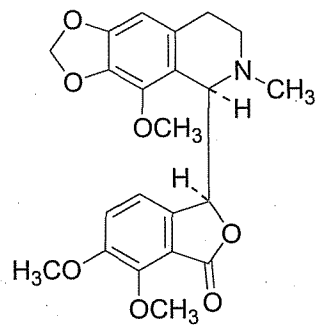
Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{21}N$ in the tablets.

Storage. Store protected from light and moisture.

Noscapine

Narcotine



$C_{22}H_{23}NO_7$ Mol. Wt. 413.4

Noscapine is (3*S*)-6,7-dimethoxy-3-[(5*R*)-5,6,7,8-tetrahydro-4-methoxy-6-methyl-1,3-dioxolo[4,5-*g*]isoquinolin-5-yl]phthalide, an alkaloid obtained from opium.

Noscapine contains not less than 98.5 per cent and not more than 100.5 per cent of $C_{22}H_{23}NO_7$, calculated on the dried basis.

Category. Cough suppressant.

Dose. 15 to 30 mg three to four times daily.

Description. Colourless crystals or a white crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *noscapine RS* or with the reference spectrum of noscapine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *methanol* shows absorption maxima at about 291 nm and 310 nm; ratio of absorbance at the maximum at about 310 nm to that at the maximum at about 291 nm, 1.2 to 1.3.

C. To 0.1 g in a porcelain dish add a few drops of *sulphuric acid* and stir; a greenish-yellow solution is formed which on warming becomes red and finally violet.

D. Dissolve 50 mg in 5 ml of *5 M hydrochloric acid*, add 10 ml of a mixture of equal volumes of *ethanol (95 per cent)* and a saturated solution of *sodium acetate*, mix and allow to stand for about 3 minutes; shining crystals separate.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *acetone* examined immediately after preparation is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Specific optical rotation (2.4.22). +42.0° to +48.0°, determined at 20° in a solution prepared by dissolving 0.5 g in sufficient *0.1 M hydrochloric acid* to produce 25.0 ml.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination with gentle heating in 14 ml of *methanol*, cool, and dilute to 20 ml with *phosphate buffer solution pH 6.0*.

Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *papaverine hydrochloride* in 50 ml of the test solution. Dilute 1.0 ml of this solution to 20 ml with the mobile phase.

Reference solution (c). Dissolve 1.5 mg of *papaverine hydrochloride* in 10 ml of the test solution and dilute to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of *phosphate buffer pH 6.0*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to noscapine and papaverine (noscapine impurity A) is not less than 2.0. The relative retention time with reference to noscapine for noscapine impurity A is about 1.3.

Inject the test solution, reference solution (a) and (b). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to noscapine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of the any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), and the sum of the areas of secondary peaks other than noscapine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Morphine. Dissolve 0.1 g in 10 ml of *0.1 M hydrochloric acid*. To 1 ml of the resulting solution add a mixture of 1 ml of *potassium ferricyanide solution*, 0.05 ml of *ferric chloride test solution* and 4 ml of *water*; no blue or dark green colour develops within 1 minute.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.35 g, dissolve in 40 ml of *anhydrous glacial acetic acid*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04134 g of $C_{22}H_{23}NO_7$.

Storage. Store protected from light and moisture.

Noscapine Linctus

Narcotine Linctus

Noscapine Linctus is a solution of Noscapine in a suitable flavoured vehicle. It may contain up to 1 per cent w/v solution of Citric Acid.

Noscapine Linctus contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of noscapine, $C_{22}H_{23}NO_7$.

Usual strength. 7 mg per 5 ml.

Identification

To a quantity containing 60 mg of Noscapine add 20 ml of *water*, 2 g of *sodium chloride* and 2 ml of *5 M sodium hydroxide*. Extract with successive quantities of 50, 50, 25 and 25 ml of *ether*. Combine the extracts, wash with three quantities, each of 5 ml, of *water* and evaporate to dryness. Dissolve the residue in 20 ml of *chloroform*. Wash with three quantities, each of 20 ml, of *water*, dry the chloroform layer with *anhydrous sodium sulphate*, filter and evaporate the solvent. If necessary, induce crystallisation by scratching with a glass rod. The crystals comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *noscapine RS* or with the reference spectrum of noscapine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *methanol* shows absorption maxima at about 291 nm and 310 nm; ratio of absorbance at the maximum at about 310 nm to that at the maximum at about 291 nm, 1.2 to 1.3.

Tests

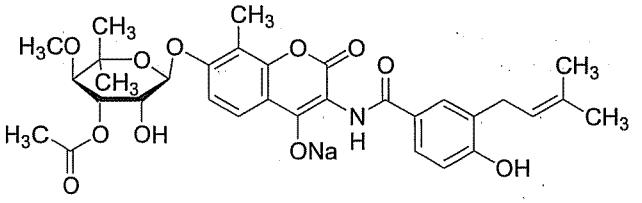
Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity containing 60 mg of Noscapine, add 20 ml of *water*, 2 g of *sodium chloride* and 2 ml of *5 M sodium hydroxide* and extract with successive quantities of 50, 50, 25 and 25 ml of *ether*. Combine the extracts, wash with three quantities, each of 5 ml, of *water* and evaporate to dryness. To the residue add 50.0 ml of *0.1 M hydrochloric acid*, warm on a water-bath to dissolve and to remove any traces of ether and dilute to 100.0 ml with *water*. Dilute 3.0 ml to 50.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 310 nm (2.4.7). Calculate the content of $C_{22}H_{23}NO_7$ taking 90.7 as the specific absorbance at 310 nm.

Determine the weight per ml of the linctus (2.4.29), and calculate the content of $C_{22}H_{23}NO_7$, weight in volume.

Storage. Store protected from light and moisture.

Novobiocin Sodium



$C_{31}H_{35}N_2NaO_{11}$ Mol. Wt. 634.6

Novobiocin Sodium is the monosodium salt of novobiocin, *N*-[7-{3-*O*-(aminocarbonyl)-6-deoxy-5-*C*-methyl-4-*O*-methyl-β-L-*lyxo*-hexopyranosyl}-oxy-4-hydroxy-8-methyl-2-oxo-2*H*-1-benzopyran-3-yl]-4-hydroxy-3-(3-methyl-2-butenyl)benzamide, an antimicrobial substance produced by the growth of certain strains of *Streptomyces niveus* or related organisms or by other means.

Novobiocin Sodium contains the equivalent of not less than 850 µg of novobiocin per mg, calculated on the dried basis.

Category. Antibacterial.

Dose. The equivalent of 1 to 2 g of novobiocin daily, in divided doses.

Description. A white or yellowish white, crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 75 volumes of *chloroform*, 25 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Dissolve a quantity of the substance under examination in *methanol* so as to obtain a solution containing 0.1 per cent w/v solution of novobiocin.

Reference solution. A 0.1 per cent w/v solution of *novobiocin RS* in *methanol*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydroxide* shows an absorption maximum only at about 307 nm.

C. The residue obtained by igniting it gives the tests for sodium salts (2.3.1).

Tests

pH (2.4.24). 6.6 to 8.5, determined in a 2.5 per cent w/v solution.

Specific optical rotation (2.4.22). –50.0° to –58.0°, determined in a 5.0 per cent w/v solution in *methanol* containing 1 per cent v/v of *hydrochloric acid.*

Loss on drying (2.4.19). Not more than 6 per cent, determined on 0.2 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10), and express the results in µg of novobiocin per mg.

Novobiocin Sodium intended for use in the manufacture of Parenteral Preparations complies with the following additional requirements.

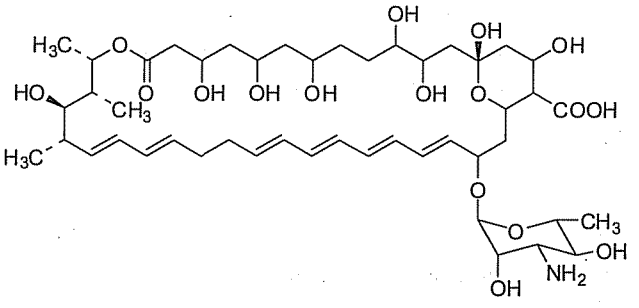
Bacterial endotoxins (2.2.3). Not more than 0.7 Endotoxin units per mg.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from light and moisture at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Nystatin



$C_{47}H_{75}NO_{17}$ Mol. Wt. 926.1

Nystatin is an antifungal substance produced by the growth of certain strains of *Streptomyces noursei* or by any other means. It consists mainly of polyenes, the principal component being nystatin A_1.

Nystatin has a potency of not less than 4400 Units per mg, calculated on the dried basis.

Category. Antifungal.

Dose. In the treatment of alimentary candidiasis, 500,000 to 1,000,000 Units daily, in divided doses.

Description. A yellow to slightly brown powder; odour, characteristic; hygroscopic.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), the final solution obtained in the test for Light absorption shows absorption maxima at about 230 nm, 291 nm, 305 nm and 319 nm. The ratios of the absorbances at the maxima at about 291 nm and about 319 nm to the absorbance at the maximum at about 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use as the blank a solution prepared in the same manner without the substance under examination.

B. Shake 30 mg with 5 ml of *water* for 2 minutes, add 2 ml of *sodium molybdotungstophosphate solution*, and allow to stand for 1 hour; the green colour produced is darker than that produced by repeating the test without the substance under examination.

C. Shake 30 mg with 5 ml of *water* for 2 minutes, add 2 ml of a solution prepared by dissolving 0.1 g of *pyrogallol* in 100 ml of *decolorised magenta solution*, heat on a water-bath until a dark pink colour is produced, cool and allow to stand for 1 hour; the pink colour is retained.

D. To 2 mg add 0.1 ml of *hydrochloric acid*; a brown colour is produced.

E. To 2 mg add 0.1 ml of *sulphuric acid*; a brown colour is produced which becomes violet on standing.

Tests

pH (2.4.24). 6.5 to 8.0, determined in a 3.0 per cent w/v suspension in *water*.

Light absorption (2.4.7). Dissolve 0.1 g in a mixture of 5.0 ml of *glacial acetic acid* and 50 ml of *methanol*, add sufficient *methanol* to produce 100.0 ml and dilute 1.0 ml of the resulting solution to 100.0 ml with *methanol*. Absorbance of the resulting solution, measured within 30 minutes of preparation, at the maximum at about 305 nm, not less than 0.60. Use as the blank a solution prepared in the same manner without the substance under examination.

Composition. Determine by liquid chromatography (2.4.14).

Note—Carry out the test protected from light.

Test solution. Dissolve 20 mg of the substance under examination in 50 ml of *dimethyl sulphoxide.*

Reference solution (a). A 0.04 per cent w/v solution of *nystatin RS* in *dimethyl sulphoxide.*

Reference solution (b). Dissolve 20 mg of the substance under examination in 25 ml of the *methanol* and dilute to 50 ml with

water. To 10.0 ml of this solution add 2.0 ml of *dilute hydrochloric acid*. Allow to stand at room temperature for 1 hour.

Reference solution (c). Dilute 1.0 ml of reference solution (a) in 100.0 ml of *dimethyl sulphoxide*. Dilute 1.0 ml of this solution to 10.0 ml with *dimethyl sulphoxide*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 29 volumes of *acetonitrile* and 71 volumes of 0.385 per cent w/v solution of *ammonium acetate*,
  B. a mixture of 40 volumes of 0.385 per cent w/v solution of *ammonium acetate* and 60 volumes of *acetonitrile*.
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 305 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent w/v)	Mobile phase B (per cent w/v)
0-25	100	0
25-35	100-0	0-100
35-45	0	100
45-50	0-100	100-0
50-55	100	0

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.5.

Inject the test solution, reference solution (a) and (c). In the chromatogram obtained with the test solution the area of the peak due to nystatin A1 is not less than 85 per cent the area of the principal peak in the chromatogram obtained with reference solution (a), the area of the any other compound is not more than 4.0 per cent the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak with a retention time of less than 2 minutes.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 3.5 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.1 kPa for 3 hours.

Assay. *Protect the solution from light throughout the assay.*

Weigh accurately about 75 mg and dissolve in sufficient *dimethylformamide* to produce 50.0 ml; dilute 10.0 ml of the resulting solution to 200.0 ml with *buffer solution No 4* (2.2.10). Determine by the microbiological assay of antibiotics (2.2.10).

Nystatin intended for oral administration complies with the following additional requirement.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity, using a quantity containing not less than 600 Units suspended in not more than 0.5 ml of a 0.5 per cent w/v solution of *acacia* and injecting the suspension intraperitoneally.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the number of Units of Nystatin per mg.

Nystatin Ointment

Nystatin Ointment is a dispersion of Nystatin in microfine powder in a suitable ointment basis.

Nystatin Ointment contains not less than 90.0 per cent and not more than 130.0 per cent of the stated number of Units of nystatin.

Usual strength. 100,000 Units per g.

Identification

Disperse a quantity containing 25,000 Units in 10 ml of *chloroform*, add 40 ml of *methanol* and shake. Filter and dilute 1 ml of the filtrate to 25 ml with *methanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 291 nm, 305 nm and 319 nm. The ratios of the absorbances at the maxima at about 291 nm and about 319 nm to the absorbance at the maximum at about 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use as the blank a solution prepared exactly in the same manner without the substance under examination.

Tests

Other tests. Complies with the tests stated under Ointments.

Assay. *Protect the solution from light throughout the assay.*

Weigh accurately a quantity containing 400,000 Units, disperse in 20 ml of *ether* in a stoppered flask, add 70 ml of *dimethylformamide*, shake for a few minutes, add 10 ml of *water*, shake vigorously for a few minutes and add sufficient *dimethylformamide* to produce 100.0 ml. Mix well, filter and dilute 10.0 ml of the filtrate to 100.0 ml with *buffer solution No 4* (2.2.10).

Determine by the microbiological assay of antibiotics (2.2.10).

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the number of Units of Nystatin per g.

Nystatin Pessaries

Nystatin Vaginal Tablets

Nystatin Pessaries contain not less than 90.0 per cent and not more than 130.0 per cent of the stated number of Units of nystatin.

Usual strength. 100,000 Units.

Identification

Extract a quantity of the powdered pessaries containing 300,000 Units with a mixture of 50 ml of *methanol* and 5 ml of *glacial acetic acid*, add sufficient *methanol* to produce 100 ml and filter. Dilute 1 ml of the filtrate to 100 ml with *methanol*. The resulting solution complies with the follwing test.

Disperse a quantity containing 25,000 Units in 10 ml of *chloroform*, add 40 ml of *methanol* and shake. Filter and dilute 1 ml of the filtrate to 25 ml with *methanol*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 291 nm, 305 nm and 319 nm. The ratios of the absorbances at the maxima at about 291 nm and about 319 nm to the absorbance at the maximum at about 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use as the blank a solution prepared exactly in the same manner without the substance under examination.

Tests

Other tests. Comply with the tests stated under Pessaries.

Loss on drying (2.4.19). Not more than 5 per cent, determined on 1.0 g of the powdered pessaries by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. *Protect the solution from light throughout the assay.*

Weigh and powder 20 pessaries. Weigh accurately a quantity of the powder containing 200,000 Units and shake with 50.0 ml of *dimethylformamide* for 1 hour. Centrifuge, dilute 10.0 ml of the clear, supernatant liquid to 200.0 ml with *buffer solution No 4* (2.2.10).

Determine by the microbiological assay of antibiotics (2.2.10).

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the number of Units of Nystatin.

Nystatin Tablets

Nystatin Tablets contain not less than 90.0 per cent and not more than 130.0 per cent of the stated number of Units of nystatin. The tablets are coated.

Usual strength. 500,000 Units.

Identification

Extract a quantity of the powdered tablets containing 300,000 Units with a mixture of 50 ml of *methanol* and 5 ml of *glacial acetic acid*, add sufficient *methanol* to produce 100 ml and filter. Dilute 1 ml of the filtrate to 100 ml with *methanol*. The resulting solution complies with the follwing test.

When examined in the range 230 nm to 360 nm (2.4.7), the solution shows absorption maxima at about 291 nm, 305 nm and 319 nm. The ratios of the absorbances at the maxima at about 291 nm and about 319 nm to the absorbance at the maximum at about 305 nm are 0.61 to 0.73 and 0.83 to 0.96, respectively. Use as the blank a solution prepared exactly in the same manner without the substance under examination.

Tests

Disintegration (2.5.1). 30 minutes, but using a 0.6 per cent v/v solution of *hydrochloric acid* in place of *water*. If the tablets fail to disintegrate, wash them rapidly by immersion in *water* and continue the test using *phosphate buffer pH 6.8*; the tablets then disintegrate within a further 30 minutes.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g of the powdered tablets by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 200,000 Units and shake with 50.0 ml of *dimethylformamide* for 1 hour. Centrifuge, dilute 10.0 ml of the clear, supernatant liquid to 200.0 ml with *buffer solution No 4* (2.2.10).

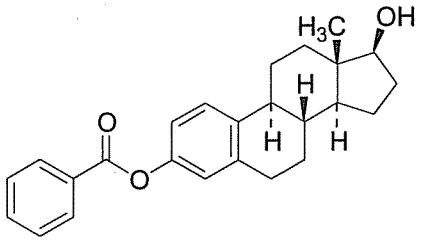
Determine by the microbiological assay of antibiotics (2.2.10).

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the number of Units of Nystatin.

O

Oestradiol Benzoate



$C_{25}H_{28}O_3$ Mol. Wt. 376.5

Oestradiol Benzoate is 17β-hydroxyestra-1,3,5(10)-trien-3-yl benzoate.

Oestradiol Benzoate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{25}H_{28}O_3$, calculated on the dried basis.

Category. Oestrogen.

Dose. By intramuscular injection, 1 to 2 mg daily.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oestradiol benzoate RS* or with the reference spectrum of oestradiol benzoate.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b) when examined in daylight and in ultraviolet light at 365 nm.

C. To about 1 mg add 0.5 ml of a 5 per cent w/v solution of *ammonium molybdate* in *sulphuric acid*; a yellowish green colour develops which exhibits an intense green fluorescence when examined in ultraviolet light at 365 nm. Add 1 ml of *sulphuric acid* and 9 ml of *water*; the solution becomes pink with a yellowish fluorescence.

Tests

Specific optical rotation (2.4.22). +57.0° to +63.0°, determined in a 1.0 per cent w/v solution in *dioxan*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethanol (95 per cent)*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of the same solvent mixture.

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *oestradiol benzoate RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until odour of the solvent is no longer detectable, heat at 110° for 10 minutes, spray the plate while hot with *ethanolic sulphuric acid (20 per cent)*, heat again at 110° for 10 minutes and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.2 per cent, determined on 0.5 g.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 25 mg, dissolve in sufficient *ethanol (95 per cent)* to produce 250.0 ml. Dilute 10.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 231 nm (2.4.7). Calculate the content of $C_{25}H_{28}O_3$ taking 500 as the specific absorbance at 231 nm.

Storage. Store protected from light and moisture.

Oestradiol Injection

Oestradiol Benzoate Injection

Oestradiol Injection is a sterile solution of Oestradiol Benzoate in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these. It may contain suitable alcohols.

Oestradiol Benzoate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oestradiol benzoate, $C_{25}H_{28}O_3$.

Usual strength. 1 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene* and 20 volumes of *ethyl acetate*.

Test solution. Add 10 ml of *2,2,4-trimethylpentane* to a volume of the injection containing 2 mg of Oestradiol Benzoate and extract with three quantities, each of 10 ml, of *ethanol (70 per cent)*. Wash the combined extracts with 15 ml of *2,2,4-trimethylpentane*, evaporate the ethanolic extract to dryness using a rotary evaporator and dissolve the residue in 2 ml of *chloroform*.

Reference solution. A 0.1 per cent w/v solution of *oestradiol benzoate RS* in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent)*, heat at 105° for 10 minutes and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution (a). Dilute an accurately measured quantity of the injection containing about 1 mg of Oestradiol Benzoate to 10.0 ml with a mixture of 90 volumes of *cyclohexane* and 10 volumes of *dioxan*.

Test solution (b). Add 1 ml of a solution prepared by dissolving 15 mg of *4-hydroxybenzaldehyde* (internal standard) in 10.0 ml of *dioxan*, adding sufficient *cyclohexane* to produce 100.0 ml (solution A), to an accurately measured quantity of the injection containing about 1 mg of Oestradiol Benzoate and dilute to 10.0 ml with sufficient of a mixture of 90 volumes of *cyclohexane* and 10 volumes of *dioxan*.

Reference solution. Add 10 ml of solution A to 10 mg of *oestradiol benzoate RS*, accurately weighed, and dilute to 100.0 ml with the same solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with porous silica particles (10 µm),
- mobile phase: 90 volumes of *cyclohexane* and 10 volumes of *dioxan*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

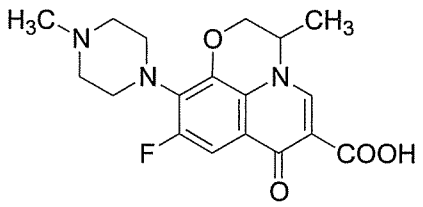
Inject test solutions (a), (b) and the reference solution. The assay is not valid unless the resolution between the peaks due to benzyl alcohol (if present) and oestradiol benzoate and between the peaks due to oestradiol benzoate and the internal standard is more than 1.5.

Calculate the content of $C_{25}H_{28}O_3$ in the injection.

Storage. Store protected from light.

Labelling. The label states (1) the nature and composition of the solvent; (2) that it is meant for intramuscular injection only; (3) that any solid matter that may have separated on standing should be redissolved by warming before use.

Ofloxacin



$C_{18}H_{20}FN_3O_4$ Mol. Wt. 361.4

Ofloxacin is (*RS*)-9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3,-*de*]-1,4-benzoxazine-6-carboxylic acid

Ofloxacin contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{18}H_{20}FN_3O_4$, calculated on the dried basis.

Category. Antibacterial.

Dose. 200 to 400 mg daily.

Description. A pale yellow or bright yellow, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ofloxacin RS* or the spectrum obtained with the reference solution.

Tests

Light absorption. Absorbance at 440 nm (2.4.7), of 0.5 per cent w/v solution in *0.1 M hydrochloric acid* is not more than 0.25.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.1 per cent w/v solution of *ofloxacin RS* in *methanol*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with *methanol*.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *phosphate buffer pH 2.4* prepared by dissolving 27.2 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjust the pH to 2.4 with

orthophosphoric acid,
- flow rate. 2 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the column efficiency in not less than 1400 theoretical plates.

Inject the test solution and reference solution (b). Run the chromatogram three times of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy Metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.3 g, dissolve in 100 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03614 g of $C_{18}H_{20}FN_3O_4$.

Storage. Store protected from light and moisture.

Ofloxacin Infusion

Ofloxacin Infusion is a sterile solution of Ofloxacin in 5 per cent Dextrose Injection or in Sodium Chloride Injection.

Ofloxacin Infusion contain not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of ofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strengths. 25 mg; 50 mg; 100 mg; 200 mg; 400 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.8 to 7.5.

Other tests. Complies with the tests stated under Parenteral Preparation (Infusions).

Assay. Determine by liquid chromatography (2.4.14).

NOTE —Protect the solutions from the light.

Test solution. Measure accurately a volume containing 50 mg of Ofloxacin in 100.0 ml with mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with mobile phase.

Reference solution. A 0.005 per cent w/v solution of *ofloxacin RS* in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 0.47 g *sodium 1-hexane sulphonate* in 1000 ml of *water*, add 1 ml of *triethylamine* and adjust the pH to 3.0 with *orthophosphoric acid* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$ in infusion.

Ofloxacin Ophthalmic Solution

Ofloxacin Ophthalmic Solution is a sterile aqueous solution of Ofloxacin.

Ofloxacin Ophthalmic Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strength. 0.3 per cent w/w.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 7.2.

Sterility (2.2.11). Comply with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Phosphate buffer pH 7.25 prepared by dissolving 3.56 g of *disodium hydrogen phosphate* in 1000 ml *water*, adjusted pH to 7.25 using *orthophosphoric acid* or *sodium hydroxide solution*.

Test solution. Measure accurately a volume of Opthalmic Solution containing 30 mg of Ofloxacin in 100.0 ml of solvent mixture. Dilute 1.0 ml of the solution to 10.0 with solvent mixture.

Reference solution. A 0.003 per cent w/v solution of *ofloxacin RS* in solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as TSK GEL),
- mobile phase: a mixture of 20 volumes of *acetonitrile,* 80 volumes of phosphate buffer pH 7.25 prepared by dissolving 2.54 g of *tetrabutyl ammonium hydrogen sulphate* and 3.56 g of *disodium hydrogen phosphate* in 1000 ml *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$.

Storage. Store protected from light.

Ofloxacin Tablets

Ofloxacin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ofloxacin, $C_{18}H_{20}FN_3O_4$.

Usual strengths. 200 mg; 400 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloride acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the medium if necessary, at the maximum at about 294 nm (2.4.7). Calculate the content of $C_{18}H_{20}FN_3O_4$ in the medium from the absorbance obtained from a solution of known concentration of *ofloxacin RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{18}H_{20}FN_3O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 100 mg of Ofloxacin, disperse in 60 ml of *methanol* and dilute to 100 ml with *methanol* and filter.

Reference solution (a). A 0.1 per cent w/v solution of *ofloxacin RS* in *methanol.*

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with *methanol.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 8 volumes of *acetonitrile* and 92 volumes of *phosphate buffer pH 2.4* prepared by dissolving 27.2 g of *monobasic potassium phosphate* in 1000 ml of *water,* adjust the pH to 2.4 with *orthophosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the column efficiency in not less than 1400 theoretical plates.

Inject the test solution and reference solution (b). Run the chromatogram three times of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 25 mg of Ofloxacin, disperse in 60 ml of *methanol* and dilute to 100 ml with *methanol* and filter.

Reference solution. A 0.025 per cent w/v solution of *ofloxacin RS* in *methanol.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 92 volumes of buffer solution prepared by dissolving 27.2 g of *potassium dihydrogen*

phosphate in 1000 ml of *water* and adjust the pH to 2.4 with *orthophosphoric acid* and 8 volumes of *acetonitrile,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 10 µl.

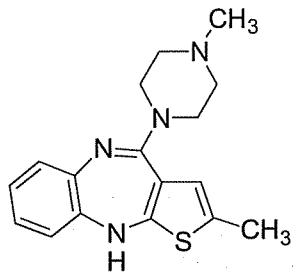
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}FN_3O_4$.

Storage. Store protected from light and moisture.

Olanzapine



$C_{17}H_{20}N_4S$ Mol. Wt. 312.4

Olanzapine is 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine.

Olanzapine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{17}H_{20}N_4S$, calculated on the anhydrous basis.

Category. Antipsychotic.

Dose. 10 mg daily.

Description. A yellow crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *olanzapine RS* or with the reference spectrum of olanzapine.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *water* and 60 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 25 ml of solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *olanzapine RS* in solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a mixture of 80 volumes of *buffer solution pH 6.8* prepared by dissolving 4.825 g of *sodium dihydrogen orthophosphate monohydrate* in 1000 ml of *water*, adjust pH to 6.8 with 10 per cent w/v of *sodium hydroxide* and 20 volumes of *acetonitrile*,
  B. *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
20	63	37
30	45	55
32	100	0
38	100	0

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.2 g, dissolve in 40 ml of *glacial acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01562 g of $C_{17}H_{20}N_4S$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Olanzapine Tablets

Olanzapine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of olanzapine, $C_{17}H_{20}N_4S$.

Usual strengths. 2.5 mg; 5 mg; 7.5 mg; 10 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

NOTE – Protect all the solutions from light.

Test solution. Use the filtrate, if necessary dilute with dissolution medium.

Reference solution. Weigh 16 mg of *olanzapine RS*, dissolve in about 2.5 ml of *acetonitrile* and dilute to 25 ml with *0.01 M hydrochloroc acid.*Dilute suitabely to get 0.00016 per cent w/v in dissolution medium.

Chromatographic system as described under Assay, using Injection volume 50 µl.

Inject the reference solution . The test is not valid unless the tailing factor is not more than 2.0. The column efficiency is not less than 2500 theoretical plates.

Inject the test solution and the reference solution.

Calculate the content of $C_{17}H_{20}N_4S$.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{20}N_4S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Take 10 intact tablets to a suitable volumetric flask, disperse in *acetonitrile* and dilute with *0.01 M hydrochloric acid* to get a final concentration of 0.01 per cent w/v of Olanzapine.

Reference solution. Weigh 10 mg of *olanzapine RS*, dissolve in about 25 ml of *acetonitrile* and dilute to 100 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *buffer solution* prepared by dissolving 3 g of *ammonium dihydrogen orthophosphate* in 900 ml *water*, add 2 ml of *triethylamine* and dilute to 1000 ml with *water.* Adjust the pH to 2.5 with *orthophosphoric acid* and 30 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

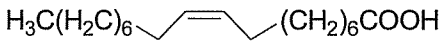
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2500 theoretical plates. The relative standard deviation for replicate injections is not more than 2 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{17}H_{20}N_4S$.

Storage. Store protected from light and moisture, at a temperature not exceeding 25°.

Oleic Acid



$C_{18}H_{34}O_2$ Mol. Wt. 282.5

Oleic Acid consists mainly of (*Z*)-octadec-9-enoic acid, $C_{18}H_{34}O_2$, together with varying amounts of saturated and other unsaturated fatty acids and is obtained by the hydrolysis of fats or fixed oils and separation of the liquid acids by expression or other suitable means. It may contain a suitable antioxidant.

Category. Pharmaceutical aid (emulsion adjuvant).

Description. A clear, yellowish to pale brown, oily liquid; odour, characteristic. On exposure to air it darkens in colour and the odour becomes more pronounced.

Identification

A.To 1 ml add 1 ml of *ethanol (95 per cent);* the solution is clear. It turns orange or red on addition of 0.1 ml of *methyl orange solution.*

B.Take a mixture of 1 ml of *nitric acid* and 1 ml of *water* in a test-tube with an internal diameter of about 12.5 mm and add 1 ml of the substance under examination on the surface of the mixture. Introduce 0.5 g of *copper* turnings into the lower layer and allow to stand under a hood for 4 hours; the upper layer solidifies.

C.Complies with the test for Iodine value (2.3.28).

Tests

Weight per ml (2.4.29). 0.889 g to 0.895 g.

Peroxide value (2.3.35). Not more than 10.0.

Acid value (2.3.23). 195 to 202, determined on 0.5 g.

Iodine value (2.3.28). 85 to 95, determined by Method A.

Water-soluble acids. Shake 5 ml with 5 ml of *water* for 2 minutes, allow the liquids to separate and filter the aqueous layer through paper moistened with *water*. To the filtrate add 0.05 ml of *methyl orange solution*; the liquid does not become red.

Neutral fats and mineral oils. Boil 1 ml with 5 ml of *0.5 M sodium carbonate* and 25 ml of water in a large flask. The solution, while still hot, is not more opalescent than opalescence standard OS2 (2.4.1).

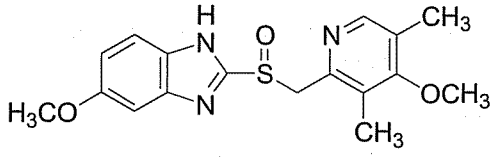
Congealing point. Dry about 10 ml by heating to 110° with frequent stirring, transfer to a test-tube about 20 mm in diameter, cool and when at 15° immerse the tube in a suitable water-bath so that the cooling takes place at the rate of 2° per minute. Stir the sample with a thermometer; it does not become cloudy until the temperature has fallen to 10°. On further cooling it congeals to a white solid or semi-solid mass at about 4°.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from light and moisture in a refrigerator (8° to 15°).

Labelling. The label states (1) where applicable, that it is used for external use only; (2) the name and concentration of any added antioxidant.

Omeprazole



$C_{17}H_{19}N_3O_3S$ Mol. Wt. 345.4

Omeprazole is 5-methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulfinyl]-1*H*-benzimidazole.

Omeprazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{19}N_3O_3S$, calculated on the dried basis.

NOTE — Perform the tests and assay in subdued light and use low-actinic glassware.

Category. Antiulcerative (proton pump inhibitor).

Dose. For duodenal and gastric ulcers and for reflux oesophagitis, 20 to 40 mg daily; for Zollinger-Ellison syndrome, initially 60 mg once daily; usual range, 20 to 120 mg daily.

Description. A white or almost white powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *omeprazole RS* or with the reference spectrum of omeprazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 276 nm and 305 nm; the ratio of the absorbance at about 305 nm to that at about 276 nm, 1.6 to 1.8.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *dichloromethane* is clear (2.4.1).

Light absorption (2.4.7). Absorbances of a freshly prepared 2.0 per cent w/v solution in *dichloromethane* at 400 nm, 500 nm and 600 nm are not more than 0.25, 0.10 and 0.10 respectively.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *benzene*, 30 volumes of *ethyl acetate* and 10 volumes of *methanol*.

Test solution. Dissolve 0.4 g of the substance under examination in 100 ml of *ethanol*.

Reference solution (a). A 0.4 per cent w/v solution of *omeprazole RS* in *ethanol*.

Reference solution (b). A 0.004 per cent w/v solution of *omeprazole RS* in *ethanol*.

Reference solution (c). A 0.002 per cent w/v solution of *omeprazole RS* in *ethanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c) and the total intensity of all such spots in the chromatogram obtained with the test solution is not more than the intensity of the spot obtained with reference solution (b).

B. In the Assay, the sum of the areas of all the secondary peaks is not greater than 1.5 per cent of the total area of all peaks.

Heavy metals (2.3.13). The residue obtained in the test for Sulphated ash, complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.005 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *omeprazole RS* in the mobile phase.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *phosphate buffer pH 7.4* and 35 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 20 µl.

Inject the reference solution. Repeat the procedure at least five times and measure the peak responses of the peak due to omeprazole. The relative standard deviation of the replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{17}H_{19}N_3O_3S$.

Storage. Store protected from light and moisture in a refrigerator (2° to 8°).

NOTE — A combination of elevated temperatures (37°-50°) and high humidity degrades Omeprazole. It rapidly degrades under acidic conditions.

Omeprazole Capsules

Omeprazole Capsules contain enteric-coated granules of Omeprazole.

Omeprazole Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of omeprazole, $C_{17}H_{19}N_3O_3S$.

NOTE — Perform the tests and assay in subdued light and use low-actinic glassware.

Usual strength. 20 mg.

Identification

A.To a quantity of the contents of the capsules containing 50 mg of Omeprazole in a 100-ml volumetric flask add about 70 ml of *0.1 M sodium hydroxide*. Mix in an ultrasonic bath for about 5 minutes and heat on a water-bath for 10 minutes. Cool, make up to volume with *0.1 M sodium hydroxide* and filter. Dilute 2 ml of the filtrate to 100 ml with *0.1 M sodium hydroxide*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 276 nm and 305 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to omeprazole in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

A. Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 2 hours.

Tap the granules from a capsule slightly with a glass rod to make them settle to the bottom. Rotate the paddle at 100 rpm for 2 hours, drain the solution slowly without losing any granules. Transfer them quantitatively to a 100-ml volumetric flask, add 20 ml of *0.1 M sodium hydroxide* and mix with the aid of ultrasound. Dilute to volume with *0.1 M sodium hydroxide*, centrifuge about 15 ml for 5 minutes and dilute 5.0 ml of the clear supernatant liquid to 50.0 ml with the mobile phase. Using the resulting solution as the test solution, carry out the determination as described in the Assay. Calculate the content of $C_{17}H_{19}N_3O_3S$ in the supernatant liquid. Calculate the percentage of omeprazole released in the acid medium by subtracting the content of $C_{17}H_{19}N_3O_3S$ in the test solution from the total content of omeprazole determined in the Assay.

Not more than 15 per cent of the stated amount of $C_{17}H_{19}N_3O_3S$ is dissolved in 2 hours.

B. Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 100 rpm and 45 minutes.

Tap the granules from a capsule slightly with a glass rod to make them settle to the bottom. Rotate the paddle at 100 rpm for 45 minutes and filter the solution. Using the filtered medium as the test solution, carry out the determination as described in the Assay. Calculate the content of $C_{17}H_{19}N_3O_3S$ in the medium.

D. Not less than 70 per cent of the stated amount of $C_{17}H_{19}N_3O_3S$.

Other tests. Comply with the tests stated under Capsules.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 0.5 g of the contents of the capsules by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Weigh and transfer the granules containing about 20 mg of Omeprazole to a 100-ml volumetric flask, add 20 ml of *0.1 M sodium hydroxide*, mix with the aid of ultrasound and dilute to volume with *0.1 M sodium hydroxide*. Centrifuge for 5 minutes and dilute 5.0 ml of the clear supernatant liquid to 50.0 ml with the mobile phase.

Reference solution. Take 20 mg of *omeprazole RS* in a dry, stoppered test-tube, add 20.0 ml of *0.1 M sodium hydroxide*, shake vigorously for 5 minutes and dilute 1.0 ml of the solution with the mobile phase to produce 50.0 ml.

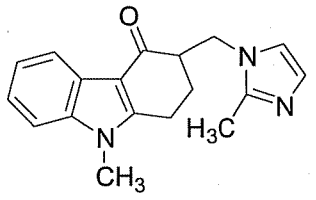
Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *phosphate buffer pH 7.4* and 35 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 302 nm,
- injection volume. 20 µl.

Inject the reference solution. Repeat the procedure at least five times and measure the peak responses of the peak due to omeprazole. The relative standard deviation of the replicate injections is not more than 2.0 per cent.

Calculate the content of $C_{17}H_{19}N_3O_3S$ in the capsules.

Storage. Store protected from light and moisture.

Ondansetron Hydrochloride



, HCl, $2H_2O$

$C_{18}H_{19}N_3O$, HCl, $2H_2O$ Mol. Wt. 365.9

Ondansetron Hydrochloride is (*RS*)-9-methyl-3-[(2-methyl-1*H*-imidazol-1-yl)methyl]-1,2,3,9-tetrahydro-4*H*-carbazol-4-one hydrochloride dihydrate.

Ondansetron Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{19}N_3O$,HCl, calculated on the anhydrous basis.

Category. Antiemetic. (5-HT_3 receptor antagonist)

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6).Compare the spectrum with that obtained with *ondansetron hydrochloride RS* or with the reference spectrum of ondansetron hydrochloride.

B. It gives the reactions of chlorides (2.3.12).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, the test solution and reference solution (b) described under Assay.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 9.0 to 10.5 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *ondansetron hydrochloride RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v of *ondansetron hydrochloride RS* and 0.002 per cent w/v of *3[Dimethylaminomethyl]-1, 2, 3, 9-tetrahydro-9-methyl-4H-carbozol-4-one RS* (*ondansetron impurity A RS*) in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica (5 µm) with chemically bonded nitrile groups (Such as Supelco LC-CN),
- mobile phase: a mixture of 50 volumes of *0.02 M monobasic potassium phosphate* with the pH previously adjusted to 5.4 with *1 M sodium hydroxide* and 50 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention time for ondansetron is about 1.0 and for ondansetron impurity A is about 1.1. The resolution between ondansetron and ondansetron impurity A is not less than 1.5.

Inject reference solution (a). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{18}H_{19}N_3O$,HCl.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ondansetron Injection

Ondansetron Hydrochloride Injection

Ondansetron Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ondansetron, $C_{18}H_{19}N_3O$.

Usual strengths. 2 mg; 4 mg; 8 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

pH (2.4.24). 3.3 to 4.0.

Related substances. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, test solution and reference solution (b) described under Assay.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent).

Bacterial endotoxins (2.2.3). Not more than 9.9 Endotoxin Units per mg of ondansetron.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution.Transfer an accurately measured volume of the injection containing about 2 mg of ondansetron to a 25-ml volumetric flask, dilute to volume with the mobile phase and mix.

Reference solution (a). A 0.01 per cent w/v solution of *ondansetron hydrochloride RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v of *ondansetron hydrochloride RS* and 0.005 per cent w/v of *3[Dimethylaminomethyl]-1, 2, 3, 9-tetrahydro-9-methyl-4H-carbozol-4-one RS (ondansetron impurity A RS)* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with porous silica (5 µm) bonded to nitrile groups (such as Supelco LC-CN),
- mobile phase: a mixture of 50 volumes of *0.02 M monobasic potassium phosphate* with the pH previously adjusted to 5.4 with *1 M sodium hydroxide* and 50 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention time for ondansetron is about 1.0 and for ondansetron impurity A is about 1.1. The resolution between ondansetron and ondansetron impurity A is not less than 1.5.

Inject reference solution (a). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{18}H_{19}N_3O$ in the injection.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ondansetron.

Ondansetron Orally Disintegrating Tablets

Ondansetron Orally Disintegrating Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ondansetron, $C_{18}H_{19}N_3O$.

Usual strength. 4 mg; 8 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 10 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with the dissolution medium if necessary, at the maximum at about 310 nm (2.4.7). Calculate the content of $C_{18}H_{19}N_3O$ in the medium from the absorbance obtained by using a solution of known concentration of *ondansetron RS* in same medium.

D. Not less than 80 per cent of the stated amount of $C_{18}H_{19}N_3O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 40 mg of ondansetron with 100.0 ml of the mobile phase. Centrifuge a portion of this solution at 3000 rpm for 10 minutes. Use the supernatant.

Reference solution (a). A 0.004 per cent w/v solution of *1,2,3,9-tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one RS (ondansetron impuirty D RS)* in *acetonitrile.*

Reference solution (b). A 0.004 per cent w/v solution of *2-methylimidazole* in *acetonitrile.*

Reference solution (c). A 0.004 per cent w/v solution of *ondansetron RS* in *acetonitriile.*

Reference solution (d). Dilute 5.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Reference solution (e). Dilute 10.0 ml of reference solution (c) to 100.0 ml with the mobile phase.

Reference solution (f). Transfer 5.0 ml of each reference solution (a), (b) and (c) to a 100-ml volumetric flask, dilute to volume with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica chemically bonded to nitrile groups (5 µm),
- mobile phase: a mixture of 80 volumes of phosphate buffer prepared by dissolving 2.72 g of *monobasic potassium phosphate* in 1000 ml of *water*, adjusted to pH 5.4 with *1 M sodium hydroxide* and 20 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 20 µl.

Inject reference solution (f). The test is not valid unless the resolution between ondansetron and adjacent peak is not less than 1.5, the theoretical plates is not less than 8000 for ondansetron and tailing factor is not more than 2.0.

Inject reference solution (e). The signal to noise ratio for the ondansetron peak is not less than 15. The relative retention time with reference to ondansetron for 2-methylimidazole is about 0.16 and for ondansetron impurity D is about 0.45.

Inject reference solution (d) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to 2-methylimidazole is not more than 0.15 per cent, multiply with response factor 0.5, the area of peak due to ondansetron impurity D is not more than 0.12 per cent, multiply with response factor 1.2. The area of any other secondary peak is not more than 0.1 per cent and the sum of all the secondary peaks is not more than 0.5 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 1.0 g.

Other tests. Comply with the tests stated under tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 40 mg of ondansetron with 100.0 ml *0.01 M hydrochloric acid.* Dilute 1.0 ml of the solution to 10.0 ml with *0.01 M hydrochloric acid.*

Reference solution (a). A 0.004 per cent w/v solution of *ondansetron RS* in *0.01 M hydrochloric acid.*

Reference solution (b). A 0.014 per cent w/v solution of *3[Dimethylamino methyl]-1,2,3,9-tetrahydro-9-methyl-H-carbozol-4-one-RS (ondansetron impurity A RS)* in *0.01 M hydrochloric acid.*

Reference solution (c). Transfer 8.0 ml each of reference solution (a) and (b) to 50-ml volumetric flask and dilute to volume with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with porous silica bonded to nitrile groups (5 µm),
- mobile phase: a mixture of 52 volumes of 0.272 per cent w/v solution of *monobasic potassium phosphate,* adjusted to pH 5.4 with *1 M sodium hydroxide* and 48 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to ondansetron and ondansetron impurity A is not less than 1.5. The relative retention time with reference to ondansetron for ondansetron impurity A is about 1.1. The tailing factor is not more than 2.0 for ondansetron peak.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{18}H_{19}N_3O$ in the tablets.

Storage. Store protected form light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the active ingredient in terms of equivalent amount of ondansetron.

Ondansetron Oral Solution

Ondansetron Hydrochloride Oral Solution

Ondansetron Oral Solution is a solution of Ondansetron Hydrochloride in a suitable vehicle.

Ondansetron Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ondansetron, $C_{18}H_{19}N_3O$.

Usual strength. 2 mg per 5 ml; 4 mg per 5 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 50 volumes of *ethyl acetate*, 40 volumes of *methanol* and 1 volume of *ammonium hydroxide*.

Test solution. Dilute an accurately measured volume of the oral solution containing about 20 mg of ondansetron to 100.0 ml in a mixture of 50 volumes of *methanol* and 50 volumes of *water*.

Reference solution. A 0.025 per cent w/v solution of *ondansetron RS* in *methanol*.

Apply 10 µl of each solution. Allow the solvent front to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The chromatogram obtained with the test solution corresponds to that obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.3 to 4.0.

Ondansetron impurity D. Not more than 0.1 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute an accurately measured volume of oral solution to obtain a solution having a concentration of 0.08 per cent w/v of ondansetron in the mobile phase.

Reference solution (a). A 0.00005 per cent w/v solution of *1,2,3,9-tetrahydro-9-methyl-3-methylene-4H-carbazol-4-one RS* (*ondansetron impurity D RS*) in the mobile phase.

Reference solution (b). A solution containing 0.00005 per cent w/v of *ondansetron impurity D RS* and 0.0002 per cent w/v of *N-methyl[2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride RS (ondansetron impurity C RS)* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with porous silica bonded to nitrile groups (5 µm),
- mobile phase: a mixture of 80 volumes of *0.02 M monobasic potassium phosphate,* previously adjusted to pH 5.4 and 20 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 328 nm,
- injection volume. 20µl.

Inject reference solution (b). The test is not valid unless the resolution between ondansetron impurity D and ondansetron impurity C is not less than 2.0, the tailing factor for ondansetron impurity D is not more than 2.0 and the relative standard deviation for replicate injections is not more than 4.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of ondansetron impurity D in the oral solution.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay with the following modifications.

Test solution. Weigh accurately a quantity of oral solution containing about 45 mg of Ondansetron Hydrochloride in 50-ml volumetric flask, dissolve and dilute with the mobile phase and mix. Dilute 5.0 ml of this solution to 50 ml with the mobile phase.

Inject reference solution (c). The test is not valid unless the resolution between ondansetron impuirty A and ondansetron is not less than 1.5, the tailing factor is not less than 2.0 and the relative standard deviation for replicate injections is not more than 1.5 per cent. The relative retention time with reference to ondansetron for ondansetron impurity A is about 1.1.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of the peak due to ondansetron impurity D is not more than 0.1 per cent, the area of each peak due to imidazole, 2-methyl imidazole, des-(-methyl)ondansetron hydrochloride, N-Desmethyl ondansetron maleate, ondansetron impurity A is not more than 0.2 per cent. The area of any other secondary peak is not more than 0.2 per cent and the sum of all the secondary peaks is not more than 0.5 per cent.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the oral solution containing about 9 mg of ondansetron to 100.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *ondansetron hydrochloride RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v of *ondansetron hydrochloride RS* and 0.002 per cent w/v of *3[Dimethylaminomethyl]-1, 2, 3, 9-tetrahydro-9-methyl-4H-carbozol-4-one RS* (*ondansetron impurity A RS*) in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica (5 µm) with chemically bonded nitrile groups (such as Supelco LC-CN),
- mobile phase: a mixture of 50 volumes of *0.02 M monobasic potassium phosphate* with the pH previously adjusted to 5.4 with *1 M sodium hydroxide* and 50 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention time for ondansetron is about 1.0 and for ondansetron impurity A is about 1.1. The resolution between ondansetron and ondansetron impurity A is not less than 1.5.

Inject reference solution (a). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{18}H_{19}N_3O$, HCl in the oral solution.

Microbial contamination (2.2.9). Complies with the microbial contamination test. The total aerobic count is not more than 100 cfu per g, *Enterobacteriaceae* count is not more than 10 cfu per g and the total combined molds and yeasts count is not more than 50 cfu per g.

Storage. Store protected from light and moisture.

Ondansetron Tablets

Ondansetron Hydrochloride Tablets

Ondansetron Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ondansetron, $C_{18}H_{19}N_3O$.

Usual strengths. 2 mg; 4 mg; 8 mg

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

Tests

Dissolution (2.5.2)

Apparatus No. 1,

Medium. 500 ml of *water*,

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 310 nm (2.4.7). Calculate the content of $C_{18}H_{19}N_3O$ in the medium from the absorbance obtained from a solution of known concentration of *ondansetron hydrochloride dihydrate RS* in the same medium.

1 mg of $C_{18}H_{19}N_3O$ is equivalent to 1.25 mg of $C_{18}H_{19}N_3O,HCl,2H_2O$.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{19}N_3O$.

Related substances. Determine by liquid chromatography (2.4.14).

Use the chromatographic system, test solution and reference solution (b) described under Assay.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions and reference solution (a) described in the Assay.

Test solution. Disperse one tablet in the mobile phase and dilute to obtain a solution containing 0.01 per cent w/v of Ondanseteron Hydrochloride and filter.

Calculate the content of $C_{18}H_{19}N_3O$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 50 mg of ondansetron, disperse in 50 ml of the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *ondansetron hydrochloride RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Reference solution (c). A solution containing 0.01 per cent w/v of *ondansetron hydrochloride RS* and 0.002 per cent w/v of *3[Dimethylaminomethyl]-1, 2, 3, 9-tetrahydro-9-methyl-4H-carbozol-4-one RS* (*ondansetron impurity A RS*) in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica (5 µm) bonded to nitrile groups (such as Supelco LC-CN),
- mobile phase: a mixture of 50 volumes of *0.02 M monobasic potassium phosphate* with the pH previously adjusted to 5.4 with *1 M sodium hydroxide* and 50 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention time for ondansetron is about 1.0 and for ondansetron impurity A is about 1.1. The resolution between ondansetron and ondansetron impurity A is not less than 1.5.

Inject reference solution (a). The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{18}H_{19}N_3O$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ondansetron.

Oral Rehydration Salts

ORS Powder

Oral Rehydration Salts are dry, homogeneously mixed powders containing Dextrose, Sodium Chloride, Potassium Chloride and either Sodium Bicarbonate or Sodium Citrate for use in oral rehydration therapy after being dissolved in the requisite amount of water.

They may contain suitable flavouring agents and, where necessary, suitable flow agents in the minimum quantity required to achieve a satisfactory product but may not contain artificial sweetening agents like mono- and/or poly-saccharides. If saccharin/saccharin sodium or aspartame is used in preparations meant for paediatric use, the concentration of saccharin should be such that its daily intake is not more than 5 mg/kg of body weight and that of aspartame should be such that its daily intake is not more than 40 mg/kg of body weight.

Category: Replacement solution for diarrhoeal rehydration.

Usual strength. A formulation of reduced osmolarity (given below) recommended by the World Health Organization (WHO) for the Diarrhoeal Diseases Control Programme, and of the United Nations Children's Emergency Fund (UNICEF).

Composition of the formulation in terms of the amount, in g, to be dissolved in sufficient water to produce 1000 ml.

Sodium Chloride	2.6
Dextrose (anhydrous)	13.5
or	
Dextrose Monohydrate	14.85
Potassium Chloride	1.5
Sodium Citrate	2.9

The molar concentrations of sodium, potassium, chloride and citrate ions in terms of millimoles per litre are given below:

	mmol/l
Sodium	75
Potassium	20
Chloride	65
Citrate	10
Dextrose	75

The total osmolar concentration of the solution in terms of mOsmol per litre is 245.

Oral Rehydration Salts contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Dextrose (anhydrous) or Dextrose Monohydrate (as appropriate) and of the requisite amounts of sodium, Na, potassium, K, chloride, Cl, and citrate, $C_6H_5O_7$, calculated from the stated amounts of the relevant constituents.

Description. A white to creamy-white, amorphous or crystalline powder; odourless.

Identification

A. When heated, melting and charring occurs and an odour of burnt sugar is produced.

B. Add a few drops of the solution prepared as directed in the label to 5 ml of *potassium cupri-tartrate solution*; a copious red precipitate is produced on boiling.

C. Gives reaction A of sodium salts, reaction A of potassium salts and reaction A of chlorides (2.3.1).

D. A quantity containing about 50 mg of *citric acid* gives the reactions of citrates (2.3.1).

Tests

Uniformity of weight. Comply with the test for contents of packaged dosage forms (2.5.6).

Seal test (*only for sachets*). Loosely bundle 10 sachets with a rubber band and submerge the bundle under water in a vacuum desiccator maintained at a pressure not exceeding 18 kPa for one minute. Examine the bundle for any fine stream of bubbles. Re-establish normal pressure and open the bundle. No penetration of water is observed in any sachet.

Other tests. Comply with the tests stated under Oral Powders.

Assay. Carry out the following assays on the well-mixed contents of an individual sachet or on a suitable sample from the well-mixed contents of a bulk container. Where the amount in an individual sachet is insufficient to carry out all the assays, take a separate sachet for the Assay for citrate and for the Assay for dextrose. For the Assays for total sodium, for potassium and for total chloride weigh accurately about 8.0 g and dissolve in sufficient *water* to produce 500.0 ml (solution A).

For total sodium — Dilute a suitable volume of solution A with a sufficient volume of a solution of *strontium chloride* such that the final solution contains a 1500 to 2000-fold excess of strontium ions and determine by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution AAS*, suitably diluted with the *strontium chloride solution*, for the standard solutions or, alternately by Method A for flame photometry (2.4.4).

1 g of Sodium Chloride, and of Sodium Citrate is equivalent to 0.3934 and 0.2345 g of Na respectively.

For potassium — Dilute a suitable volume of solution A with a sufficient volume of solution of *strontium chloride* such that the final solution contains a 1500 to 2000-fold excess of strontium ions and determine by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution AAS*, suitably diluted with the *strontium chloride solution*, for the standard solutions or, alternatively by Method A for flame photometry (2.4.4).

1 g of Potassium Chloride is equivalent to 0.5245 g of K.

For total chloride — Titrate 50 ml of solution A with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of Cl.

1 g of Sodium Chloride, and of Potassium Chloride is equivalent to 0.6066 and 0.4756 g of Cl respectively.

For citrate — Weigh accurately about 2.0 g and dissolve in 50 ml of *anhydrous glacial acetic acid* by heating at about 50°. Cool, allow to stand for 10 minutes. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.006303 g of $C_6H_5O_7$.

1 g of Sodium Citrate is equivalent to 0.6430 g of $C_6H_5O_7$.

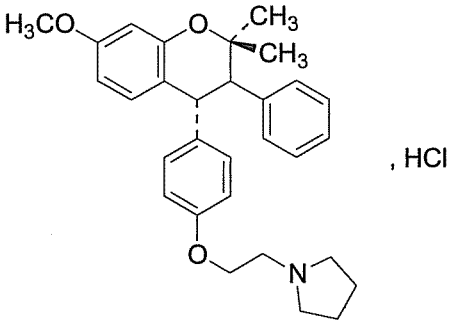
For dextrose — Weigh accurately about 7.5 g, dissolve in 40 ml of *water*, add 0.5 ml of *dilute ammonia solution*, and dilute to 50 ml with *water*. Mix well, allow to stand for 30 minutes, filter the solution, if turbid, and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation, in degrees, multiplied by 0.9477 and 1.0424 represents the weight in g of $C_6H_{12}O_6$ and $C_6H_{12}O_6,H_2O$ respectively, as appropriate, in the weight taken for the Assay.

Storage. Store protected from moisture in sachets, preferably made of aluminum foil, containing sufficient powder for a single dose or for a day's treatment or for use in hospitals, in bulk containers containing sufficient quantity to produce a volume of solution appropriate to the daily requirements of the hospital concerned.

Labelling. The label states (1) for sachets, the total weights, in g, of each constituent; (2) for bulk containers, the weights, in g, of each constituent in a stated quantity, in g, of the oral powder; (3) the molar concentration in millimoles per litre of sodium, potassium, chloride and citrate ions, and of dextrose as well as the total osmolar concentration in mOsmol per litre of the solution prepared from the oral powder; (4) the total weight of the contents of the container; (5) the directions for use; (6) that any portion of the solution prepared from the oral powder that remains unused for 24 hours after preparation should be discarded; (7) the storage conditions. g, of the oral powder.

Ormeloxifene Hydrochloride

Centchroman Hydrochloride; Centchroman



, HCl

$C_{30}H_{35}NO_3,HCl$ Mol. Wt. 493.5

Ormeloxifene Hydrochloride is *trans*-7-methoxy-2,2-dimethyl-3-phenyl-4[4-(2-pyrrolidinoethoxy)phenyl]chroman hydrochloride.

Ormeloxifene Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{30}H_{35}NO_3,HCl$, calculated on the dried basis.

Category. Antifertility agent (female oral contraceptive).

Dose. 120 mg twice a week, 3 days apart, for three months and thereafter once a week.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ormeloxifene hydrochloride RS* or with the reference spectrum of ormeloxifene.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *methanol* shows absorption maxima at about 278 and 282 nm.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of *chloroform.*

Reference solution. A 0.25 per cent w/v solution of *ormeloxifene hydrochloride RS* in *chloroform.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, until the odour of the solvents is no longer detectable and spray with a 0.3 per cent w/v solution of *potassium permanganate.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 1 ml of a 2 per cent w/v solution in *ethanol (95 per cent)* add 1 ml of a saturated solution of *picric acid* in *water*, stir well and set aside for 5 minutes. The yellow precipitate obtained after washing with *water* and drying at 60° for 4 hours melts at 212° to 218° (2.4.21).

Tests

Total basic substances. Weigh accurately 0.5 g, dissolve in 25 ml of *anhydrous glacial acetic acid*, add 10 ml of a 5 per cent w/v solution of *mercuric acetate* in *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator to a bluish green end-point. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04935 g of $C_{30}H_{35}NO_3,HCl$.

cis-Isomer. *Not more than 1.5 per cent of the total content of hydrochlorides of trans-and cis-isomers determined in the Assay.*

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.004 per cent w/v solution of the substance under examination in the mobile phase.

Reference solution. A solution containing 0.004 per cent w/v of *trans-ormeloxifene hydrochloride RS* and 0.0006 per cent w/v of *cis-ormeloxifene hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 80 volumes of *acetonitrile* and 20 volumes of *water* containing 0.04 per cent w/v solution of *tetramethylammonium hydroxide* adjusted to pH 7.6 with *phosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the reference solution and use an attenuation such that the response for the principal peak due to trans-ormeloxifene hydrochloride is more than 50 per cent of the full-scale deflection of the recorder. Make a total of six injections. When the chromatograms are recorded under the conditions described above, trans-ormeloxifene hydrochloride is eluted before cis-ormeloxifene hydrochloride.The test is not valid unless the relative standard deviation of six replicate injections is not greater than 6 per cent and the resolution between the peaks due to cis-ormeloxifene hydrochloride and trans-ormeloxifene hydrochloride is greater than 1. If necessary, adjust the proportions and the flow rate of the mobile phase to obtain proper resolution. Inject a suitable volume of test solution and record the response. From the average peak areas of the six replicate analyses calculate the content of trans-ormeloxifene hydrochloride and cis-ormeloxifene hydrochloride in the substance under examination by comparing with the peak responses for trans-ormeloxifene hydrochloride and cis-ormeloxifene hydrochloride respectively obtained with reference solution.

Storage. Store protected from moisture.

Ormeloxifene Hydrochloride Tablets

Centchroman Hydrochloride Tablets; Centchroman Tablets

Ormeloxifene Hydrochloride Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Ormeloxifene hydrochloride, $C_{30}H_{35}NO_3,HCl$.

Usual strength. 30 mg.

Identification

Shake a quantity of the powdered tablets containing 0.1 g of Ormeloxifene Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness at a pressure not exceeding 0.7 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ormeloxifene*

hydrochloride RS or with the reference spectrum of ormeloxifene.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *methanol* shows absorption maxima at about 278 and 282 nm.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of *chloroform.*

Reference solution. A 0.25 per cent w/v solution of *ormeloxifene hydrochloride RS* in *chloroform.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, until the odour of the solvents is no longer detectable and spray with a 0.3 per cent w/v solution of *potassium permanganate.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *water,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{30}H_{35}NO_3$,HCl in the medium from the absorbance obtained from a solution of known concentration of *Ormeloxifene hydrochloride RS* in *water.*

D. Not less than 70 per cent of the stated amount of $C_{30}H_{35}NO_3$,HCl.

cis-Isomer. *Not more than 1.5 per cent of the total content of ormeloxifene hydrochlorides of trans- and cis-isomers determined in the Assay.*

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.17).

Test solution. Weigh and powder 20 tablets. Shake a quantity of the powder containing 0.1 g of Ormeloxifene Hydrochloride with three quantities, each of 5 ml, of *methanol,* centrifuge each extract, dilute the combined extracts to 25 ml with *methanol* and then dilute 250 µl of the resulting solution to 25 ml with the mobile phase.

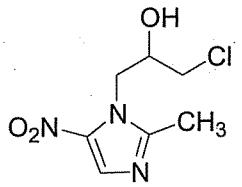
Reference solution. A solution containing 0.004 per cent w/v of *trans-ormeloxifene hydrochloride RS* and 0.0006 per cent w/v of cis-*ormeloxifene hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 80 volumes of *acetonitrile* and 20 volumes of *water* containing 0.04 per cent w/v solution of *tetramethylammonium hydroxide* adjusted to pH 7.6 with *phosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the reference solution and use an attenuation such that the response for the principal peak due to trans-ormeloxifene hydrochloride is more than 50 per cent of full-scale deflection of the recorder. Make a total of six injections. When the chromatograms are recorded under the conditions described above, trans-ormeloxifene hydrochloride is eluted before cis-ormeloxifene hydrochloride.The test is not valid unless the relative standard deviation of six replicate injections is not greater than 6 per cent and the resolution between the peaks due to cis-ormeloxifene hydrochloride and trans-ormeloxifene hydrochloride is greater than 1. If necessary, adjust the proportions and the flow rate of the mobile phase to obtain proper resolution. Inject a suitable volume of test solution and record the response. From the average peak areas of the six replicate analyses calculate the content of trans-ormeloxifene hydrochloride and cis-ormeloxifene hydrochloride in the substance under examination.

Storage. Store protected from moisture.

Ornidazole



$C_7H_{10}ClN_3O_3$ Mol. Wt. 219.6

Ornidazole is (*RS*)-1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol.

Ornidazole contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_{10}ClN_3O_3$, calculated on the anhydrous basis.

Category. Antiamoebic.

Dose. 500 mg twice a day orally for five days.

Description. A white to yellowish white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ornidazole RS* or with the reference spectrum of ornidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 277 nm; absorbance at 277 nm, 0.580 to 0.630.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the mobile phase.

Reference solution (b). A 0.001 per cent w/v solution of *ornidazole RS* in the mobile phase.

Reference solution (c). Dilute 20.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 200.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *0.01M potassium dihydrogen orthophosphate* and 30 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 318 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to 2-methyl-5-nitroimidazole and ornidazole is at least 1.5.

Inject the test solution, reference solutions (a) and (b). In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-methyl-5-nitroimidazole is not more than 0.2 times the area of the peak obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.15 g, dissolve in 50 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent of 0.02196 g of $C_7H_{10}ClN_3O_3$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Ornidazole Tablets

Ornidazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of ornidazole, $C_7H_{10}ClN_3O_3$.

Usual strength. 500 mg.

Identification

Extract a quantity of the powdered tablets containing 0.1 g of Ornidazole with 40 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ornidazole RS* or with the reference spectrum of ornidazole.

B. Heat a quantity of the powdered tablets containing 0.1 g of Ornidazole with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml *hydrochloric acid* for 5 minutes, cool in ice, add 0.5 ml of *sodium nitrite solution* and remove the excess of nitrite with *sulphuric acid*. Add 0.5 ml of *2-naphthol solution* and 2 ml of *5 M sodium hydroxide solution*. An orange-red colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate with *0.1 M hydrochloric acid*. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of ornidazole, $C_7H_{10}ClN_3O_3$ in the medium from the absorbance obtained from a solution of known concentration of *ornidazole RS*.

D. Not less than 80 per cent of the stated amount of $C_7H_{10}ClN_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of ornidazole in 25.0 ml of the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *2-methyl-5-nitroimidazole RS* in the mobile phase.

Reference solution (b). A 0.001 per cent w/v solution of *ornidazole RS* in the mobile phase.

Reference solution (c). Dilute 20.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *0.01M potassium dihydrogen orthophosphate* and 30 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 318 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to 2-methyl-5-nitroimidazole and ornidazole is at least 2.0.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-methyl-5-nitroimidazole is not more than 0.2 times the area of the peak obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

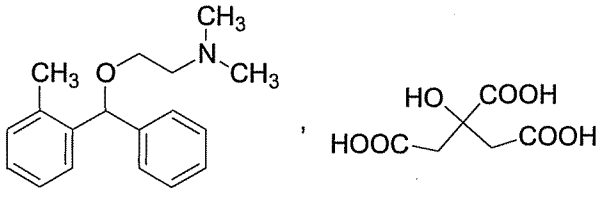
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.2 g of Ornidazole, transfer to a sintered-glass crucible and extract with 10 ml of hot *acetone*. Repeat the extraction 6 times with hot *acetone*. Cool, add to the combined extracts 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02196 g of $C_7H_{10}ClN_3O_3$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Orphenadrine Citrate



$C_{18}H_{23}NO,C_6H_8O_7$ Mol. Wt. 461.5

Orphenadrine Citrate is (*RS*)-dimethyl[2-(2-methylbenzhydryloxy)ethyl]amine dihydrogen citrate.

Orphenadrine Citrate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{23}NO$, $C_6H_8O_7$, calculated on the dried basis.

Category. Skeletal muscle relaxant.

Dose. By intramuscular or by slow intravenous injection (over 5 minutes), 60 mg repeated after 12 hours if necessary.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry,(2.4.6). Compare the spectrum with that obtained with *orphenadrine citrate RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.06 per cent w/v solution in *ethanol (95 per cent)* shows absorption maxima at 258 nm, 264 nm and 271 nm; absorbances at the maxima are about 0.68, 0.72 and 0.47 respectively.

C. Dissolve 50 mg in 10 ml of *ethanol (50 per cent),* add 10 ml of *picric acid solution* and allow to stand. The precipitate after recrystallisation from *ethanol (95 per cent),* melts at about 89° or at about 107°(2.4.21).

D. Dissolve 5 mg in 2 ml of *sulphuric acid*; an orange-red colour is produced.

E. To 1 g add 10 ml *water* and 2 ml of *5 M sodium hydroxide,* shake with two quantities, each of 10 ml, of *chloroform* and discard the chloroform. Heat the aqueous solution to boiling with an excess of *mercuric sulphate solution,* filter if necessary and boil the resulting solution with 0.2 ml of *dilute potassium permanganate solution;* the solution is decolorised and a white precipitate is produced.

Tests

Quaternary ammonium salt. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* G.

Mobile phase. A mixture of 50 volumes of *2-propanol,* 30 volumes of *butyl acetate.* 15 volumes of *water* and 5 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml *methanol.*

Reference solution. A 0.005 per cent *w/v solution* of *ethyldimethyl [2-(2-methylbenzhydryloxy)ethyl]ammonium chloride RS.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with *dilute potossium*

iodobismuthate solution. Any spot corresponding to ethyldimethyl [2-(2-methylbenzhydryloxy) ethyl] ammonium chloride in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Secondary amine. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 96 volumes of *1-butanol* and 4 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml *methanol.*

Reference solution. A 0.02 per cent *w/v* solution of *methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride RS.*

Apply to the plate 10 µl of each solution. After development, dry in air and examine in ultra-violet light at 254 nm. Spray the plate with *dilute potassium iodobismuthate solution* and examine again. By each method of visualisation any spot corresponding to methyl [2-(2-methylbenzhydryloxy) ethyl]amine hydrochloride in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm). Use 2 ml of lead standard solution (*10 ppm Pb*) to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 1.0 g, dissolve in 30 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04615 g of $C_{18}H_{23}NO, C_6H_8O_7$.

Storage. Store protected from light.

Orphenadrine Hydrochloride

$C_{18}H_{23}NO,HCl$ Mol. Wt.305.8

Orphenadrine Hydrochloride is (*RS*)-dimethyl [2-(2methylbenzhydryloxy) ethyl]amine hydrochloride.

Orphenadrine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{18}H_{23}NO,HCl$. calculated on the dried basis.

Category. Antiparkinsonian.

Dose. 150 mg daily, in divided doses, gradually increased; maximum 400 mg daily.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Tests A and F may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A, E and F are carried out.

A. Determine by infrared absorption spectrophotometry,(2.4.6). Compare the spectrum with that obtained with *orphenadrine hydrochloride RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.06 per cent *w/v* solution in *ethanol (95 per cent)* shows three absorption maxima at about 258 nm,264 nm and 271 nm; absorbances at the maxima, about 1.07, 1.13 and 0.73 respectively.

C. Dissolve about 5 mg in 2 ml of *sulphuric acid;* an orange-red colour is produced.

D. Dissolve about 50 mg in 10 ml of *ethanol (95per cent),* add 10 ml of *picric acid solution* and allow to stand. The precipitate, after recrystallisation from *ethanol* (95 per cent) melts at about 89° or at about 107°(2.4.21).

Tests

Quaternary ammonium salt. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel* G.

Mobile phase. A mixture of 50 volumes of *2-propanol,* 30 volumes of *butyl acetate*, 15 volumes of *water* and 5 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol.*

Reference solution. A 0.005 per cent *w/v solution* of *ethyldimethyl [2-(2-methylbenzhydryloxy) ethyl] ammonium chloride RS.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with *dilute potassium iodobismuthate solution.* Any spot corresponding to ethyldimethyl [2-(2-methylbenzhydryloxy) ethyl] ammonium chloride in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Secondary amine. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 96 volumes of *l-butanol* and 4 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol.*

Reference solution. A 0.02 per cent *w/v* solution of *methyl [2-(2-methylbenzhydryloxy)ethyl]amine hydrochloride RS.*

Apply to the plate 10 µl of each solution. After development, dry in air and examine in ultra-violet light at 254 nm. Spray the plate with *dilute potassium iodobismuthate solution* and examine again. By each method of visualisation any spot corresponding to methyl [2-(2-methylbenzhydryloxy) ethyl]amine hydrochloride in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm). Use 2 ml of lead standard solution (*10 ppm Pb*) to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.3.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 1.0 g, dissolve in 20 ml of *anhydrous glacial acetic acid,* add 20 ml of *mercuric acetate solution* and titrate with *0.1 M perchloric acid* using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03058 g of $C_{18}H_{23}NO,HCl$.

Storage. Store protected from light.

Orphenadrine Tablets

Orphenadrine Hydrochloride Tablets

Orphenadrine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of orphenadrine hydrochloride, $C_{18}H_{23}NO,HCl$.

Usual strength. 50 mg.

Identification

Extract a quantity of the powdered tablets containing about 0.15 g of Orphenadrine Hydrochloride with *chloroform,* filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Dissolve 5 mg in 2 ml of *sulphuric acid;* an orange-red colour is produced.

B. Dissolve 50 mg in 10 ml of *ethanol (50 per cent),* add 10 ml of *picric acid solution* and allow to stand. The precipitate after recrystallisation from *ethanol (95 per cent),* melts at about 89° or at about 107° (2.4.21).

C. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

Tests

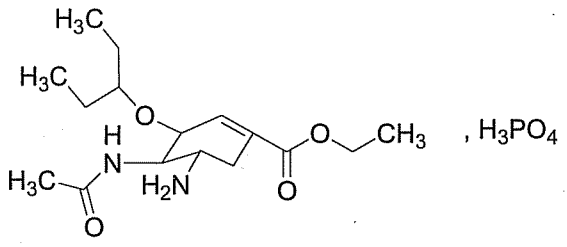
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 70 mg Orphenadrine Hydrochloride and dissolve as completely as possible in a mixture of 5 ml of *water* and 5 ml of *2 M hydrochloric acid.* Without delay extract with four quantities, each 15 ml, of *chloroform,* filter the combined extracts and evaporate to about 20 ml. Add 30 ml of *anhydrous glacial acetic* and 2 ml of *mercuric acetate solution* and titrate with *0.02 M perchloric acid* determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.006117 g of $C_{18}H_{23}NO,HCl$.

Storage. Store protected from light and moisture.

Oseltamivir Phosphate



$C_{16}H_{28}N_2O_4,H_3PO_4$ Mol. Wt. 410.4

Oseltamivir Phosphate is phosphoric acid salt of ethyl (3*R*,4*R*,5*S*)-4-(acetylamino)-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate.

Oseltamivir Phosphate contain not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{28}N_2O_4.H_3PO_4$, calculated on the dried basis.

Category. Antiviral.

Description. A white or off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oseltamivir phosphate RS.*

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). - 42.0° to - 48.0°, determined in a 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 25 mg of the substance under examination in 25 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as YMC pack PRO C8),
- column temperature 50°,
- mobile phase: a mixture of 66 volumes of a buffer solution prepared by dissolving 6.8 g of *anhydrous monobasic potassium phosphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *dilute sodium hydroxide,* 24.5 volumes of *methanol* and 23.5 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.5 per cent) and the sum of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Phosphoric acid. 23.4 to 24.4 per cent, calculated on a dried basis.

Weigh accurately about 0.2 g and dissolve in 40 ml of distilled *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.0049 g of phosphoric acid.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method A (20 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as YMC pack PRO C8),
- column temperature. 50°,
- mobile phase: a mixture of 66 volumes of *buffer solution* prepared by dissolving 6.8 g of *anhydrous monobasic potassium phosphate* in 1000 ml of *water*, adjusting the pH to 6.0 with *dilute sodium hydroxide,* 24.5 volumes of *methanol* and 23.5 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{28}N_2O_4,H_3PO_4$.

Storage. Store protected from light and moisture.

Oseltamivir Capsules

Oseltamivir Phosphate Capsules

Oseltamivir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oseltamivir, $C_{16}H_{28}N_2O_4$.

Usual strength. 75 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. Dissolve 50 mg of *oseltamivir phosphate RS* in 20 ml of the mobile phase and dilute to 100 ml with the dissolution medium. Dilute 5 ml of the solution to 25 ml with the dissolution medium.

Use the chromatographic system described under Assay.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{28}N_2O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of oseltamivir, disperse in 50 ml of mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (such as YMC pack PRO C8),
- mobile phase: a mixture of 66 volumes of a buffer solution prepared by dissolving 6.8 g of *anhydrous monobasic potassium phosphate* in 1000 ml of *water*, adjusting the pH to 6.0 with *dilute sodium hydroxide*, 24.5 volumes of *methanol* and 23.5 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 200 mg of oseltamivir, disperse in 100.0 ml of the mobile phase and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 66 volumes of a buffer solution prepared by dissolving 6.8 g of *anhydrous monobasic potassium phosphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *dilute sodium hydroxide*, 24.5 volumes of *methanol* and 23.5 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{28}N_2O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of oseltamivir.

Oseltamivir Oral Suspension

Oseltamivir Phosphate Oral Suspension

Oseltamivir Oral Suspension is a mixture consisting of Oseltamivir Phosphate with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specified volume of water just before issue.

Oseltamivir Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oseltamivir $C_{16}H_{28}N_2O_4$.

The contents of the sealed container comply with the following requirement.

Usual strength. 15 mg per ml.

Water (2.3.43). Not more than 2.5 per cent, determined on 1.0 g.

The constituted suspension complies with the requirements stated under Oral liquids and with the following requirements.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (b).

B. To 2 ml of the reconstituted suspension, add 2 ml of *dilute nitric acid* and 4 ml of a 10 per cent w/v solution of *ammonium*

molybdate and warm the solution. A bright yellow precipitate is formed.

Tests

pH (2.4.24). 3.0 to 5.0.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the suspension containing 25 mg of oseltamivir, dissolve in 25 ml of the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature 35°,
- mobile phase: 70 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *dilute sodium hydroxide,* 15 volumes of *methanol* and 15 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

Other tests. Complies with the tests stated under Oral liquids.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the suspension containing 60 mg of oseltamivir, dissolve in 250.0 ml of the mobile phase and filter. Dilute 10.0 ml of the solution to 25.0 ml with the mobile phase and filter.

Reference solution. A 0.0125 per cent w/v solution of *oseltamivir phosphate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and adjusting the pH to 6.0 with *dilute sodium hydroxide,* 24.5 volumes of *methanol* and 23.5 volumes of *acetonitrile,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

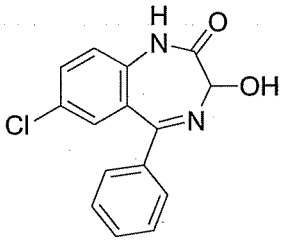
Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{28}N_2O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of active ingredient in terms of the equivalent amount of oseltamivir; (b) the temperature of storage and the period during which the constituted suspension may be expected to be satisfactory for use.

Oxazepam



$C_{15}H_{11}ClN_2O_2$ Mol. Wt. 286.7

Oxazepam is 7-chloro-3-hydroxy-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one.

Oxazepam contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{15}H_{11}ClN_2O_2$, calculated on the dried basis.

Category. Anxiolytic.

Dose. 15 to 30 mg, 2 to 3 times daily.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxazepam RS.*

B. Prepare the solutions immediately before use, protected from light.

Dissolve about 20 mg in sufficient *ethanol (95 per cent)* to produce 100 ml. Dilute 10 ml of the solution to 50 ml with *ethanol (95 per cent)* (solution A). Dilute 10 ml of solution A to 100 ml with *ethanol (95 per cent)* (solution B).

When examined in the range 230 nm to 360 nm (2.4.7), solution A shows an absorption maximum at about 316 nm. When examined in the range 220 nm to 250 nm, solution B shows an absorption maximum at about 229 nm; absorbance at about 229 nm, 1.220 to 1.300.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve about 20 mg in a mixture of 5 ml of *hydrochloric acid* and 10 ml of *water.* Heat to boiling for 5 minutes and cool. Add 2 ml of a 0.1 per cent *w/v* solution of *sodium nitrite* and allow to stand for 1 minute. Add 1 ml of a 0.5 per cent w/v solution of *sulphamic acid,* mix and allow to stand for 1 minute. Add 1 ml of 0.1 per cent w/v solution of a *N- (1-naphthyl) ethylenediamine dihydrochloride;* a red colour is produced.

Tests

Related substances. *Carry out the test protected from light.*

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Wash the plate with methanol before use.

Mobile phase. A mixture of 100 volumes of *dichloromethane* and 10 volumes of *methanol.*

Test solution (a). Dissolve 50 mg of the substance under examination in sufficient *acetone* to produce 10 ml.

Test solution (b). Dilute 2 ml of test solution (a) to 10 ml with *acetone.*

Reference solution (a). Dissolve 10 mg of *oxazepam RS* in sufficient *acetone* to produce 10 ml.

Reference solution (b). Dissolve 10 mg each of *oxazepam RS and bromazepam RS* in sufficient *acetone* to produce 10 ml.

Reference solution (c). Dilute 1 ml of reference solution (a) to 100 ml with *acetone.*

Reference solution (d). Dilute 5 ml of solution (d) to 10 ml with *acetone.*

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 17 cm in the same direction as the washing with methanol. Dry in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) and at most one such spot is more intense than the spot in the chromatogram obtained with reference solution (d). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.25 g and dissolve in a mixture of 10 ml of *anhydrous glacial acetic acid* and 90 ml of *acetic anhydride* and titrate with *0.1 M perchloric acid* determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02867 g of $C_{15}H_{11}ClN_2O_2$.

Storage. Store protected from light and moisture.

Oxazepam Tablets

Oxazepam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oxazepam, $C_{15}H_{11}ClN_2O_2$.

Usual strengths. 10 mg; 15 mg; 30 mg.

Identification

A. Extract a quantity of the powdered tablets containing 20 mg of Oxazepam with 25 ml of *chloroform,* filter, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 0.7 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxazepam RS or* with the *reference spectrum* of oxazepam.

B. When examined in the range 210 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 230 nm and 316 nm.

Tests

Related substances. *Carry out the test protected from light.*

Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF 254.*

Wash the plate with *methanol* before use.

Mobile phase. A mixture of 100 volumes of *dichloromethane* and 10 volumes of *methanol.*

Test solution. Shake a quantity of powdered tablets containing 30 mg of Oxazepam with 6 ml of *acetone* and centrifuge.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *acetone.*

Reference solution (b). Dilute 1 volume of the test solution to 500 volumes with *acetone.*

Reference solution (c). A solution containing 0.1 per cent w/v each of *oxazepam RS* and *bromazepam RS.*

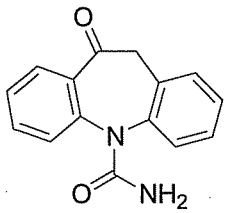
Apply to the plate 20 µl of each of the solutions. Allow the mobile phase to rise 17 cm in the same direction as in the washing with methanol. Dry in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (1 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Oxazepam and shake with 150 ml of *ethanol (95 per cent)* for 30 minutes. Add sufficient *ethanol (95 per cent)* to produce 250.0 ml and centrifuge. Dilute 5.0 ml of the supernatant liquid to 100.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 230 nm (2.4.7). Calculate the content of $C_{15}H_{11}ClN_2O_2$ taking 1250 as the specific absorbance at 230 nm.

Storage. Store protected from light at a temperature not exceeding 30°.

Oxcarbazepine



$C_{15}H_{12}N_2O_2$, Mol. Wt. 252.3

Oxcarbazepine is 10,11-dihydro-10-oxo-5*H*-dibenz[*b,f*]azepine-5-carboxamide.

Oxcarbazepine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{15}H_{12}N_2O_2$, calculated on the dried basis.

Category. Non-opioid analgesics.

Dose. 300 mg twice a day.

Description. An off-white to yellow crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxcarbazepine RS* or with the reference spectrum of oxcarbazepine.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Note — Use freshly prepared solutions.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *oxcarbazepine RS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil C18),
- column temperature 50°,
- mobile phase: a mixture of 22 volumes of *methanol*, 16 volumes of *acetonitrile* and 62 volumes of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, adding 2 ml of *triethylamine* and adjusting the pH to 6.0,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatograms for about 60 minutes. The relative retention times with reference to oxcarbazepine are 1.7 for dibenz[b,f]azepine-5-carboxylic acid amide (oxcarbazepine impurity A), 7.9 for 10-methoxy-5H-dibenz[b,f]azepine (oxcarbazepine impurity B), and 2.5 for 10-methoxy-5H-dibenz[b,f]azepine-5-carboxylic acid amide (oxcarbazepine impurity C).

Calculate the contents of the impurities in the test solution using the correction factors, 0.68 for oxcarbazepine impurity A, 0.76 for oxcarbazepine impurity B and 0.80 for oxcarbazepine impurity C. Not more than 1.0 per cent of oxcarbazepine impurity A and not more than 0.1 per cent each of oxcarbazepine impurity B and oxcarbazepine impurity C and not more than 0.1 per cent of any other impurity is found. The sum of all the impurities is not more than 1.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14) as described under related substances using the following solution.

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *oxcarbazepine RS* in the mobile phase.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{12}N_2O_2$.

Storage. Store protected from moisture.

Oxcarbazepine Tablets

Oxcarbazepine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oxcarbazepine, $C_{15}H_{12}N_2O_2$.

Usual strengths. 150 mg; 300 mg; 450 mg; 600 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

B. Extract a quantity of the powdered tablets containing about 50 mg of oxcarbazepine with 100 ml with *methanol* and filter. Diluted 2.0 ml of this solution to 100.0 ml with *methanol*. When examined in the range 200 nm to 350 nm (2.4.7), the solution shows an absorption maximum at about 254 nm.

Tests

Dissolution (2.5.2).

Apparatus No. 1

Medium. 900 ml of 0.75 per cent w/v solution of *sodium lauryl sulphate* in *water*,

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 305 nm (2.4.7). Calculate the content of $C_{15}H_{12}N_2O_2$ in the medium from the absorbance obtained from a solution of known concentration of *oxcarbazepine RS* prepared by dissolving in minimum volume of *methanol* and then diluting with the dissolution medium.

D. Not less than 75 per cent of the stated amount of $C_{15}H_{12}N_2O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Note — Use freshly prepared solutions.

Test solution (a). Weigh accurately a quantity of the powdered tablets containing 50 mg of Oxcarbazepine add 15 ml *methanol*, mix with the aid of ultrasound for 15 minutes and dilute to 100.0 ml with *methanol*. Mix and centrifuge.

Test solution (b). Dilute 5.0 ml of test solution (a) to 25.0 ml with the mobile phase.

Reference solution (a). Dissolve 25.0 mg of *oxcarbazepine RS* in *methanol* with the aid of ultrasound and dilute to 50.0 ml with *methanol*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 25.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- column temperature 50°,
- mobile phase: a mixture of 66 volumes of *0.05M potassium dihydrogen phosphate*, 14 volumes of *acetonitrile* and 20 volumes of *methanol*, add 0.1 ml of *triethylamine* and adjust the pH to 6.0 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject test solution (a) and reference solution (c). Run the chromatograms for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (c) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (c) (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

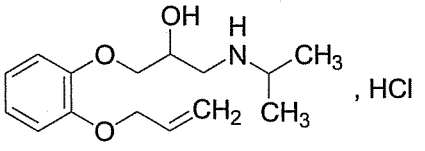
Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject test solution (b) and reference solution (b).

Calculate the content of $C_{15}H_{12}N_2O_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Oxprenolol Hydrochloride



$C_{15}H_{23}NO_3,HCl$ Mol. Wt. 301.8

Oxprenolol Hydrochloride is (*RS*)-1-(2-allyloxyphenoxy)-3-isopropylaminopropan-2-ol hydrochloride.

Oxprenolol Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{15}H_{23}NO_3,HCl$, calculated on the dried basis.

Category. Beta-adrenoceptor antagonist (antihypertensive; antianginal; antiarrhythmic).

Dose. As antihypertensive, initially 80 mg twice daily, increased as required at weekly intervals; maximum 480 mg daily. As antianginal, 40 to 160 mg thrice daily. As antiarrhythmic, initially 20 to 40 mg thrice daily, increased as necessary.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxprenolol RS* or with the reference spectrum of oxprenolol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

C. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution GYS6 (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a freshly prepared 10.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 88 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution.*

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Test solution (b). Dissolve 0.5 g of the substance under examination in 100 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Reference solution (c). A 0.5 per cent w/v solution of *oxprenolol hydrochloride RS* in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 13 cm. Dry the plate in warm air for 10 minutes, allow to cool, spray with *anisaldehyde solution*, heat at 105° for 10 minutes and examine in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 6 hours.

Assay. Weigh accurately about 0.25 g, dissolve in 60 ml of *anhydrous glacial acetic acid*, add 5 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03018 g of $C_{15}H_{23}NO_3,HCl$.

Storage. Store protected from light and moisture.

Oxprenolol Tablets

Oxprenolol Hydrochloride Tablets

Oxprenolol Hydrochloride Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of oxprenolol hydrochloride, $C_{15}H_{23}NO_3,HCl$. The tablets are coated.

Usual strengths. 40 mg; 80 mg.

Identification

A.To a quantity of the powdered tablets containing 50 mg of Oxprenolol Hydrochloride add 10 ml of *water* and 2 ml of *dilute sodium hydroxide solution*, extract with 10 ml of *chloroform* and reserve the aqueous layer for test C. Dry the chloroform extract over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxprenolol hydrochloride RS* or with the reference spectrum of oxprenolol hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum only at about 273 nm.

C. The aqueous layer obtained in test A gives reaction A of chlorides (2.3.1).

D. The residue obtained in test A melts at about 76° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 88 volumes of *chloroform*, 12 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution. Extract a quantity of the powdered tablets containing 0.25 g of Oxprenolol Hydrochloride with 5 ml of *water*, centrifuge and use the supernatant liquid.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 13 cm. Dry the plate in warm air for 10 minutes, allow to cool, spray with *anisaldehyde solution*, heat at 105° for 10 minutes and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Oxprenolol Hydrochloride, add 25 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 250.0 ml. Mix with the aid of ultrasound for 5 minutes, shake for 15 minutes and filter. Measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of $C_{15}H_{23}NO_3,HCl$ taking 74.5 as the specific absorbance at 273 nm.

Storage. Store protected from moisture.

Oxygen

O_2 Mol. Wt. 32.0

Oxygen contains not less than 99.0 per cent v/v of O_2.

This monograph applies to oxygen for medicinal use only.

Description. A colourless gas; odourless.

Identification

A. A glowing splinter of wood bursts into flame on contact with the gas.

B. Shake with *alkaline pyrogallol solution*; the gas under examination is absorbed and the solution becoming dark brown.

C. When tested as described under Assay, not more than 1.0 ml of gas remains.

Tests

Carbon dioxide. Not more than 300 ppm v/v, determined by using a carbon dioxide detector tube (2.1.1).

Carbon monoxide. Not more than 5 ppm v/v, determined by using a carbon monoxide detector tube (2.1.1).

Water vapour. Not more than 67 ppm v/v, determined by using a water vapour detector tube (2.1.1).

Assay (2.3.33). Use 100 ml of the gas under examination and place spirals of freshly cleaned copper wire and 125 ml of *ammonia buffer pH 10.9* in the pipette. The volume of the residual gas in the burette is not more than 1.0 ml.

Storage. Store under pressure in metal cylinders of the type conforming to the appropriate safety regulations. Valves and taps should not be lubricated with oil or grease.

Labelling. The shoulder of the metal cylinder should be painted white and the remainder should be painted black. The cylinder should carry a label stating "Oxygen". In addition, "Oxygen" or the symbol "O_2" should be stencilled in paint on the shoulder of the cylinder.

Oxygen 93 Per Cent

Oxygen 93 per Cent contains not less than 90.0 per cent and not more than 96.0 per cent, v/v of O_2, the remainder consisting mostly of argon and nitrogen. It is produced from air by the molecular sieve process.

Description. A colourless gas; odourless.

Identification

A. A glowing splinter of wood bursts into flame on contact with the gas.

B. Shake with alkaline pyrogallol solution; the gas under examination is absorbed and the solution becomes dark brown.

C. When tested as described under Assay, not more than 10.0 ml and not less than 4.0 ml of gas remain.

Tests

Carbon dioxide. Not more than 300 ppm v/v, determined by using a *carbon dioxide detector tube* (2.1.1).

Carbon monoxide. Not more than 5 ppm v/v, determined by using a *carbon monoxide detector tube* (2.1.1).

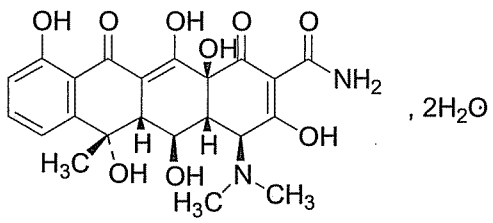
Assay (2.3.33). Use 100 ml of the gas under examination and place spirals of freshly cleaned copper wire and 125 ml of *ammonia buffer pH 10.9* in the pipette. The volume of the residual gas in the burette is not more than 10.0 ml and not less than 4.0 ml.

Storage. Store in cylinders or in a low pressure collecting tank. Containers used for Oxygen 93 Per Cent must not be treated with any toxic, sleep-inducing or narcosis-producing compounds and must not be treated with any compound that will be irritating to the respiratory tract when the Oxygen 93 Per Cent is used.

Labelling. Label each outlet "Oxygen 93 Per Cent", when it is piped directly from the collecting tank to the point of use. If it is stored in cylinders, reduce the pressure by means of a regulator. Measure the gases with a gas volume meter downstream from the detector tube in order to minimise contamination or change of the specimens. The shoulder of the cylinder should be painted white and the remainder should be painted black. The cylinder should carry a label stating "Oxygen 93 Per Cent" and "For medicinal use".

Oxytetracycline Dihydrate

Oxytetracycline



$C_{22}H_{24}N_2O_9,2H_2O$ Mol. Wt. 496.5

Oxytetracycline is (4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-dimethyl amino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide dihydrate, a substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means. It contains a variable quantity of water.

Oxytetracycline has a potency not less than 900 µg of $C_{22}H_{24}N_2O_9$, per mg, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. Orally, 250 to 500 mg every 6 hours; by intramuscular injection, 1 to 2 g daily.

Description. A tan yellow or light yellow (with or without a greenish tinge), crystalline powder; odourless.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Dissolve 0.05 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To about 0.5 mg add 2 ml of *sulphuric acid*; a red colour is produced. Add the solution to 1 ml of *water*; the colour changes to yellow.

C. Dissolve about 2 mg in 5 ml of a 1 per cent w/v solution of *sodium carbonate* and add 2 ml of *diazotised sulphanilic acid solution*; an orange-red to brownish-red colour is produced.

Tests

pH (2.4.24). 4.5 to 7.5, determined in a 1.0 per cent w/v suspension in freshly boiled and cooled *water*.

Specific optical rotation (2.4.22). –203° to –216°, determined at 20° in a 1.0 per cent w/v solution in *0.1 M hydrochloric acid*, after allowing the solution to stand protected from light for 30 minutes before measurement.

Light absorption. Absorbance of a 0.002 per cent w/v solution in *buffer solution pH 2.0* at the maximum at about 353 nm, 0.58 to 0.62 (2.4.7).

Light-absorbing impurities. A. Dissolve 20 mg in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 430 nm, when measured within 1 hour of preparing the solution, not more than 0.25, calculated on the anhydrous basis (2.4.7).

B. Dissolve 0.1 g in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 490 nm, when measured within 1 hour of preparing the solution, not more than 0.20, calculated on the anhydrous basis (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 25 ml of *0.01 M hydrochloric acid*.

Reference solution (a). Dissolve 20 mg of *oxytetracycline RS* in 25 ml of *0.01 M hydrochloric acid*.

Reference solution (b). Dissolve 20 mg of *4-epioxytetracycline RS* in 25 ml of *0.01 M hydrochloric acid*.

Reference solution (c). Dissolve 20 mg of *tetracycline hydrochloride RS* in 25 ml of *0.01 M hydrochloric acid*.

Reference solution (d). Dilute a mixture of 1.5 ml of reference solution (a), 1.0 ml of reference solution (b) and 3.0 ml of reference solution (c) to 25 ml with *0.01 M hydrochloric acid*.

Reference solution (e). Dilute a mixture of 1.0 ml of reference solution (b) and 4.0 ml of reference solution (c) to 200 ml with *0.01 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene- divinylbenzene co-polymer (8 to 10 µm),
- column temperature. 60°,
- mobile phase: add 60 g of *2-methyl-2-propanol* to a volumetric flask with the aid of 200 ml of *water*, add 60 ml of *0.33 M phosphate buffer pH 7.5*, 50 ml of a 1.0 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and 10 ml of a 0.04 per cent w/v solution of *disodium edetate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and dilute to 1000.0 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Adjust the sensitivity so that the heights of the peaks in the chromatogram obtained with reference solution (d) are at least 50 per cent of the full-scale deflection of the recorder.

The test is not valid unless (a) the resolution between the first peak (4-epioxytetracycline) and the second peak (oxytetracycline) is at least 4.0 (b) the resolution between the second peak and the third peak (tetracycline) is at least 5.0 (the content of *2-methyl-2-propanol* in the mobile phase may be adjusted if necessary) and (c) the symmetry factor for the second peak is at most 1.25.

Inject reference solution (a) six times. The test is not valid unless the relative standard deviation of the area of the peak due to oxytetracycline is not greater than 1.0 per cent.

Inject the test solution and reference solution (e). In the chromatogram obtained with the test solution the area of any peak corresponding to 4-epioxytetracycline or tetracycline is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (e). In the chromatogram obtained with the test solution the area of any peak appearing on the tail of the principal peak is not greater than 4.0 times that of the peak due to 4-epioxytetracycline in the chromatogram obtained with reference solution (e).

Heavy metals (2.3.13). 0.4 g complies with limit the test for heavy metals, Method B (50 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). 4.0 to 9.0 per cent, determined on 0.5 g.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the results in µg of oxytetracycline, $C_{22}H_{24}N_2O_9$, per mg.

Oxytetracycline intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg of Oxytetracycline.

Oxytetracycline intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Oxytetracycline Injection

Oxytetracycline Injection is a sterile solution of oxytetracycline with or without one or more suitable buffering agents, anaesthetics, preservatives, antioxidants, complexing agents and solvents.

Oxytetracycline Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of anhydrous oxytetracycline, $C_{22}H_{24}N_2O_9$.

Usual strengths. 50 mg per ml; 125 mg per ml.

Description. A clear, yellow to tan yellow solution. It may have a greenish tinge.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Shake a quantity containing 10 mg of anhydrous oxytetracycline with 20 ml of *methanol*, centrifuge if necessary and use the clear supernatant liquid.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Add 0.1 ml to 2 ml of *sulphuric acid*; a red colour is produced. Add the solution to 1 ml of *water*; the colour changes to yellow.

Tests

pH (2.4.24). 8.0 to 9.0.

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg of Oxytetracycline.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the result in mg of oxytetracycline, $C_{22}H_{24}N_2O_9$, per ml.

Storage. Store protected from light.

Labelling. The label states (1) the strength in mg of anhydrous oxytetracycline per ml; (2) that the contents are to be used for intramuscular use only; (3) the names of any preservatives used.

Oxytetracycline Hydrochloride

$C_{22}H_{24}N_2O_9,HCl$ Mol. Wt. 496.9

Oxytetracycline Hydrochloride is (4*S*,4a*R*,5*S*,5a*R*,6*S*, 12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide hydrochloride, a substance produced by the growth of certain strains of *Streptomyces rimosus* or obtained by any other means.

Category. Antibacterial.

Dose. Orally, 250 to 500 mg every 6 hours; by intravenous infusion, in a concentration of 0.1 per cent w/v, 1 to 2 g daily.

Description. A pale yellow, crystalline powder; odourless; hygroscopic.

Oxytetracycline Hydrochloride has a potency not less than 835 µg of $C_{22}H_{24}N_2O_9$, per mg, calculated on the anhydrous basis.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Dissolve 0.05 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol.*

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To about 0.5 mg add 2 ml of *sulphuric acid*; a red colour is produced. Add the solution to 1 ml of *water*; the colour changes to yellow.

C. Dissolve about 2 mg in 5 ml of a 1 per cent w/v solution of *sodium carbonate* and add 2 ml of *diazotised sulphanilic acid solution*; an orange-red to brownish-red colour is produced.

D. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 2.0 to 3.0, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). –188° to –200°, determined at 20° in a 1 per cent w/v solution in *0.1 M hydrochloric acid.*

Light absorption (2.4.7). Absorbance of a 0.002 per cent w/v solution in *chloride buffer solution pH 2.0* at the maximum at about 353 nm, 0.54 to 0.58.

Light-absorbing impurities. A. Dissolve 20 mg in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 430 nm, when measured within 1 hour of preparing the solution, not more than 0.50, calculated on the anhydrous basis (2.4.7).

B. Dissolve 0.1 g in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 490 nm, when measured within 1 hour of preparing the solution, not more than 0.20, calculated on the anhydrous basis (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 25 ml of *0.01 M hydrochloric acid.*

Reference solution (a). Dissolve 20 mg of *oxytetracycline RS* in 25 ml of *0.01 M hydrochloric acid.*

Reference solution (b). Dissolve 20 mg of *4-epioxytetracycline RS* in 25 ml of *0.01 M hydrochloric acid.*

Reference solution (c). Dissolve 20 mg of *tetracycline hydrochloride RS* in 25 ml of *0.01 M hydrochloric acid.*

Reference solution (d). Dissolve 20 mg of *α-apo-oxytetracycline RS* in 20 ml of *0.01 M sodium hydroxide* and dilute to 250 ml with *0.01 M hydrochloric acid.*

Reference solution (e). Dissolve 20 mg of *β -apo-oxytetracycline RS* in 20 ml of *0.01 M sodium hydroxide* and dilute to 250 ml with *0.01 M hydrochloric acid.*

Reference solution (f). Dilute a mixture of 1.5 ml of reference solution (a), 1.0 ml of reference solution (b), 3.0 ml each of reference solutions (c) (d) and (e) to 25 ml with *0.01 M hydrochloric acid.*

Reference solution (g). Dilute a mixture of 1.0 ml of reference solution (b), 4.0 ml of reference solution (c) and 40.0 ml of reference solution (e) to 200 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with styrene-divinylbenzene co-polymer (8 to 10 µm),
- column temperature. 60°,
- mobile phase: transfer separately 30 g (for mobile phase A) or 100 g (for mobile phase B) of *2-methyl-2-propanol* to volumetric flasks with the aid of 200 ml of *water*; to each flask add 60 ml of *0.33 M phosphate buffer pH 7.5*, 50 ml of a 1.0 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and 10 ml of a 0.04 per cent w/v solution of *disodium edetate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and dilute each solution to 1000.0 ml with *water.*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Carry out a one-step gradient elution in the following manner. Pump a mixture containing 30 volumes of mobile phase B and 70 volumes of mobile phase A for 15 minutes, then pump a mixture containing 30 volumes of mobile phase A and 70 volumes of mobile phase B for 15 minutes and finally equilibrate with the first mixture. Adjust the sensitivity so that the heights of the peaks in the chromatogram obtained with reference solution (f) are at least 50 per cent of full-scale deflection of the recorder.

The test is not valid unless, in the chromatogram obtained with reference solution (f), (a) the resolution between the first peak *(4-epioxytetracycline)* and the second peak (*oxytetracycline*) is at least 4.0, (b) the resolution between the second peak and the third peak (tetracycline) is at least 5.0, (c) the resolution between the fourth peak (*α-apo-oxytetracycline*) and the fifth peak (*β-apo-oxytetracycline*) is at least 3.5, and (d) the symmetry factor of the second peak

is at most 1.25. If necessary adjust the proportions of the mobile phases used to produce the one-step gradient elution. Adjust the time-programme for the one-step gradient elution if necessary.

Inject reference solution (a) six times. The test is not valid if the relative standard deviation of the area of the peak due to oxytetracycline is greater than 1.0 per cent. If necessary, adjust the integrator parameters.

Inject the test solution and reference solution (g). In the chromatogram obtained with the test solution the area of any peak corresponding to *4-epioxytetracycline* or *tetracycline* is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (g) and the total area of the peaks corresponding to α-*apo-oxytetracycline* and to β -*apo-oxytetracycline* and any peak between the latter two is not greater than the area of the peak due to β -*apo-oxytetracycline* in the chromatogram obtained with reference solution (g). In the chromatogram obtained with the test solution the area of any peak appearing on the tail of the principal peak is not greater than 4.0 times that of the peak due to 4-epioxytetracycline in the chromatogram obtained with reference solution (g).

Heavy metals (2.3.13). 0.4 g complies with the limit test for heavy metals, Method B (50 ppm).

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 2.0 per cent w/w, determined on 0.5 g.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the result in µg of oxytetracycline, $C_{22}H_{24}N_2O_9$, per mg.

Oxytetracycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg.

Oxytetracycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility. Complies with test for sterility (2.2.11).

Storage. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Oxytetracycline Capsules

Oxytetracycline Hydrochloride Capsules

Oxytetracycline Capsules contain not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl.

Usual strengths. 250 mg; 500 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Extract a quantity of the contents of the capsules containing 10 mg of Oxytetracycline Hydrochloride with 20 ml *methanol*, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To 0.5 mg of the contents of the capsules add 2 ml of *sulphuric acid*; a red colour is produced. Add the solution to 1 ml of *water*; the colour changes to yellow.

C. Dissolve about 2 mg of the contents of the capsules in 5 ml of a 1 per cent w/v solution of *sodium carbonate* and add 2 ml of *diazotised sulphanilic acid solution*; a light brown colour is produced.

D. The contents of the capsules give the reactions of chlorides (2.3.1).

Tests

Light-absorbing impurities. Dissolve a portion of the mixed contents of five capsules as completely as possible in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce two solutions of Oxytetracycline Hydrochloride containing (1) 0.2 per cent w/v and (2) 1.0 per cent w/v and filter each solution. Absorbance of the filtrate obtained from solution (1) at about 430 nm, when measured within 1 hour of preparing the solution, not greater than 0.75 and of the filtrate obtained from solution (2) at about 490 nm, not more than 0.40 (2.4.7).

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 353 nm (2.4.7). Calculate the content of $C_{22}H_{24}N_2O_9$,HCl in the medium taking 282 as the specific absorbance at 353 nm.

D. Not less than 75 per cent of the stated amount of $C_{22}H_{24}N_2O_9$,HCl.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g of the mixed contents of the capsules by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 0.25 g of Oxytetracycline Hydrochloride, add 250.0 ml of *water*, shake, filter.

Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the results in mg of oxytetracycline hydrochloride per capsule taking each mg of oxytetracycline to be equivalent to 1.079 mg of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl.

Storage. Store protected from light and moisture.

Oxytetracycline Eye Ointment

Oxytetracycline Hydrochloride Eye Ointment

Oxytetracycline Eye Ointment contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl.

Usual strength. 1 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. A solution prepared by heating a quantity containing 20 mg of Oxytetracycline Hydrochloride with 20 ml of *methanol* for 20 minutes, cooling in ice, filtering, carefully evaporating the filtrate to dryness and dissolving the residue in 20 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

Tests

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Other tests. Complies with the tests stated under Eye Ointments.

Assay. Weigh accurately about 1.0 g and transfer to a separating funnel. Add 25 ml of *peroxide-free ether*, shake well and extract with five quantities, each of 20 ml, of *0.1 M hydrochloric acid*. Combine the extracts and dilute to 200.0 ml with *0.1 M hydrochloric acid*. Dilute a suitable volume of the resulting solution with *buffer solution No 3* (2.2.10), to produce a solution containing 1 µg of oxytetracycline per ml.

Determine by the microbiological assay of antibiotics, Method B (2.2.10), and express the results as a percentage of oxytetracycline hydrochloride taking each mg of oxytetracycline to be equivalent to 1.079 mg of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9$,HCl.

Storage. Store protected from light and moisture.

Oxytetracycline Hydrochloride Injection

Oxytetracycline Hydrochloride Injection is a sterile material consisting of Oxytetracycline Hydrochloride with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution may be used within three days of preparation when stored in a refrigerator (2° to 8°).

Oxytetracycline Hydrochloride Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of oxytetracycline, $C_{22}H_{24}N_2O_9$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. The equivalent of 250 mg and 500 mg of oxytetracycline.

Description. A pale yellow, crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with the substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *chloroform* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Dissolve 0.05 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS, oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution, freshly prepared. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To about 0.5 mg add 2 ml of *sulphuric acid*; a red colour is produced. Add the solution to 1 ml of *water*; the colour changes to yellow.

C. Dissolve about 2 mg in 5 ml of a 1 per cent w/v solution of *sodium carbonate* and add 2 ml of *diazotised sulphanilic acid solution*; an orange-red to brownish-red colour is produced.

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1) and yellow.

pH (2.4.24). 2.0 to 3.0, determined in a 1.0 per cent w/v solution.

Light-absorbing impurities. A. Dissolve 20 mg in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 430 nm, when measured within 1 hour of preparing the solution, not more than 0.50, calculated on the anhydrous basis (2.4.7).

B. Dissolve 0.1 g in sufficient of a mixture of 1 volume of *1 M hydrochloric acid* and 99 volumes of *methanol* to produce 10 ml. Absorbance of the resulting solution at about 490 nm, when measured within 1 hour of preparing the solution, not more than 0.20, calculated on the anhydrous basis (2.4.7).

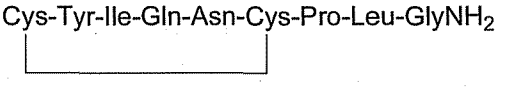
Assay. Determine by the microbiological assay of antibiotics, method A or B (2.2.10), and express the result in µg of oxytetracycline, $C_{22}H_{24}N_2O_9$, per mg.

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg.

Storage. Store protected from light and moisture.

Labelling. The label states (1) the quantity of Oxytetracycline Hydrochloride contained in it in terms of the equivalent amount of oxytetracycline; (2) that the contents are to be used for intravenous injection only; (3) the names of the buffering agents used.

Oxytocin



$C_{43}H_{66}N_{12}O_{12}S_2$ Mol. Wt.1007.2

Oxytocin is a cyclic nonapeptide hormone obtained by a process of fractionation from the posterior lobe of the pituitary

gland of healthy oxen or other mammals or by synthesis that has the property of stimulating contraction of the uterus and milk ejection in receptive animals. It may be presented as a solid or as a solution in a solvent containing an appropriate antimicrobial preservative such as 0.2 per cent w/v solution of chlorbutol.

If it is derived from animal species, Oxytocin contains not less than 95.0 per cent and not more than 105.0 per cent of the stated number of Units of oxytocic activity. If it is a synthetic product presented as a solid, it contains not less than 560 Units per mg, calculated with reference to the peptide content and when presented as a liquid, it contains not less than 150 Units per ml.

Category. Oxytocic.

Description. When presented as a solid, a white or almost white powder. When presented as a liquid, a clear colourless liquid.

Identification

Test B may be omitted if tests A and C are carried out. Test C may be omitted if tests A and B are carried out.

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis.

For hydrolysis—Acid hydrolysis using *hydrochloric acid* containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis solution. 6 M hydrochloric acid containing 0.1 per cent to 1.0 per cent of *phenol.*

Procedure

Liquid phase hydrolysis. Place the protein or peptide sample in a hydrolysis tube, and dry (the sample is dried so that water in the sample will not dilute the acid used for the hydrolysis). Add 200 µl of hydrolysis solution per 500 µg of lyophilised protein. Freeze the sample tube in a dry ice-acetone bath, and flame seal in vacuum. Samples are typically hydrolysed at 110° for 24 hours in vacuum or in an inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g. 48 hours and 72 hours) are investigated if there is a concern that the protein is not completely hydrolysed.

Vapour phase hydrolysis. This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimised by using vapour phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of hydrolysis solution. The hydrolysis solution does not come in contact with the test sample. Apply an inert atmosphere or vacuum (less than 200 µm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110° for a 24 hours hydrolysis time. Acid vapour hydrolysis the dried sample. Any condensation of the acid in the sample vials is to be minimised. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

For analysis—Post-column ninhydrin derivatisation . Ion-exchange chromatography with post-column ninhydrin derivatisation is one of the most common methods employed for quantitative amino acid analysis. As a rule, a lithium-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster sodium-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has a characteristic purple or yellow colour. Amino acids, except imino acid, give a purple colour, and show an absorption maximum at 570 nm. The imino acids such as proline give a yellow colour, and show an absorption maximum at 440 nm. The post-column reaction between ninhydrin and amino acids eluted from the column is monitored at 440 nm and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition.

The detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for the proline derivative. Response linearity is obtained in the range of 20-500 pmol with correlation coefficients exceeding 0.999. To obtain good composition data, samples larger than 1 µg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1.

The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

C. For biological response, dissect out the uterus from a virgin female rat weighing between 120 to 200 g and in diestrus stage. Immediately before injection confirm the uterine stage of the rat by vaginal smear. Suspend one horn of the uterus in a

organ bath containing 9.0 g of *sodium chloride*, 0.42 g of *potassium chloride*, 0.16 g of *calcium chloride*, 0.50 g of *sodium bicarbonate*, 0.25 g of *dextrose*, and 0.0053 g of *magnesium chloride* per litre of the solution. Maintain the bath temperature at 32° or any other suitable temperature at which spontaneous contractions of the uterus are abolished and the preparations maintain its sensitivity. Oxygenate the bath solution with a mixture of 95 per cent *oxygen* and 5 per cent *carbon dioxide*. Record the contractions of the uterine muscle on a recorder, using a suitable instrument producing linear response (for example an isotonic liver with a load of not exceeding 2 g or isotonic and linear transducer). Add to the bath two appropriate dilutions of the oxytocin reference solution, and record the contraction of the muscle following each dilution. The appropriate dilutions (doses) are those dilutions of the reference solution which produce clearly distinctive submaximal contractions. The required dilution normally lies between 0.1 to 5 micro units per ml of the bath solution. When maximal contraction is reached, replace the bath solution by a fresh solution and wait until the muscle is relaxed completely and the pointer of the recorder returns to the base line. The doses of the different reference solutions should be added at regular intervals depending upon the rate of the recovery of the uterine muscle. Dissolve or dilute the preparation to be tested in a suitable diluent (preferably using bath solution) to obtain responses on the addition of two dilutions similar to the one used with the oxytocin reference solution. The two selected dilutions of the reference solution and preparation under examination should be applied according to a randomised block or Latin square design and at least three responses to each dilution should be recorded. The magnitude of contractions obtained with the reference solution is comparable to the contractions obtained with the test solution.

Tests

Peptide. 90.0 to 110.0 per cent of the stated amount of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$ expressed per mg for the solid, and in mg per ml for the liquid,

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 3.5 mg of the substance under examination in sufficient of a 1.56 per cent w/v solution of *sodium dihydrogen phosphate* to produce 10.0 ml or use the liquid preparation as appropriate.

Reference solution. Dissolve 3.5 mg of *oxytocin RS* in sufficient of a 1.56 per cent w/v solution of *sodium dihydrogen phosphate* to produce 10.0 ml.

Chromatographic system

- a stainless steel column 12 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: appropriate proportions of a 1.56 per cent w/v solution of *sodium dihydrogen phosphate* (mobile phase A) and a mixture of equal volumes of *acetonitrile* and *water* (mobile phase B),
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl

Equilibrate the column with a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B and record the chromatograms as follows. Operate by gradient elution increasing continuously and linearly the proportion of mobile phase B by 1.0 per cent v/v per minute for 30 minutes. Finally elute using the same mixture for 15 minutes to re-equilibrate the column.

Calculate the content of the peptide, $C_{43}H_{66}N_{12}O_{12}S_2$.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 100 ml of mobile phase A.

Reference solution. A 0.025 per cent w/v solution of *oxytocin RS* in mobile phase A.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 1.6 per cent w/v solution of *sodium dihydrogen phosphate*,
  B. a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,

injection volume. 25 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 30	70 → 40	30 → 60
30 – 30.1	40 → 70	60 → 30
30.1 – 45	70	30

Inject the test solution and the reference solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$.

Oxytocin intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Less than 0.5 Endotoxin Units per Unit of oxytocin.

Oxytocin intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture. If the substance is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units of oxytocic activity per mg (for solid) or per ml (for liquid); (2) either the animal species from which it is obtained or whether it is synthetic, as appropriate; (3) whether or not the contents are intended for use in the manufacture of parenteral preparations.

Oxytocin Injection

Oxytocin Injection is a sterile solution of Oxytocin in Water for Injections.

Oxytocin Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated number of Units of oxytocin activity.

Usual strengths. 5 Units per ml; 10 Units per ml.

Description. A clear colourless liquid.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.0

Bacterial endotoxins (2.2.3). Less than 0.5 Endotoxin Units per Unit of oxytocin.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Use the injection under examination.

Reference solution. Dissolve the contents of one vial of *oxytocin RS* in a 1.65 per cent w/v solution of *sodium dihydrogen orthophosphate* to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the injection.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a 0.2 per cent v/v solution of *orthophosphoric acid* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 50,000.

Inject the test solution and the reference solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ in the injection.

Oxytocin Injection containing Oxytocin of natural origin obtained by extraction and purification complies with the following additional requirement.

Vasopressin impurity. Not more than 0.5 Unit per ml.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 5.0 g of chlorobutanol in 5.0 ml of *glacial acetic acid*, add 1.1 g of *sodium acetate*, 5.0 g of *ethanol*, and dilute to 1000 ml with *water* and mix.

Test solution. Dilute 2.0 ml of injection under examination to 25 ml with 0.25 per cent w/v of *glacial acetic acid* and mix.

Reference solution. Dissolve the contents of one vial of *vasopressin RS* in a known volume of solvent mixture. If necessary dilute the prepared solution to a working concentration range.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 87 volumes of 6.6 per cent v/v solution of *dibasic ammonium phosphate*, adjusted to pH 3.0 with *orthophosphoric acid* and 13 volumes of *acetonitrile*, filter through 0.45 µm nylon membrane,
- flow rate. 1 ml per minute,
- spectrophometer set at 220 nm,
- injection volume. 20 µl.

Equilibrate the column at least for one hour. Run the chromatogram minimum of 60 minutes.

Inject the reference solution. The test is not valid unless the resolution between vasopressin and the adjacent peak is not less than 1.5 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Labelling. The label states (1) the number of Units of oxytocin activity per ml; (2) either the animal spieces from which it is obtained or whether it is synthetic, as appropriate.

Oxytocin Nasal Solution

Oxytocin Nasal Solution is a solution of Oxytocin in a suitable solvent containing an appropriate antimicrobial preservative.

Oxytocin Nasal Solution contains not less than 85.0 per cent and not more than 120.0 per cent of the stated number of Units of oxytocic activity.

Usual strength. 40 Units per ml.

Description. A clear, colourless solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.0

Other tests. Complies with the tests stated under Nasal Preparations.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Use the nasal solution under examination.

Reference solution. Dissolve the contents of one vial of *oxytocin RS* in a 1.65 per cent w/v solution of *sodium dihydrogen orthophosphate* to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the nasal solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a 0.2 per cent v/v solution of *orthophosphoric acid* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 50,000.

Inject the test solution and the reference solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ in the nasal solution.

Oxytocin Nasal Solution containing Oxytocin of natural origin obtained by extraction and purification complies with the following additional requirement.

Vasopressin impurity. Not more than 0.5 Unit per ml.

Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 5.0 g of chlorobutanol in 5.0 ml of *glacial acetic acid*, add 1.1 g of *sodium acetate*, 5.0 g of *ethanol*, and dilute to 1000 ml with *water* and mix.

Test solution. Dilute 2.0 ml of injection under examination to 25 ml with 0.25 per cent w/v of *glacial acetic acid* and mix.

Reference solution. Dissolve the contents of one vial of *vasopressin RS* in a known volume of solvent mixture. If necessary dilute the prepared solution to a working concentration range.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 87 volumes of 6.6 per cent v/v solution of *dibasic ammonium phosphate*, adjusted to pH 3.0 with *orthophosphoric acid* and 13 volumes of *acetonitrile*, filter through 0.45 µm nylon membrane,
- flow rate. 1 ml per minute,
- spectrophometer set at 220 nm,
- injection volume. 20 µl.

Equilibrate the column at least for one hour. Run the chromatogram minimum of 60 minutes.

Inject the reference solution. The test is not valid unless the resolution between vasopressin and the adjacent peak is not less than 1.5 and relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

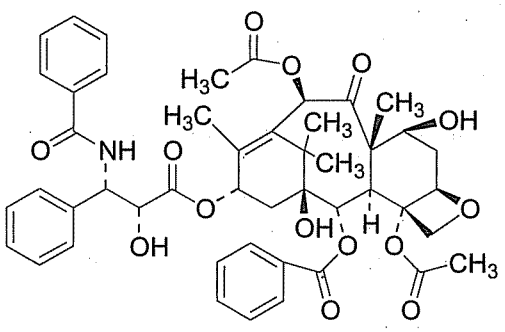
Storage. Store at a temperature not exceeding 30°.

Labelling. The label states (1) the number of Units of oxytocic activity per ml; (2) either the animal species from which it is obtained or whether it is synthetic, as appropriate; (3) that the preparation is intended for intranasal administration only.

P

Paclitaxel

Taxol



$C_{47}H_{51}NO_{14}$ Mol. Wt. 853.9

Paclitaxel is 5β,20-epoxy-1,2α4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with(2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

A taxane derivative first isolated from the bark of the Pacific yew tree, *Taxus brevifolia*.

Paclitaxel contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{47}H_{51}NO_{14}$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white or almost white powder.

CAUTION — Paclitaxel is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *paciltaxel RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). −49.0° to −55.0°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of substance under examination in 10 ml of *acetonitrile*.

Reference solution (a). A 0.001 per cent w/v solution of *paclitaxel RS* in *acetonitrile*.

Reference solution (b). A solution containing 0.008 per cent w/v of 10 deacetyl-7-epipaclitaxel and 0.1 per cent w/v of *paclitaxel RS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3μm),
- column temperature. 35°,
- mobile phase: A. a mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*,
  B. *acetonitrile*
- flow rate. 1.2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 227nm,
- injection volume. 10 μl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
20	100	0
60	10	90
62	100	0
70	100	0

Inject reference solution (b). The test is not valid unless the resolution between the peak due to paclitaxel and 10-deacetyl-7-epipaclitaxel is not less than 1.2. The relative retention time for 10-deacetyl-7-epipaclitaxel is about 0.94 in respect to paclitaxel.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (a) (2.0 per cent).

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g by coloumetry method.

Bacterial endotoxins (2.2.3). Not more than 0.4 Endotoxin Unit per mg of paclitaxel.

Microbial contamination (2.2.9). The total viable aerobic count does not exceed 100 cfu per g. It meets the requirements of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella species*, and *Escherichia coli*.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 200 μl of *glacial acetic acid* in 1000 ml of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml in solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *paclitaxel RS* in solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with pentafluoro phenyl groups bonded to porous silica (5 µm),
- column temperature 35°,
- mobile phase: a mixture of 11 volumes of *water* and 9 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 227nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{47}H_{51}NO_{14}$.

Storage. Store protected from light, at a temperature not exceeding 25°.

Paclitaxel Injection

Paclitaxel Injection is a sterile solution of Paclitaxel suitable for dilution,for intravenous use.

Paclitaxel Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of paclitaxel, $C_{47}H_{51}NO_{14}$.

Description. A clear colourless to slight yellow viscous solution.

Identification

In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

pH (2.4.24). 3.0 to 7.0, determined in a 10 per cent v/v solution in *water.*

Light absorption. Absorbance of the injection at about 425 nm, not more than 0.1.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Accurately measure the volume of injection containing 12 mg of Paclitaxel, dilute to 10 ml with *acetonitrile.*

Reference solution (a). A 0.0012 per cent w/v solution of *paclitaxel RS* in *acetonitrile.*

Reference solution (b). A solution containing 0.006 per cent w/v of *10 deacetyl -7-epipaclitaxel RS* and 0.12 per cent w/v of *paclitaxel RS* in *acetonitrile.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- column temperature. 35°,
- mobile phase: A. a mixture of 60 volumes of *water* and 40 volumes of *acetonitrile*,
  B. *acetonitrile,*
- flow rate. 1.2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 227 nm,
- injection volume. 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
26	100	0
66	17	83
67	100	0

Inject reference solution (b). The test is not valid unless the resolution between the peak due to paclitaxel and 10-deacetyl-7-epipaclitaxel is not less than 1.2. The relative retention time for 10-deacetyl-7-epipaclitaxel is about 0.94 in respect to paclitaxel.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.8 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.8 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (a) (2.0 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.67 Endotoxin Unit per mg of paclitaxel.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 200 µl of *glacial acetic acid* in 1000 ml of *methanol.*

Test solution. Accurately measure the volume of injection containing 6 mg of Paclitaxel and dissolve in 10 ml of solvent mixture.

Reference solution. A 0.06 per cent w/v solution of *paclitaxel RS* in solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with penta fluro phenyl group chemically bonded to porous silica (5 µm),
- column temperature 35°,
- mobile phase: a mixture of 11 volumes of *water* and 9 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 227nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{47}H_{51}NO_{14}$.

Storage. Store protected from light, at a temperature not exceeding 25°.

Pancreatin

Pancreatin is a preparation of mammalian pancreas containing protease, lipase and amylase activity. It may contain Sodium Chloride.

Pancreatin contains not less than the minimum protease activity, amylase activity and lipase activity determined under the conditions of the Assay.

Category. Digestive enzyme.

Dose. 500 mg to 1 g.

Description. A white or buff-coloured, amorphous powder; odour, meaty and not unpleasant.

Identification

A. Triturate 0.5 g with 10 ml of *water* and adjust to pH 8.0 by the addition of *1 M sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. To each add a few shreds of *congo red fibrin*, warm to 39° ± 1° and maintain at this temperature for 1 hour. Solution (2) is stained red and solution (1) is colourless or not more than slightly pink.

B. Triturate 0.25 g with 10 ml of *water* and adjust to pH 8.0 by the addition of *1 M sodium hydroxide* using *cresol red solution* as indicator. Divide the resulting solution into two equal portions. Boil one portion [solution (1)] and leave the other untreated [solution (2)]. Dissolve 0.1 g of *soluble starch* in 100 ml of boiling *water*, boil for 2 minutes, cool and dilute to 150 ml with *water*. Add solution (1) to half the starch mucilage and solution (2) to the remainder and maintain the mixtures at 39° ± 1° for 5 minutes. To 1 ml of each mixture add 10 ml of *iodinated potassium iodide solution*. The liquid containing solution (2) retains the colour of the solution of iodine and the liquid containing solution (1) acquires an intense blue colour.

Tests

Fat. Not more than 5.0 per cent, determined by the following method. Extract 1 g with *light petroleum (40° to 60°)* for 3 hours in an apparatus for the continuous extraction of drugs (2.1.8), evaporate the extract and dry the residue at 105° for 2 hours.

Microbial contamination (2.2.9). 1 g is free from *Escherichia coli*; 10 g is free from *Salmonellae*.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by dying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. *For protease activity* — Weigh accurately 4.0 g of purified casein and dissolve in about 90 ml of *water* containing 3 ml of *1 M sodium hydroxide*, adjust the pH of the solution to 8.7 and add sufficient *water* to produce 100.0 ml. Weigh accurately about 0.5 g of the substance under examination, triturate with *water* and add sufficient *water* to produce 300.0 ml to give the test solution. Dilute 15.0 ml of the *casein solution* with 30 ml of *water*, warm to 55° and add 10.0 ml of the unfiltered test solution. Heat rapidly to 55° and keep at this temperature for 20 minutes. Cool rapidly to room temperature. Dilute a further portion of 15.0 ml of the casein solution with 30 ml of *water*, add 10.0 ml of the unfiltered test solution, previously boiled and cooled, heat rapidly to 55° and keep at this temperature for 20 minutes. Cool to room temperature. To each solution add 0.75 ml of *phenolphthalein solution* and 10 ml of *formaldehyde solution*. Titrate each solution with *0.1 M sodium hydroxide* until the colour of the solution matches that produced by mixing 10 ml of *buffer solution pH 8.7* and 0.15 ml of *phenolphthalein solution*. The difference between the two titrations is not less than 4.5 ml.

For lipase activity — To 95 ml of *water*, add 6.5 ml of *triacetin* and 0.2 ml of a 0.1 per cent w/v solution of *bromocresol purple*, neutralise with *0.5 M sodium hydroxide* and add sufficient *water* to produce 110 ml. Place 50 ml of this solution in each of two large tubes 3 cm x 20 cm A and B contained in a thermostat at 30°. Insert in each tube a rubber stopper having two holes, one for the tip of a burette and the other for a short glass tube through which passes a thread operating a glass stirring coil. Stir the contents of the tube until they attain the temperature of the thermostat. Prepare a solution of 0.1 g of the substance under examination in 10.0 ml of *water*. To tube A add 1.0 ml of the solution, to tube B add 1.0 ml of the solution previously boiled. Adjust and maintain the pH of the solutions in the two

tubes to 6.2 to 6.4 by the addition of *0.05 M sodium hydroxide* dropwise, stirring frequently. After 30 minutes, the difference between the volumes of *0.05 M sodium hydroxide* added to the two tubes is not less than 1.0 ml.

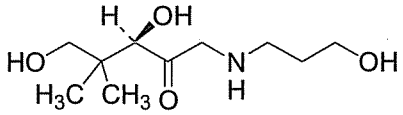
For amylase activity—Not less than 100 Units per g. Dissolve 0.1 g or a quantity containing 10 Units, accurately weighed, in sufficient *buffer solution pH 6.8* to produce 1000.0 ml. Filter if necessary (1 ml of the test solution should be capable of digesting about 10 mg of dry soluble maize or corn starch). Into each of six stoppered test-tubes add 5.0 ml of *starch substrate* without touching the sides of the test-tube. Place the test-tubes in a *water*-bath maintained at 40° ± 0.1°. When the temperature of the solution in the tubes has reached 40°, add 0.35 ml, 0.4 ml, 0.45 ml, 0.5 ml, 0.55 ml and 0.6 ml of the test solution to each of the test-tubes marked 1 to 6 respectively and record the time of addition. Mix thoroughly and replace the tubes in the *water*-bath. After exactly 60 minutes remove the tubes and cool rapidly in cold water. Add to each tube 0.05 ml of *0.02 M iodine* and mix well. Note the tube containing the lowest volume of test solution, which does not show a bluish or violet tinge (if there is doubt, warm the solution slightly, when the colour distinction is prominent). From this volume calculate the number of grams of dry soluble maize or corn starch digested by 1.0 g of the substance under examination. This represents the number of Units of amylase activity per g.

Storage. Store protected from moisture.

Labelling. The label states the name of any added substance.

D-Panthenol

Pantothenol; Dextro-pantothenyl Alcohol



$C_9H_{19}NO_4$ Mol. Wt. 205.3

D-Panthenol is (*R*)-2,4-dihydroxy-*N*-(3-hydroxypropyl)-3, 3-dimethylbutanamide.

D-Panthenol contains not less than 6.60 per cent and not more than 6.95 per cent of nitrogen, N.

Category. Vitamin B (enzyme co-factor).

Dose. 250 to 500 mg.

Description. A clear, colourless or slightly yellow, viscous liquid; odourless.

Identification

Boil 50 mg with 5 ml of *0.1 M sodium hydroxide* for 1 minute, cool and add 5 ml of *1 M hydrochloric acid* and 0.1 ml of *ferric chloride test solution*; a deep yellow colour is produced.

Tests

pH (2.4.24). Not more than 10.5, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). +28.2° to +30.2°, determined at 20° in a 5.0 per cent w/v solution.

Refractive index (2.4.27). 1.490 to 1.498, determined at 20°.

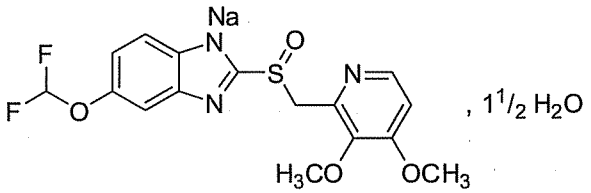
Heavy metals (2.3.13). 1.0 g dissolved in 25 ml of *water* complies with the limit test for heavy metals, Method A (20 ppm).

Assay. Weigh accurately about 0.5 g and carry out Method A for the determination of nitrogen (2.3.30).

1 ml of *0.05 M sulphuric acid* is equivalent to 0.001401 g of N.

Storage. Store protected from moisture.

Pantoprazole Sodium



$C_{16}H_{14}F_2N_3NaO_4S, 1½ H_2O$ Mol. Wt. 432.4

Pantoprazole Sodium is sodium 5-(difluromethoxy)-2[[(3,4 dimethoxy-pyridin-2-yl)methyl]sulphinyl]benzimidazol-1-ide, sesquihydrate.

Pantoprazole Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{16}H_{14}F_2N_3NaO_4S$, calculated on the anhydrous basis.

Category. Antiulcer.

Description. A white to off-white powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pantoprazole sodium RS* or with the reference spectrum of pantoprazole sodium.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. Gives the reaction (a) of sodium (2.3.1).

Tests

Optical rotation (2.4.22). -0.40° to +0.40°, determined on 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *0.001 M sodium hydroxide.*

Test solution. Dissolve about 23 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution. A 0.06 per cent w/v solution of *pantoprazole sodium RS* in the solvent mixture. Dilute 2.5 ml of the solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- column temperature. 40°,
- mobile phase: A. dissolve 1.74 g of *dibasic potassium phosphate* to 1000 ml with *water*, adjusted to pH 7.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-40	80→20	20→80
40-45	20	80
45-55	20→80	80→20

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of the peak due to impurity C determined at wavelength 305 nm at relative retention time about 0.6 is not more than 0.77 times the area of the principal peak at 290 nm in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any secondary peak is not more than 0.077 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not less than 5.0 per cent and not more than 8.0 per cent, determined on 0.15 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture (a). Dilute 25.0 ml of *ammonium hydroxide* to 500.0 ml with *water.*

Solvent mixture (b). Equal volumes of *acetonitrile* and *water.*

Test solution. Dissolve about 20 mg of the substance under examination 10.0 ml of solvent mixture (b) and dilute to 50.0 ml with solvent mixture (a). Dilute 3.0 ml of this solution to 20.0 ml with solvent mixture (a).

Reference solution. Dissolve 20 mg of *pantoprazole sodium RS* in 10.0 ml of solvent mixture (b) and dilute to 50.0 ml with solvent mixture (a). Dilute 3.0 ml of this solution to 20.0 ml with solvent mixture (a).

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (4 µm) (Such as Inertsil ODS-3),
- column temperature. 30°,
- sample temperature. 4°,
- mobile phase: A. a mixture of 85 volumes of buffer solution prepared by dissolving 1.32 g of *dibasic ammonium phosphate* to 1000 ml with *water*, adjusted to pH 7.5 with *orthophosphoric acid* and 15 volumes of *acetonitrile*,
  B. a mixture of 7 volumes of *acetonitrile* and 3 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 285 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-10	86	14
35	42	58
36	86	14
46	86	14

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{16}H_{14}F_2N_3NaO_4S$.

Storage. Store protected from light and moisture, between 2° to 8°.

Pantoprazole Sustained-release Tablets

Pantoprazole Sodium Sustained-release Tablets

Pantoprazole Sustained-release Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pantoprazole, $C_{16}H_{15}F_2N_3O_4S$.

Usual strenth. 40 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima at about 289 nm.

Tests

Dissolution (2.5.2).

NOTE—Prepare the solutions immediately before use. Protect the solutions from light.

A. Apparatus No. 1,
Medium. 1000 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 120 minutes.

Determine by liquid chromatography (2.4.14).

Test solution. Withdraw the medium completely and disperse the intact tablet in 100 ml of the mobile phase and filter.

Reference solution. Dissolve an accurately weighed quantity of *pantoprazole sodium RS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ released in the acid medium by subtracting the content of $C_{16}H_{15}F_2N_3O_4S$ in the test solution from the total content of Pantoprazole, $C_{16}H_{15}F_2N_3O_4S$ determined in the Assay.

D. Not more than 10 per cent of the stated amount of $C_{16}H_{15}F_2N_3O_4S$.

B. Apparatus No.1,

Medium. 1000 ml of *tris-acetate buffer solution pH 8.5,*
Speed and time. 75 rpm and 60 minutes.

Run method A on another 6 tablets and discard the medium completely and fill the empty vessel with the dissolution medium. Withdraw a suitable volume of the medium and filter. Dilute the filtrate, if necessary, with the dissolution medium. Measure the absorbance at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ in the medium from the absorbance obtained from a solution of known concentration of *pantoprazole sodium RS.*

D. Not less than 75 per cent of the stated amount of $C_{16}H_{15}F_2N_3O_4S$.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described in the Assay using the following solutions.

Test solution. Disperse 1 intact tablet in 100.0 ml of the mobile phase and filter.

Reference solution. Dissolve an accurately weighed quantity of *pantoprazole sodium RS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the test solution.

Inject the reference solution and the test solution.

Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use. Protect the solutions from light.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 20 mg of Pantoprazole Sodium, disperse in 100 ml of the mobile phase and filter.

Reference solution. A 0.02 per cent w/v solution of *pantoprazole sodium RS* in the mobile phase.

Chromatographic system
- stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 50 volumes of buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 1 g of *hexane sulphonic acid sodium salt* in 1000 ml of *water,* adjusted to pH 7.3 with *1 M sodium hydroxide* and 50 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

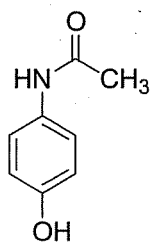
Calculate the content of $C_{16}H_{15}F_2N_3O_4S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the amount of Pantoprazole.

Paracetamol

Acetaminophen



$C_8H_9NO_2$ Mol. Wt. 151.2

Paracetamol is 4-hydroxyacetanilide.

Paracetamol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_9NO_2$, calculated on the dried basis.

Category. Analgesic; antipyretic.

Dose. 500 mg to 1 g every 4 to 6 hours, upto 4 g daily, in divided doses

Description. White crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *paracetamol RS* or with the reference spectrum of paracetamol.

B. Dissolve 50 mg in sufficient *methanol* to produce 100 ml. To 1 ml of this solution add 0.5 ml of *0.1 M hydrochloric acid* and dilute to 100 ml with *methanol*. Protect the resulting solution from bright light and immediately measure the absorbance at the maximum at about 249 nm; absorbance at 249 nm, about 0.44 (2.4.7).

C. Boil 0.1 g in 1 ml of *hydrochloric acid* for 3 minutes, add 10 ml of *water* and cool; no precipitate is produced. Add 0.05 ml of *0.0167 M potassium dichromate*; a violet colour develops which does not turn red.

D. Gives the reaction of acetyl groups (2.3.1).

Tests

4-Aminophenol. Dissolve 0.5 g in sufficient *methanol (50 per cent)* to produce 10 ml. Add 0.2 ml of freshly prepared *alkaline sodium nitroprusside solution*, mix and allow to stand for 30 minutes. Any blue colour in the solution is not more intense than that in 10 ml of a solution prepared at the same time and in the same manner containing 0.5 g of *4-aminophenol-free paracetamol* and 0.5 ml of a 0.005 per cent w/v solution of *4-aminophenol* in *methanol (50 per cent)* (50 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Note—Prepare the solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in 2.5 ml of *methanol* containing 0.46 per cent w/v of *tetrabutylammonium hydroxide solution* (40 per cent w/v) and dilute to 10.0 ml with the solution containing equal volumes of 1.79 per cent w/v of *disodium hydrogen phosphate* and 0.78 per cent w/v of *sodium dihydrogen phosphate*.

Reference solution (a). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). A solution containing 0.025 per cent w/v each of *4-aminophenol*, *paracetamol RS* and *chloroacetanilide* in *methanol*. Dilute 1.0 ml of this solution to 250.0 ml with the mobile phase.

Reference solution (d). Dissolve 20 mg of *4-nitrophenol* in 50.0 ml of *methanol*. Dilute 1.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: a mixture of 37.5 volumes of a 1.79 per cent w/v solution of *disodium hydrogen phosphate* , 37.5 volumes of a 0.78 per cent w/v solution of *sodium dihydrogen phosphate* and 25 volumes of *methanol* containing 0.46 per cent v/v of *tetrabutylammonium hydroxide solution (40 per cent w/v)*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to 4-aminophenol (paracetamol impurity K) and paracetamol is not less than 4.0 and the signal-to-noise ratio of the peak due to chloroacetanilide (paracetamol imputity J) is not less than 50. The relative retention time with reference to paracetamol for paracetamol impurity K is about 0.8, for 4-nitrophenol (paracetamol impurity F) is about 3.0 and for paracetamol impurity J is about 7.0.

Inject the test solution, reference solution (a), (b) and (c). Run the chromatogram 12 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to paracetamol impurity J is not more than 0.2 times the area of the corresponding peak in the chromatogram obtained with reference solution (c) (10 ppm)

and the area of the peak due to paracetamol impurity K is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (50 ppm). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent). The sum of areas of other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in a mixture of 10 ml of *water* and 50 ml of *1 M sulphuric acid*. Boil under a reflux condenser for 1 hour, cool and dilute to 100.0 ml with *water*. To 20.0 ml of the solution add 40 ml of *water*, 40 g of *water* in the form of ice, 15 ml of *2 M hydrochloric acid* and 0.1 ml of *ferroin solution* and titrate with *0.1 M ceric ammonium sulphate* until a yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M ceric ammonium sulphate* is equivalent to 0.00756 g of $C_8H_9No_2$.

Storage. Store protected from light and moisture.

Paracetamol Syrup

Paracetamol Oral Solution; Acetaminophen Syrup

Paracetamol Syrup is a solution of Paracetamol in a suitable flavoured vehicle.

Paracetamol Syrup contains not less than 95.0 per cent and not more than 105.0 per cent w/v solution of the stated amount of paracetamol, $C_8H_9NO_2$.

Usual strength. 125 mg per 5 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 65 volumes of *chloroform*, 25 volumes of *acetone*, 10 volumes of *toluene* and 0.5 volumes of *glacial acetic acid*.

Test solution. Dilute a volume containing 25 mg of Paracetamol to 10 ml with *methanol* and filter if necessary.

Reference solution. A 0.25 per cent w/v solution of *paracetamol RS* in *methanol*

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air, examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

4-Aminophenol. Determine by liquid chromatography (2.4.14).

Test solution. Shake 5 ml of the preparation under examination with 15 ml of the mobile phase, dilute to 25 ml with the mobile phase and filter if necessary.

Reference solution. A 0.0025 per cent w/v solution of *4-aminophenol* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: *0.01 M sodium butanesulphonate* in a mixture of 85 volumes of *water*, 15 volumes of *methanol* and 0.4 volume of *formic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

In the chromatogram obtained with the test solution the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution peaks with a long retention time may occur due to preservatives in the preparations.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix an accurately weighed quantity of the preparation under examination containing 25 mg of Paracetamol in 100 ml of the mobile phase, dilute to 200.0 ml with the mobile phase and filter if necessary.

Reference Solution. A 0.0125 per cent w/v solution of *paracetamol RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: *0.01 M sodium butanesulphonate* in a mixture of 85 volumes of *water*, 15 volumes of *methanol* and 0.4 volume of *formic acid*,
- flow rate. 2 ml per minute,

- spectrophotometer set at 243 nm,
- injection volume. 20 µl.

Determine the weight per ml (2.4.29) of the syrup and calculate the percentage content of $C_8H_9NO_2$, weight in volume.

Storage. Store protected from light and moisture.

Paracetamol Tablets

Acetaminophen Tablets

Paracetamol Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of paracetamol, $C_8H_9NO_2$.

Usual strengths. 300 mg; 500 mg.

Identification

Extract a quantity of the powdered tablets containing 0.5 g of Paracetamol with 20 ml of *acetone*, filter, evaporate the filtrate to dryness and dry at 105°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *paracetamol RS* or with the reference spectrum of paracetamol.

B. Boil 0.1 g in 1 ml of *hydrochloric acid* for 3 minutes, add 10 ml of *water* and cool; no precipitate is produced. Add 0.05 ml of *0.0167 M potassium dichromate*; a violet colour develops which does not turn red.

Tests

4-Aminophenol. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 1 g of Paracetamol with 15 ml of *methanol*, dilute to 100 ml with *water* and filter.

Reference solution. A 0.001 per cent w/v solution of *4-aminophenol* in *methanol (15 per cent)*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: *0.01 M sodium butanesulphonate* in a mixture of 85 volumes of *water*, 15 volumes of *methanol* and 0.4 volume of *formic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

In the chromatogram obtained with the test solution the area of any peak corresponding to 4-aminophenol is not greater than the area of the peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution peaks with a long retention times may occur due to excipients.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 0.2 g of Paracetamol in 10.0 ml of the mobile phase, filter.

Reference solution (a). Dilute 1 ml of the test solution to 20 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase.

Reference solution (b). A solution containing 0.002 per cent w/v each of *4-aminophenol* and *paracetamol RS* in the mobile phase.

Reference solution (c).) A 0.00002 per cent w/v solution of *4-chloroacetanilide* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Zorbax Rx C8),
- column temperature. 35 °,
- mobile phase: a mixture of 25 volumes of *methanol* containing 1.15 g of *tetrabutylammonium hydroxide solution* (40 per cent w/v), with 37.5 volumes of *0.05 M disodium hydrogen orthophosphate* and 37.5 volumes of *0.05 M sodium dihydrogen orthophosphate,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 4.0.

Inject the test solution and reference solution (a), (b) and (c). Run the chromatogram 12 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of peak corresponding to 4-aminophenol is not more than the area of the corresponding peak in reference solution (b) (0.1 per cent), the area of peak corresponding to 4- chloroacetanilide is not more than the area of the principal peak in reference solution (c) (10 ppm) and the area of any other secondary peak is not more than the area of the principal peak obtained with reference solution (a) (0.25 per cent).

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of *phosphate buffer pH 5.8*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at

about 243 nm (2.4.7). Similarly measure the absorbance of a solution of known concentration of *paracetamol RS*. Calculate the content of $C_8H_9NO_2$.

D. Not less than 80 per cent of the stated amount of $C_8H_9NO_2$.

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Paracetamol, add 50 ml of *0.1 M sodium hydroxide*, dilute with 100 ml of *water*, shake for 15 minutes and add sufficient *water* to produce 200.0 ml. Mix, filter and dilute 10.0 ml of the filtrate to 100.0 ml with *water*. To 10.0 ml of the resulting solution add 10 ml of *0.1 M sodium hydroxide*, dilute to 100.0 ml with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 257 nm (2.4.7). Calculate the content of $C_8H_9NO_2$ taking 715 as the specific absorbance at 257 nm.

Storage. Store protected from light and moisture.

Hard Paraffin

Hard Paraffin is a purified mixture of solid hydrocarbons obtained from petroleum or from shale oil.

Category. Pharmaceutical aid (stiffening agent).

Description. A white or colourless, translucent mass, frequently showing a crystalline structure; odourless even when freshly cut; slightly greasy to the touch. Burns with a luminous flame. When melted, the liquid is free from fluorescence by daylight.

Tests

Acidity or alkalinity. To 10.0 g add 20 ml of boiling *water*, heat in a water-bath for 5 minutes, shake vigorously for 1 minute, cool, allow to separate and filter the aqueous layer. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Congealing range (2.4.10). 50° to 65°.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Liquid Paraffin

White Mineral Oil; Liquid Petrolatum

Liquid Paraffin is a purified mixture of liquid hydrocarbons obtained from petroleum to which not more than 10 ppm of tocopherol or of butylated hydroxytoluene may be added.

Category. Laxative; faecal softener.

Dose. 10 to 30 ml.

Description. A transparent, colourless, oily liquid, free from fluorescence by daylight; odourless or almost odourless.

Tests

Weight per ml (2.4.29). 0.860 g to 0.904 g.

Dynamic viscosity (2.4.28). 110 mPas to 230 mPas, determined at 20° ±1° by method B or **Viscosity** (2.4.28). 10 cps to 40 cps, determined at 30° ±1° by method C using LV1 spindle at 30 rpm.

Acidity or alkalinity. To 10.0 g add 20 ml of boiling *water*, heat in a water-bath for 5 minutes, shake vigorously for 1 minute, cool, allow to separate and filter the aqueous layer. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Light absorption. When examined in the range 240 nm to 280 nm (2.4.7), a 2.0 per cent w/v solution in *2,2,4-trimethylpentane* shows an absorption of not more than 0.1.

Readily carbonisable substances. Place 5 ml in a dry, heat-resistant glass-stoppered test-tube (125 mm x 18 mm) previously rinsed with chromic acid solution, then with *water* and dried. Add 5 ml of *nitrogen-free sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4), insert the stopper and shake as vigorously as possible in the longitudinal direction of the tube for 5 seconds. Loosen the stopper, immediately place the tube in a bath of boiling *water*, supporting it so as to prevent contact of the tube with the bottom or side of the bath and heat for 10 minutes. At the end of the second, fourth, sixth, and eighth minutes, remove the tube from the bath and shake as vigorously as possible in the longitudinal direction of the tube for 5 seconds. At the end of 10 minutes from the time the tube was placed in the bath remove the tube and allow to stand for 10 minutes. The lower acid layer is not more intensely coloured than a mixture of 3 ml of FCS, 1.5 ml of CCS and 0.5 ml of CSS (2.4.1), overlaid with 5 ml of liquid paraffin. If the sulphuric acid remains dispersed in the molten paraffin, the colour of the emulsion is not darker than that of the standard mixture when shaken vigorously.

Solid paraffins. Place a suitable quantity, previously dried by heating at 100° for 2 hours and cooled in a desiccator over sulphuric acid, in a glass cylindrical vessel having an internal diameter of approximately 25 mm. Close the vessel and immerse in a mixture of ice and *water*; after 4 hours the liquid is sufficiently clear that a black line, 0.5 mm in width, held vertically behind the vessel is easily seen.

Sulphur compounds. Mix 4 ml with 2 ml of *ethanol (95 per cent)*, and 2 drops of a clear, saturated solution of *lead monoxide* in *sodium hydroxide solution* and heat at 70° for

10 minutes with frequent shaking; the mixture remains colourless.

Storage. Store protected from light.

Light Liquid Paraffin

Light Mineral Oil; Light Liquid Petrolatum

Light Liquid Paraffin is a purified mixture of liquid saturated hydrocarbons obtained from petroleum. It may contain a suitable stabiliser.

Category. Pharmaceutical aid (vehicle).

Description. A transparent, colourless, oily liquid, free from fluorescence by daylight; almost odourless when cold.

Tests

Weight per ml (2.4.29). 0.820 g to 0.880 g.

Dynamic viscosity (2.4.28). 25 mPas to 80 mPas, determined at 20° ±1° by method B or **Viscosity** (2.4.28). 10 cps to 40 cps, determined at 30° ±1° by method C using LV1 spindle at 30 rpm.

Acidity or alkalinity. To 10.0 g add 20 ml of boiling *water*, heat in a water-bath for 5 minutes, shake vigorously for 1 minute, cool, allow to separate and filter the aqueous layer. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Light absorption. When examined in the range 240 nm to 280 nm (2.4.7), a 2.0 per cent w/v solution in *2,2,4-trimethylpentane* shows an absorption of not more than 0.1.

Readily carbonisable substances. Place 5 ml in a dry, heat-resistant glass-stoppered test-tube (125 mm x 18 mm) previously rinsed with chromic acid solution, then with *water* and dried. Add 5 ml of *nitrogen-free sulphuric acid* (containing 94.5 per cent to 95.5 per cent w/w of H_2SO_4), insert the stopper and shake as vigorously as possible in the longitudinal direction of the tube for 5 seconds. Loosen the stopper, immediately place the tube in a bath of boiling *water*, supporting it so as to prevent contact of the tube with the bottom or side of the bath and heat for 10 minutes. At the end of the second, fourth, sixth, and eighth minutes, remove the tube from the bath and shake as vigorously ssas possible in the longitudinal direction of the tube for 5 seconds. At the end of 10 minutes from the time the tube was placed in the bath remove the tube and allow to stand for 10 minutes. The lower acid layer is not more intensely coloured than a mixture of 3 ml of FCS, 1.5 ml of CCS and 0.5 ml of CSS (2.4.1), overlaid with 5 ml of liquid paraffin. If the sulphuric acid remains dispersed in the molten paraffin, the colour of the emulsion is not darker than that of the standard mixture when shaken vigorously.

Solid paraffins. Place a suitable quantity, previously dried by heating at 100° for 2 hours and cooled in a desiccator over sulphuric acid, in a glass cylindrical vessel having an internal diameter of approximately 25 mm. Close the vessel and immerse in a mixture of ice and water; after 4 hours the liquid is sufficiently clear that a black line, 0.5 mm in width, held vertically behind the vessel is easily seen.

Sulphur compounds. Mix 4 ml with 2 ml of *ethanol (95 per cent)*, and 2 drops of a clear, saturated solution of *lead monoxide* in *sodium hydroxide solution* and heat at 70° for 10 minutes with frequent shaking; the mixture remains colourless.

Storage. Store protected from light.

Liquid Paraffin Emulsion

Liquid Paraffin Oral Emulsion

Liquid Paraffin Emulsion is an oral emulsion of Liquid Paraffin in Purified Water.

Liquid Paraffin Emulsion contains not less than 44.0 per cent and not more than 49.0 per cent w/w of liquid paraffin.

Category. Laxative; faecal softener.

Dose. 10 to 30 ml.

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately about 5.0 g, add 10 ml of *water*, extract with two quantities, each of 40 ml, of a mixture of 2 volumes of *ethanol (95 per cent)*, 3 volumes of *light petroleum (40° to 60°)* and 3 volumes of *ether* and then with 30 ml of a mixture of equal volumes of *light petroleum (40° to 60°)* and *ether*. Wash the combined extracts with 15 ml of *0.5 M sodium hydroxide* and then with 15 ml of *water*, evaporate the solvent, add 5 ml of *acetone* and evaporate again. Repeat the addition and evaporation of acetone until the residue is free from *water*, dry at 105° for 15 minutes and weigh.

Storage. Store protected from moisture.

White Soft Paraffin

White Petroleum Jelly

White Soft Paraffin is a purified, semi-solid mixture of hydrocarbons obtained from petroleum and bleached.

Category. Pharmaceutical aid (ointment base).

Description. A white, translucent, soft unctuous mass, retaining these characteristics on storage and when melted and allowed to cool without stirring; not more than slightly fluorescent by daylight, even melted; odourless when rubbed on the skin.

Tests

Melting range (2.4.21). 38° to 56°, determined by Method IV.

Acidity or alkalinity. To 10.0 g add 20 ml of boiling *water*, heat in a water-bath for 5 minutes, shake vigorously for 1 minute, cool, allow to separate and filter the aqueous layer. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Light absorption (2.4.7). Absorbance of a 0.05 per cent w/v solution in *2,2,4-trimethylpentane* at about 290 nm, not more than 0.5.

Fixed oils, fats and resin. Digest 10 g with 50 ml of *sodium hydroxide solution* at 100° for 30 minutes and allow the aqueous layer to separate. On acidifying the aqueous layer with *dilute sulphuric acid*, no precipitate or oily matter is produced.

Foreign organic matter. Volatilises when heated, without emitting an acrid odour.

Consistency. 100 to 300, determined by the following method.

Apparatus. The apparatus is essentially in agreement with IS 4887:1980 and comprises a penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g having a detachable steel tip of the following dimensions. The tip of the cone has an angle of 30°, the point being truncated to a diameter of 0.38 ± 0.08 mm, the base of the tip is 8.38 ± 0.13 mm in diameter and the length of the tip is 15 ± 0.25 mm. The remaining portion of the cone has an angle of 90°, is 28 to 29 mm in height, and has a maximum diameter of 65.1 mm at the base. The containers of the test are flat-bottomed metal or glass cylinders that are 102 ± 6 mm in diameter and not less than 60 mm in height.

Procedure. Melt a sufficient quantity at a temperature below 85° and pour into one or more of the containers filling to within 6 mm of the rim. Cool to 25° ± 2.5° over a period of not less than 16 hours, protected from drafts. Two hours before the test, place the containers in a water-bath at 25° ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25° ± 0.5° by placing it in a water-bath.

Without disturbing the surface of the substance under examination, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25 mm to 38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 seconds. Secure the plunger and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings and conduct further trials to a total of 10 if the individual results differ from the average by more than ± 3 per cent. The final average of the trials is not less than 10.0 mm and not more than 30.0 mm indicating a consistency value between 100 and 300.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from light and moisture.

Yellow Soft Paraffin

Yellow Petroleum Jelly

Yellow Soft Paraffin is a purified, semi-solid mixture of hydrocarbons obtained from petroleum.

Category. Pharmaceutical aid (ointment base).

Description. A pale yellow to yellow, translucent, soft unctuous mass, retaining these characteristics on storage and when melted and allowed to cool without stirring; not more than slightly fluorescent by daylight, even melted; odourless when rubbed on the skin.

Tests

Melting range (2.4.21). 38° to 56°, determined by Method IV.

Light absorption (2.4.7). Absorbance of a 0.05 per cent w/v solution in *2,2,4-trimethylpentane* at about 290 nm, not more than 0.75.

Acidity or alkalinity. To 10.0 g add 20 ml of boiling *water*, heat in a water-bath for 5 minutes, shake vigorously for 1 minute, cool, allow to separate and filter the aqueous layer. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution is colourless and not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Fixed oils, fats and resin. Digest 10 g with 50 ml of *sodium hydroxide solution* at 100° for 30 minutes and allow the aqueous layer to separate. On acidifying the aqueous layer with *dilute sulphuric acid*, no precipitate or oily matter is produced.

Foreign organic matter. Volatilises when heated, without emitting an acrid odour.

Consistency. 100 to 300, determined by the following method.

Apparatus. The apparatus is essentially in agreement with IS 4887.1980 and comprises a penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g having a detachable steel tip of the following dimensions. The tip of the cone has an angle of 30°, the point being truncated to a diameter of 0.38 ± 0.08 mm, the base of the tip is 8.38 ± 0.13 mm in diameter and the length of the tip is 15 ± 0.25 mm. The remaining portion of the cone has an angle of 90°, is 28 to 29 mm in height, and has a maximum diameter of 65.1 mm at the base. The containers of the test are flat-bottomed metal or glass cylinders that are 102 ± 6 mm in diameter and not less than 60 mm in height.

Procedure. Melt a sufficient quantity at a temperature below 85° and pour into one or more of the containers filling to within 6 mm of the rim. Cool to 25° ± 2.5° over a period of not less than 16 hours, protected from drafts. Two hours before the test, place the containers in a water-bath at 25° ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25° ± 0.5° by placing it in a water-bath.

Without disturbing the surface of the substance under examination, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25 mm to 38 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 seconds. Secure the plunger and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings and conduct further trials to a total of 10 if the individual results differ from the average by more than ± 3 per cent. The final average of the trials is not less than 10.0 mm and not more than 30.0 mm indicating a consistency value between 100 and 300.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from light and moisture.

Paraffin Ointment

Category. Pharmaceutical aid (ointment basis).

White Beeswax	20 g
Hard Paraffin	30 g
Cetostearyl Alcohol	50 g
White Soft Paraffin*	900 g

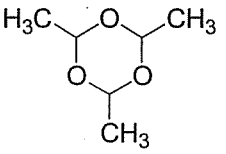
*May be replaced by Yellow Soft Paraffin if other medicaments to be incorporated are coloured.

Mix the ingredients, heat gently with stirring until homogeneous and stir until cold.

Tests

Paraffin Ointment complies with the tests stated under Ointments.

Paraldehyde



$C_6H_{12}O_3$ Mol. Wt. 132.7

Paraldehyde is 2,4,6-trimethyl-1,3,5-trioxane, the cyclic trimer of acetaldehyde. It may contain a suitable amount of antioxidant.

Category. Anticonvulsant in status epilepticus; hypnotic; sedative.

Dose. By deep intramuscular injection, as a single dose, 5 to 10 ml; by rectal injection, 5 to 10 ml, suitably diluted with physiological saline.

Description. A colourless or slightly yellow, transparent liquid; odour, strong and characteristic. Solidifies at low temperature to form a crystalline mass.

Identification

A. Heat 5 ml with 0.1 ml of *1 M sulphuric acid*; acetaldehyde, recognisable by its odour, is evolved.

B. To 5 ml of a 10 per cent v/v solution add 5 ml of *ammoniacal silver nitrate solution* in a test-tube and heat on a water-bath; metallic silver is deposited as a mirror on the sides of the tube.

C. A 10 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), but becomes turbid on warming.

Tests

Congealing range (2.4.10). 10° to 13°.

Distillation range (2.4.8). Not more than 10 per cent distils below 123° and not less than 95 per cent distils below 126°.

Refractive index (2.4.27). 1.403 to 1.406.

Relative density (2.4.29). 0.991 to 0.996.

Acetaldehyde. Shake 5 ml with a mixture of 5 ml of *ethanol (60 per cent)*, 5 ml of *hydroxylamine hydrochloride reagent in ethanol (60 per cent)* and 2 drops of *methyl orange solution* and titrate with *0.5 M sodium hydroxide* to full yellow colour; not more than 0.8 ml of *0.5 M sodium hydroxide* is required.

Acidity. Mix 5 ml with 45 ml of *carbon dioxide-free water* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator; not more than 1.5 ml is required.

Chlorides. To 5 ml of a 1 per cent v/v solution add one drop of *nitric acid* and three drops of *silver nitrate solution*; no opalescence is produced immediately.

Sulphates. To 5 ml of a 1 per cent v/v solution add one drop of *hydrochloric acid* and three drops of *barium chloride solution*; no turbidity is produced.

Peroxides. In a stoppered vessel, dissolve 5 ml in sufficient of recently boiled and cooled *water* to produce 50 ml, add 5 ml of *dilute sulphuric acid* and 10 ml of *potassium iodide solution*. Close the flask and set aside in the dark for 15 minutes. Titrate with *0.1 M sodium thiosulphate* using *starch solution* as indicator; set aside for 5 minutes and, if necessary, complete the titration. Not more than 2.0 ml of *0.1 M sodium thiosulphate* is required.

Non-volatile matter. Heat 5 ml in a small dish on a water-bath and dry at 105° for 1 hour; the residue weighs not more than 3 mg (0.06 per cent w/v).

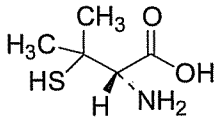
Storage. Store protected from moisture, in complete darkness and at a temperature of 8° to 15°. If solidified, the whole of the contents of the container should be liquified by warming before use.

NOTE — Do not use Paraldehyde if it has a brownish colour or an odour of acetic acid. Avoid contact with rubber and plastics.

Labelling. The label states (1) the nature and the proportion of any antioxidant added; (2) that it may decompose on standing to form potentially harmful substances.

Penicillamine

D-Penicillamine



$C_5H_{11}NO_2S$ Mol. Wt. 149.2

Penicillamine is 3-mercapto-D-valine.

Penicillamine contains not less than 98.0 per cent and not more than 101.0 per cent of $C_5H_{11}NO_2S$, calculated on the dried basis.

Category. Chelating agent in copper and lead poisoning; antirheumatoid arthritic.

Dose. In poisoning, 500 mg to 2 g daily, in divided doses or in accordance with the needs of the patient. In rheumatoid arthritis, initial dose, 125 to 250 mg daily before food, increased gradually every 4 to 12 weeks until remission occurs; usual maintenance dose, 500 to 750 mg daily.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if test B, C and D carried out. Test D may be omitted if test A, B and C are carried out.

A. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 40 volumes of *1-butanol*, 10 volumes of *glacial acetic acid* and 10 volumes of *water*.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of *water*.

Reference solution. A 0.25 per cent w/v solution of *penicillamine RS* in *water*.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 5 to 10 minutes and expose to iodine vapour for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dissolve 0.5 g in a mixture of 0.5 ml of *hydrochloric acid* and 4 ml of warm *acetone*, cool in ice and scratch the inside of the tube with a glass rod to initiate crystallisation; a white precipitate is produced. Filter under vacuum, wash the precipitate with *acetone* and dry with suction. A 1 per cent w/v solution of the dried material is dextrorotatory.

C. To 4 ml of a 1 per cent w/v solution add 2 ml of *phosphotungstic acid solution* and heat nearly to boiling; a blue colour is produced.

D. In the test for Penicillamine disulphide, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak due to penicillamine in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

pH (2.4.24). 4.5 to 5.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). –61.0° to –65.0°, determined in a 5.0 per cent w/v solution in *1 M sodium hydroxide*.

Heavy metals (2.3.13). 10 ml of solution A, complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb)* to prepare the standard.

Mercuric salts. Determine by atomic absorption spectrophotometry (2.4.2), using a solution prepared in the following manner. To 1.0 g of the substance under examination add 10 ml of *water* and 0.15 ml of *perchloric acid* and swirl until dissolution is complete. Add 1 ml of *ammonium*

pyrrolidinedithiocarbamate solution that has been washed three times immediately before use, each time with an equal volume of *4-methyl-2-pentanone*. Mix, add 2 ml of *4-methyl-2-pentanone*, shake for 1 minute, dilute to 25 ml with *water*, allow the layers to separate and use the 4-methyl-2-pentanone layer. Measure the absorbance at 254 nm using a mercury hollow-cathode lamp and an air-acetylene flame and setting the zero using a 4-methyl-2-pentanone layer obtained by repeating the procedure described above but omitting the substance under examination. For the standard solution dissolve 0.108 g of *yellow mercuric oxide* in the minimum volume of *2 M hydrochloric acid*, add sufficient *water* to produce 1000.0 ml and treat suitable volumes in the same manner as the solution of the substance under examination (10 ppm).

Penicillamine disulphide. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 40 mg of the substance under examination in 5 ml of the mobile phase, add 1 ml of a 0.0025 per cent w/v solution of *sulphanilamide* (internal standard) in the mobile phase and dilute to 10 ml with the mobile phase.

Test solution (b). Dissolve 40 mg of the substance under examination in the mobile phase and dilute to 10 ml with the same solvent.

Reference solution (a). A 0.4 per cent w/v solution of *penicillamine RS* in the mobile phase.

Reference solution (b). Add 1 ml of test solution (a) to 1 ml of a 0.04 per cent w/v solution of *penicillamine disulphide RS* in the mobile phase and dilute to 10 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 5 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: an equal volume of 0.2 per cent w/v solution of *methanesulphonic acid* and 0.01 per cent w/v solution of *disodium edetate*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

In the chromatogram obtained with test solution (a) the ratio of the area of any peak corresponding to penicillamine disulphide to the area of the peak due to the internal standard is not greater than the corresponding ratio in the chromatogram obtained with reference solution (b).

Penicillin. Carry out the following procedure in a penicillin-free atmosphere and with equipment reserved for the test. Sterilise the equipment at 180° for 3 hours and the buffer solutions at 121° for 20 minutes before use.

Liquefy a suitable nutrient medium such as that described below and inoculate at a suitable temperature with a culture of *Micrococcus flavus* (ATCC 9341) to give 5 x 10^4 micro-organisms per ml or a quantity necessary to obtain the required sensitivity and formation of clearly defined inhibition zones of suitable diameter. Immediately pour the inoculated medium into five Petri dishes (10 cm in diameter) to give uniform layers 2 to 5 mm in depth. Alternatively, the medium may consist of two layers, only the upper layer being inoculated. Store the dishes so that no appreciable growth or death of micro-organisms occurs before use and so that the surface of the medium is dry at the time of use. In each dish, place five stainless steel hollow cylinders (6 mm in diameter) on the surface of the medium evenly spaced on a circle with a radius of about 25 mm and concentric with the dish. For each dish, place in separate cylinders 0.15 ml of each of the following five solutions.

For solution (1) dissolve 1.0 g of the substance under examination in 8 ml of *phosphate buffer pH 2.5*, add 8 ml of *ether* and shake vigorously for 1 minute. Repeat the extraction and combine the ether layers. Add 8 ml of *phosphate buffer pH 2.5*, shake for 1 minute, allow to settle and separate the ether layer quantitatively, taking care to eliminate the aqueous phase completely. *(Penicillin is unstable at pH 2.5; carry out the operations at this pH within 6 to 7 minutes)*. Add 8 ml of *phosphate buffer pH 6.0*, shake for 5 minutes, allow to settle, separate the aqueous layer and check that the pH is 6.0. For solution (2) add 20 µl of *penicillinase solution* to 2 ml of solution (1) and incubate at 37° for 1 hour. For solution (3) dissolve 5 mg of *benzylpenicillin sodium* in 500 ml of phosphate buffer pH 6.0 and dilute 0.25 ml of this solution to 200 ml with *phosphate buffer pH 2.5*. Carry out the extraction procedure described under solution (1) using 8 ml of this solution and beginning at the words "add 8 ml of *ether*...". For solution (4) add 20 ml of *penicillinase solution* to 2 ml of solution (3) and incubate at 37° for 1 hour. Prepare solution (5) in the same manner as solution (1) but omitting the substance under examination.

Maintain the dishes at 30° for at least 24 hours. Measure the diameters of the zones of inhibition to within 0.1 mm. The test is not valid unless solution (3) gives a clear zone of inhibition and solutions (4) and (5) give no zones of inhibition. If solution (1) gives a zone of inhibition it is caused by penicillin provided solution (2) gives no zone of inhibition. If this is the case, the average diameter of the zones of inhibition given by solution (1) for the five Petri dishes is less than that given by solution (3) (0.1 ppm).

Nutrient medium

Peptone	5 g
Yeast extract	1.5 g
Meat extract	1.5 g
Sodium chloride	3.5 g
Agar	15 g
Distilled water	1000 ml
Adjust the pH to	6.0

Penillic acid. Absorbance of a 0.2 per cent w/v solution at about 268 nm, not more than 0.07 (2.4.7) (about 0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa.

Assay. Dissolve 0.1 g in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01492 g of $C_5H_{11}NO_2S$.

Storage. Store protected from moisture.

Penicillamine Tablets

D-Penicillamine Tablets

Penicillamine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of penicillamine, $C_5H_{11}NO_2S$. The tablets are coated.

Usual strengths. 50 mg; 125 mg; 250 mg.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Penicillamine with 4 ml of *water* and filter. Add to the filtrate 2 ml of *phosphotungstic acid solution* and allow to stand for 5 minutes; a blue colour is produced.

B. Dissolve a quantity of the powdered tablets containing 10 mg of Penicillamine in 5 ml of *water* and add 0.3 ml of *5 M sodium hydroxide* and 20 mg of *ninhydrin*; an intense blue or violet-blue colour is produced immediately.

Tests

Mercuric salts. Disperse a quantity of the powdered tablets containing 1 g of Penicillamine in 10 ml of *water* in a stoppered flask, add 0.2 ml of *9 M perchloric acid* and swirl to dissolve. Add 1 ml of *ammonium pyrrolidinedithiocarbamate solution*, mix, add 2 ml of *4-methyl-2-pentanone*, shake for 1 minute and add sufficient *water* to produce 25 ml. Determine by atomic absorption spectrophotometry (2.4.2), using a mercury hollow-cathode lamp and an air-acetylene flame and setting the zero using a *4-methyl-2-pentanone* layer obtained by repeating the procedure described above but omitting the substance under examination, measuring at 254 nm. Use *mercury solution AAS*, suitably diluted with *water*, for the standard solutions, adjusted to contain the same concentrations of *9 M perchloric acid, ammonium pyrrolidinedithiocarbamate solution* and *4-methyl-2-pentanone* as the solution under examination (40 ppm).

Penicillamine disulphide. Determine by liquid chromatography (2.4.14).

Test solution. Shake quantity of the powdered tablets containing 40 mg of Penicillamine with 10 ml of the mobile phase, filter and use the filtrate.

Reference solution. A 0.004 per cent w/v solution of *penicillamine disulphide RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 5 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: an equal volume of 0.2 per cent w/v solution of *methanesulphonic acid* and 0.01 per cent w/v solution of *disodium edetate*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

In the chromatogram obtained with the test solution the area of any peak corresponding to penicillamine disulphide is not greater than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

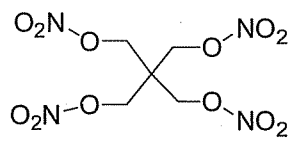
Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.1 g of Penicillamine, dissolve as completely as possible in 50 ml of *water* and filter. Add to the filtrate 5 ml of *1 M sodium hydroxide* and 0.2 ml of a 0.1 per cent w/v solution of *dithizone* in *ethanol (95 per cent)* and titrate with *0.02 M mercuric nitrate*.

1 ml of *0.02 M mercuric nitrate* is equivalent to 0.005968 g of $C_5H_{11}NO_2S$.

Storage. Store protected from moisture.

Diluted Pentaerythritol Tetranitrate



$C_5H_8N_4O_{12}$ Mol. Wt. 316.1

Diluted Pentaerythritol Tetranitrate is a dry mixture of 2,2-bis(hydroxymethyl)propane-1,3-diol tetranitrate with Lactose or Mannitol or a mixture of Lactose and Starch or any other suitable inert excipients which permit safe handling.

Diluted Pentaerythritol Tetranitrate contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pentaerythritol tetranitrate, $C_5H_8N_4O_{12}$.

Category. Antianginal.

Dose. The equivalent of 20 to 60 mg of pentaerythritol tetranitrate, three to four times daily.

Description. A white or almost white, powder; odour, faint and mild.

Identification

A. Transfer a quantity of powder containing 10 mg of pentaerythritol tetranitrate to a medium porosity sintered-glass filter, add 5 ml of dry *acetone* and collect the filtrate. Repeat with two further quantities, each of 5 ml, of dry *acetone* and evaporate the combined filtrate at a temperature not exceeding 60°, with the aid of a gentle current of air, and dry the residue at 60° for 4 hours; the residue melts at 138° to 142° (2.4.21).

B. Suspend 10 mg of the residue obtained in test A in a mixture of 2 ml of *sulphuric acid* and 1 ml of *water;* cool and carefully overlay with 3 ml of *ferrous sulphate solution*; a reddish brown colour is produced at the interface of the two liquids.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 20 volumes of *ethyl acetate* and 80 volumes of *toluene*.

Test solution. Dissolve a quantity of the substance under examination containing about 10 mg of Pentaerythritol Tetranitrate in 10 ml of *ethanol (95 per cent)*, filter.

Reference solution. Shake a quantity of *diluted pentaerythritol tetranitrate RS* containing about 10 mg of Pentaerythritol Tetranitrate with 10 ml of *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine at 254 nm, The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Impurity A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 15 volumes of *glacial acetic acid*, 30 volumes of *acetone* and 60 volumes of *toluene*.

Test solution. Dissolve about 0.1 g of the substance under examination in 5.0 ml of *ethanol (95 per cent)*, filter.

Reference solution. Dissolve about 10 mg of *potassium nitrate* in 1 ml of *water* and dilute to 100 ml with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with freshly prepared *potassium iodide* and *starch solution* and examine at 254 nm. The spot due to nitrate in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent, calculated as potassium nitrate).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Shake about 25 mg of the substance under examination in 20 ml of the mobile phase for 15 minutes and dilute to 25.0 ml with the mobile phase, filter.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve a quantity of *diluted pentaerythritol tetranitrate RS* containing about 25 mg of Pentaerythritol Tetranitrate in 20 ml of the mobile phase, sonicate for 15 minutes and dilute to 25.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the mobile phase.

Reference solution (c). Dilute 0.3 ml of reference solution (b) to 10.0 ml with the mobile phase.

Reference solution (d). Dilute 200 µl of *glyceryl trinitrate solution RS* to 25.0 ml with the mobile phase.

Reference solution (e). To 1 ml of reference solution (b), add 1 ml of reference solution (d) and dilute to 10.0 ml with the mobile phase.

Reference solution (f). Dilute 1.0 ml of reference solution (a) to 20.0 ml with the mobile phase. Dilute 0.5 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *water* and 65 volumes of *acetonitrile*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (e). The test is not valid unless the resolution between the peaks due to glyceryl trinitrate and pentaerythritol tetranitrate is not less than 3.0. The relative retention time with reference to pentaerythritol tetranitrate for pentaerythritol tetranitrate impurity B is about 0.7 and for pentaerythritol tetranitrate impurity C is about 0.3.

Inject test solution (a), reference solution (c) and (f). Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a) the area of the peak due to pentaerythritol tetranitrate impurity C is not more than the area of the principal peak in the chromatogram

obtained with reference solution (c) (0.3 per cent), the area of any other secondary peak is not more than not more than twice the area of the principal peak in the chromatogram obtained with reference solution (f) (0.1 per cent). The sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.6 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (f) (0.05 per cent).

Heavy metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Assay. Determine by liquid chromatography (2.4.14), as described in the test for Related substances.

Inject test solution (b) and reference solution (b).

Calculate the content of $C_5H_8N_4O_{12}$.

Storage. Store protected from light and moisture.

NOTE — Undiluted pentaerythritol tetranitrate is a powerful explosive. It can be exploded with percussion or excessive heat. Great care and appropriate precautions should be taken in handling and only exceedingly small amounts should be isolated.

Labelling. The label states the percentage content of pentaerythritol tetranitrate.

Pentaerythritol Tetranitrate Tablets

Pentaerythritol Tetranitrate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pentaerythritol tetranitrate, $C_5H_8N_4O_{12}$.

Usual strengths. 10 mg; 30 mg.

Identification

A. Transfer a quantity of powder containing 10 mg of pentaerythritol tetranitrate to a medium porosity sintered-glass filter, add 5 ml of dry *acetone* and collect the filtrate. Repeat with two further quantities, each of 5 ml, of dry *acetone* and evaporate the combined filtrate at a temperature not exceeding 60°, with the aid of a gentle current of air, and dry the residue at 60° for 4 hours; the residue melts at 138° to 142° (2.4.21).

Tests

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

Crush one tablet and transfer to a 50-ml volumetric flask with the aid of 15 ml of *acetone*. Add sufficient *acetone* to produce 25 ml, heat the mixture on a water-bath at a temperature not exceeding 60° and boil gently, with occasional swirling, for 5 minutes. Cool, dilute to volume with *acetone* and mix. Transfer a portion of the mixture to a glass-stoppered centrifuge tube and centrifuge at 1500 rpm for 5 minutes. Transfer 2.5 ml of the supernatant solution to a 100-ml volumetric flask and evaporate at 35° with the aid of a current of air to dryness. To the residue add 1.0 ml of *glacial acetic acid* and swirl to dissolve. Add 2 ml of *phenoldisulphonic acid solution*, mix and allow to stand for 5 minutes. Add 25 ml of *water* and 10 ml of *strong ammonia solution*, cool, dilute to volume with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 409 nm (2.4.7), using *water* as the blank.

Weigh accurately 0.130 g of *potassium nitrate*, previously dried at 105° for 4 hours, dissolve in 3 ml of *water*, dilute with sufficient *glacial acetic acid* to produce 200.0 ml and mix well. Using 1.0 ml of this solution repeat the procedure beginning at the words "Add 2 ml of *phenoldisulphonic acid solution*,.....". Calculate the content of $C_5H_8N_4O_{12}$ in the tablet.

1 ml of the potassium nitrate solution is equivalent to 0.000503 g of $C_5H_8N_4O_{12}$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of pentaerythritol tetranitrate and transfer to a 100-ml volumetric flask with the aid of about 30 ml of *acetone*. Add sufficient *acetone* to produce 50 ml and warm on a water-bath at a temperature not exceeding 60° and boil gently, with occasional swirling, for 5 minutes. Cool, dilute to volume with *acetone* and mix. Transfer a portion of the mixture to a glass-stoppered centrifuge tube and centrifuge at 1500 rpm for 5 minutes. Transfer 1.0 ml of the supernatant solution to a 100-ml volumetric flask and evaporate at 35° with the aid of a current of air to dryness. To the residue add 1.0 ml of *glacial acetic acid* and swirl to dissolve. Add 2 ml of *phenoldisulphonic acid solution*, mix and allow to stand for 5 minutes. Add 25 ml of *water* and 10 ml of *strong ammonia solution*, cool, dilute to volume with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 409 nm (2.4.7), using *water* as the blank.

Weigh accurately 0.13 g of *potassium nitrate*, previously dried at 105° for 4 hours, dissolve in 3 ml of *water*, dilute with sufficient *glacial acetic acid* to produce 200.0 ml and mix well. Using 1.0 ml of this solution repeat the procedure beginning at the words "Add 2 ml of *phenoldisulphonic acid solution*,.....".

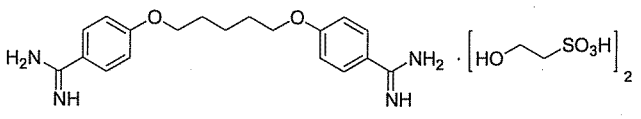
Calculate the content of $C_5H_8N_4O_{12}$ from the values of the absorbances so obtained.

1 ml of the potassium nitrate solution is equivalent to 0.000503 g of $C_5H_8N_4O_{12}$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of pentaerythritol tetranitrate.

Pentamidine Isethionate



$C_{19}H_{24}N_4O_2,2C_2H_6O_4S$ Mol. Wt. 592.7

Pentamidine Isethionate is 4,4′ -[pentane-1,5-diylbis(oxy)] bisbenzenecarboximidamide di(2-hydroxyethanesulphonate).

Pentamidine Isethionate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{24}N_4O_2$, $2C_2H_6O_4S$, calculated on the dried basis.

Category. Antiprotozoal.

Dose. By intramuscular injection, 3 to 4 mg per kg body weight daily for 10 days, maximum 200 mg.

Description. A white or almost white powder or crystals; odourless or almost odourless; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentamidine isethionate RS* or with the reference spectrum of pentamidine isethionate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 262 nm; absorbance at about 262 nm, about 0.46).

C. To 10 ml of a 0.05 per cent w/v solution add 1 ml of a 0.1 per cent w/v solution of *glyoxal sodium bisulphite* and 1 ml of a solution prepared by dissolving 4 g of *boric acid* in a mixture of 27 ml of *1 M sodium hydroxide* and sufficient *water* to produce 100 ml. Heat on a water-bath for 10 minutes; a magenta colour is produced.

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.1 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b). To 0.1 g in a conical flask, add 40 ml of *water* and glass beads, adjusted to pH 10.5 with dilute *sodium hydroxide solution* and boil under reflux for 20 minutes. Cool and dilute to 50 ml with *water.* Dilute 1.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of 3 per cent w/v solution of *ammonium acetate*, adjusted to pH 7.5 with *triethylamine*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the 2 principal peaks is not less than 2.0.

Inject the test solution and reference solution (a). Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Ammonium isethionate. To 1.0 g in a test-tube (about 4 cm in diameter) add 10 ml of *water* and 20 ml of *1 M sodium hydroxide*. Immediately attach a bung carrying a splash head and an aspirator tube (about 5 mm in diameter). Connect the splash head to two test-tubes in series, each containing 20 ml of *0.01 M sulphuric acid*. Heat the tube containing the substance under examination in a water-bath at 45° to 50° and, maintaining this temperature, draw a current of air, previously passed through *1 M sulphuric acid*, through the liquids in a series of tubes for 3 hours at such a rate that the bubbles are just too rapid to count. Titrate the combined solutions from the two absorption tubes with *0.02 M sodium hydroxide* using *methyl red-methylene blue solution* as indicator; not less than 36.5 ml of *0.02 M sodium hydroxide* is required.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.250 g in 50 ml of *dimethylformamide*. Add 0.25 ml of *thymol blue solution* and titrate with *0.1 M tetrabutylammonium hydroxide*, under a current of *nitrogen*, until the colour of the indicator changes to blue. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02963 g of $C_{23}H_{36}N_4O_{10}S_2$.

Storage. Store protected from moisture.

Pentamidine Injection

Pentamidine Isethionate Injection

Pentamidine Injection is a sterile material consisting of Pentamidine Isethionate with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Pentamidine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pentamidine isethionate, $C_{19}H_{24}N_4O_2, 2C_2H_6O_4S$.

Usual strength. 200 mg.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentamidine isethionate RS* or with the reference spectrum of pentamidine isethionate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows an absorption maximum only at about 262 nm; absorbance at about 262 nm, about 0.46.

C. To 10 ml of a 0.05 per cent w/v solution add 1 ml of a 0.1 per cent w/v solution of *glyoxal sodium bisulphite* and 1 ml of a solution prepared by dissolving 4 g of *boric acid* in a mixture of 27 ml of *1 M sodium hydroxide* and sufficient *water* to produce 100 ml. Heat on a water-bath for 10 minutes; a magenta colour is produced.

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve 0.1 g of substance under examination in 40 ml of *water*, adjusted to pH 10.5 with *2 M sodium hydroxide*, heat under a reflux condenser for 20 minutes, cool and dilute to 50 ml with *water*. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- mobile phase: a mixture of 130 volumes of *methanol* and 70 volumes of 3 per cent w/v solution of *ammonium acetate*, adjusted to pH 7.5 with *triethylamine*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 2.0.

Inject the test solution and reference solution (a). Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent).

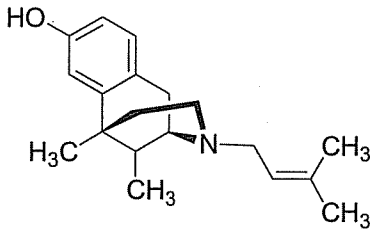
Ammonium isethionate. To 1.0 g in a test-tube (about 4 cm in diameter) add 10 ml of *water* and 20 ml of *1 M sodium hydroxide*. Immediately attach a bung carrying a splash head and an aspirator tube (about 5 mm in diameter). Connect the splash head to two test-tubes in series, each containing 20 ml of *0.01 M sulphuric acid*. Heat the tube containing the substance under examination in a water-bath at 45° to 50° and, maintaining this temperature, draw a current of air, previously passed through *1 M sulphuric acid*, through the liquids in a series of tubes for 3 hours at such a rate that the bubbles are just too rapid to count. Titrate the combined solutions from the two absorption tubes with *0.02 M sodium hydroxide* using *methyl red-methylene blue solution* as indicator; not less than 36.5 ml of *0.02 M sodium hydroxide* is required.

Assay. Dissolve 0.25 g of the mixed contents of 10 containers in 50 ml of *dimethylformamide*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02963 g of $C_{19}H_{24}N_4O_2, 2C_2H_6O_4S$.

Storage. Store in single dose containers.

Pentazocine



$C_{19}H_{27}NO$ Mol. Wt. 285.4

Pentazocine is (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol.

Pentazocine contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{27}NO$, calculated on the dried basis.

Category. Narcotic analgesic.

Dose. By subcutaneous, intramuscular or intravenous injection, 30 to 60 mg every 3 to 4 hours.

Description. A white or pale cream powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentazocine RS* or with the reference spectrum of pentazocine.

B. To 1 mg in a porcelain crucible add 0.5 ml of a solution of *sulphuric acid* containing 1 per cent w/v solution of *ammonium molybdate*; an intense blue colour is produced which changes to bluish green, green and finally, on standing, yellow.

C. Dissolve 5 mg in 5 ml of *sulphuric acid*, add 0.05 ml of *ferric chloride solution* and mix; a yellow colour is produced which deepens slightly in intensity on warming. On the addition of *0.05 ml of nitric acid* the yellow colour is unchanged.

Tests

Light absorption (2.4.7). To 0.1 g add 20 ml of *water* and 10 ml of *1 M hydrochloric acid*, shake to dissolve and add sufficient *water* to produce 100 ml. Dilute 10 ml to 100 ml with *water*. The absorbance of the resulting solution at the maximum at about 278 nm, 0.67 to 0.71.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 94 volumes of *chloroform*, 3 volumes of *2-propylamine* and 3 volumes of *methanol*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform*.

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (c). A 0.005 per cent w/v solution of the substance under examination in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a); not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) and not more than four such spots are more intense than the spot in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

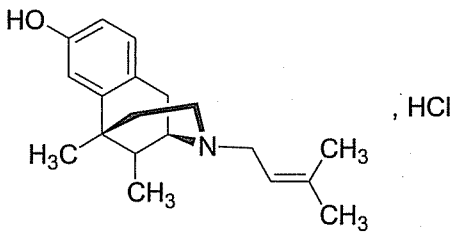
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 0.6 g, dissolve in 50 ml of *anhydrous glacial acetic acid* and titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02854 g of $C_{19}H_{27}NO$.

Storage. Store protected from light and moisture.

Pentazocine Hydrochloride



$C_{19}H_{27}NO,HCl$ Mol. Wt. 321.9

Pentazocine Hydrochloride is (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol hydrochloride.

Pentazocine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{27}NO,HCl$, calculated on the dried basis.

Category. Narcotic analgesic.

Dose. 25 to 100 mg, every 3 to 4 hours after food.

Description. A white or pale cream-coloured, crystalline powder; odourless. The material exhibits polymorphism.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentazocine hydrochloride RS* or with the reference spectrum of pentazocine hydrochloride.

B. To 1 mg in a porcelain crucible add 0.5 ml of a solution of *sulphuric acid* containing 1 per cent w/v solution of *ammonium molybdate*; an intense blue colour is produced which changes to bluish green, green and finally, on standing, yellow.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.0, determined in a 1.0 per cent w/v solution.

Light absorption (2.4.7). Dissolve 0.1 g in 10 ml of *1 M hydrochloric acid* and add sufficient *water* to produce 100 ml; dilute 10 ml to 100 ml with *water*. Absorbance of the resulting solution at the maximum at about 278 nm, 0.59 to 0.63.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 94 volumes of *chloroform*, 3 volumes of *2-propylamine* and 3 volumes of *methanol.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform.*

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (c). A 0.005 per cent w/v solution of the substance under examination in *chloroform.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a); not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) and not more than four such spots are more intense than the spot in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03219 g of $C_{19}H_{27}NO,HCl$.

Storage. Store protected from light and moisture.

Pentazocine Tablets

Pentazocine Hydrochloride Tablets

Pentazocine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pentazocine hydrochloride, $C_{19}H_{27}NO,HCl$.

Usual strength. 25 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Pentazocine Hydrochloride with 10 ml of *water*, filter, add 1 ml of *1 M sodium hydroxide* and shake the resulting solution with 20 ml of *chloroform.* Wash the chloroform extract with 5 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the chloroform using a rotary evaporator and dry the oily residue at a temperature not exceeding 25° at a pressure of 2 kPa for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentazocine RS* or with the reference spectrum of pentazocine.

B. To a quantity of the powdered tablets containing 50 mg of Pentazocine Hydrochloride add 70 ml of *water*, shake for 15 minutes, add sufficient *water* to produce 100 ml and filter. To 10 ml of the filtrate add 10 ml of *1 M sodium hydroxide* and sufficient *water* to produce 100 ml. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 238 nm and 298 nm.

C. Shake a quantity of the powdered tablets containing 25 mg of Pentazocine Hydrochloride with 5 ml of *water* and 0.5 ml of *2 M nitric acid* for 1 minute and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 94 volumes of *chloroform*, 3 volumes of *2-propylamine* and 3 volumes of *methanol.*

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Pentazocine Hydrochloride with 10 ml of *0.1 M methanolic ammonia* for 10 minutes, centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1 volume of test solution to 100 volumes with the same solvent.

Reference solution (b). Dilute 1 volume of test solution to 200 volumes with the same solvent.

Reference solution (c). Dilute 1 volume of test solution to 400 volumes with the same solvent.

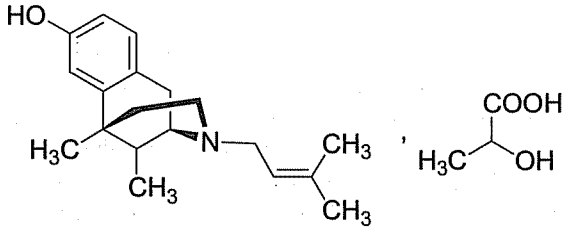
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a), not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) and not more than four such spots are more intense than the spot in the chromatogram obtained with reference solution (c).

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Pentazocine Hydrochloride, shake with 100 ml of *water* for 15 minutes, add 2.5 ml of *1 M hydrochloric acid* and sufficient *water* to produce 250.0 ml and filter. Measure the absorbance of the filtrate at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{19}H_{27}NO,HCl$ taking 61.2 as the specific absorbance at 278 nm.

Storage. Store protected from light and moisture.

Pentazocine Lactate



$C_{19}H_{27}NO,C_3H_6O_3$ Mol. Wt. 375.4

Pentazocine Lactate is (2*RS*,6*RS*,11*RS*)-6,11-dimethyl-3-(3-methylbut-2-enyl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol lactate.

Pentazocine Lactate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{27}NO,C_3H_6O_3$, calculated on the dried basis.

Category. Narcotic analgesic.

Dose. By subcutaneous, intramuscular or intravenous injection, the equivalent of 30 to 60 mg of pentazocine every 3 to 4 hours.

Description. A white or pale cream-coloured, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentazocine lactate RS* or with the reference spectrum of pentazocine lactate.

B. To 1 mg in a porcelain crucible add 0.5 ml of a solution of *sulphuric acid* containing 1 per cent w/v solution of *ammonium molybdate*; an intense blue colour is produced which changes to bluish green, green and finally, on standing, yellow.

C. Gives reaction A of lactates (2.3.1).

Tests

pH (2.4.24). 5.5 to 6.5, determined in a 1.0 per cent w/v solution.

Light absorption (2.4.7). Dissolve 0.1 g in 10 ml of *1 M hydrochloric acid* and add sufficient *water* to produce 100 ml; dilute 10 ml to 100 ml with *water.* Absorbance of the resulting solution at the maximum at about 278 nm, 0.50 to 0.54.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 94 volumes of *chloroform*, 3 volumes of *2-propylamine* and 3 volumes of *methanol.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform.*

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in *chloroform.*

Reference solution (c). A 0.005 per cent w/v solution of the substance under examination in *chloroform.*

Apply to the plate 25 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the

chromatogram obtained with reference solution (a); not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) and not more than four such spots are more intense than the spot in the chromatogram obtained with reference solution (c).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 16 hours.

Assay. Weigh accurately about 0.75 g of the substance under examination, dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03754 g of $C_{19}H_{27}NO,C_3H_6O_3$.

Storage. Store protected from light and moisture.

Pentazocine Injection

Pentazocine Lactate Injection

Pentazocine Injection is a sterile solution in Water for Injections of either Pentazocine Lactate or pentazocine lactate prepared by the interaction of Pentazocine and Lactic Acid.

Pentazocine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pentazocine, $C_{19}H_{27}NO$.

Usual strength. The equivalent of 30 mg of pentazocine per ml; the equivalent of 60 mg of pentazocine per ml.

Description. A clear, colourless or almost colourless liquid.

Identification

A. To a volume containing 90 mg of pentazocine add 5 ml of *0.1 M sodium hydroxide* and shake the resulting solution with 5 ml of *chloroform*. Wash the chloroform extract with 2 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the chloroform without applying heat and dry the oily residue at a temperature not exceeding 25° and at a pressure of 2 kPa for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentazocine RS* or with the reference spectrum of pentazocine (form B).

B. Dilute a volume containing 30 mg of pentazocine to 100 ml with *water*. To 10 ml add 10 ml of *1 M sodium hydroxide* and sufficient *water* to produce 100 ml. When examined in the range 230 nm to 360 nm (2.4.7), the solution shows absorption maxima, at about 238 nm and 298 nm.

C. To a volume containing 30 mg of pentazocine add 2 ml of *0.1 M sodium hydroxide*, extract with 2 ml of *chloroform* and evaporate 0.1 ml of the *chloroform* extract to dryness in a porcelain crucible. Add to the residue 0.5 ml of a 1 per cent w/v solution of *ammonium molybdate* in *sulphuric acid*; an intense blue colour is produced which changes to bluish green, green and finally, on standing, yellow.

Tests

pH (2.4.24). 4.0 to 5.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 94 volumes of *chloroform*, 3 volumes of *2-propylamine* and 3 volumes of *methanol*.

Test solution. Dilute a volume of the injection with sufficient *ethanol (95 per cent)* to produce a solution containing 2.0 per cent w/v solution of pentazocine.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *ethanol (95 per cent)*.

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with *ethanol (95 per cent)*.

Reference solution (c). Dilute 1 volume of the test solution to 400 volumes with *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Heat the plate at 105° for 15 minutes, allow to cool, expose to iodine vapour and re-examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a), not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) and not more than four such spots are more intense than the spot in the chromatogram obtained with reference solution (c).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

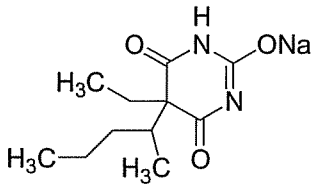
Assay. To an accurately measured volume containing about 0.15 g of pentazocine add sufficient *water* to produce 100.0 ml. To 5.0 ml add 1 ml of *1 M hydrochloric acid* and sufficient *water* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{19}H_{27}NO$, taking 69 as the specific absorbance at 278 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of pentazocine in a suitable dose-volume.

Pentobarbitone Sodium

Soluble Pentobarbitone; Pentobarbital Sodium



$C_{11}H_{17}N_2NaO_3$ Mol. Wt. 248.3

Pentobarbitone Sodium is sodium 5-ethyl-5-[(1*RS*)-1-methylbutyl]barbiturate.

Pentobarbitone Sodium contains not less than 99.0 per cent and not more than 101.5 per cent of $C_{11}H_{17}N_2NaO_3$, calculated on the dried basis.

Category. Hypnotic.

Dose. 100 to 200 mg.

Description. A white, crystalline powder or granules; hygroscopic.

Identification

A. To 10 ml of a 10 per cent w/v solution add 5 ml of *2 M acetic acid*; a white, crystalline precipitate is produced. Filter, wash the precipitate with *water* and dry at 105°. Determine the melting point of the dried precipitate (2.4.21). Mix equal parts of the dried precipitate and *phenobarbitone RS* and determine the melting point (2.4.21). The difference between the melting points (which are about 131°) is not greater than 2°.

B. Complies with the test for identification of barbiturates (2.3.1), using the dried precipitate obtained in test A for preparing the test solution.

C. To 10 mg add 10 mg of *vanillin* and 2 ml of *sulphuric acid*, mix and heat on a water-bath for 2 minutes; a reddish-brown colour is produced. Cool and add 5 ml of *ethanol*; the colour changes to violet and then blue.

D. Ignite 1 g; the residue gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Prepare freshly a 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A). Solution A is clear (2.4.1).

pH (2.4.24). 9.6 to 11.0, determined in solution A.

Related substances. Complies with the test for related substances in barbiturates (2.3.4), but applying 10 µl of each of the following solutions.

Test solution. A 2.0 per cent w/v solution of the substance under examination.

Reference solution. A 0.01 per cent w/v solution of the substance under examination.

Do not ignore any spot remaining on the line of application.

Free pentobarbitone. Not more than 3.5 per cent, determined by the following method. Weigh accurately about 2.0 g and dissolve in 75 ml of *dimethylformamide*, heating gently if necessary. Add 0.25 ml of a 1 per cent w/v solution of *thymol blue* in *dimethylformamide* and titrate with *0.1 M sodium methoxide* to a blue end-point. Carry out a blank titration.

1 ml of *0.1 M sodium methoxide* is equivalent to 0.02263 g of pentobarbitone.

Isomer. Dissolve 0.3 g in 5 ml of a 5 per cent w/v solution of *anhydrous sodium carbonate* and add 0.3 g of *4-nitrobenzyl bromide* dissolved in 10 ml of *ethanol (95 per cent)*. Heat under a reflux condenser for 30 minutes, cool to 25° scratch the side of the vessel with a glass rod if necessary to induce crystallisation and filter. Wash the residue with five quantities, each of 5 ml, of *water*. Transfer the residue as completely as possible to a small flask, add 25 ml of *ethanol (95 per cent)* and heat under a reflux condenser for 10 minutes; the solid dissolves completely. Cool to 25° and scratch the side of the flask with a glass rod to induce crystallisation. Filter, wash the residue with two quantities, each of 5 ml, of *water* and dry at 105° for 30 minutes. The dried residue melts at 136° to 148° (2.4.21).

Heavy metals (2.3.13). 0.67 g complies with the limit test for heavy metals, Method B (30 ppm).

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g, dissolve in 25 ml of a 12.75 per cent w/v solution of *silver nitrate* in *pyridine* and titrate with *0.1 M ethanolic sodium hydroxide* using 0.5 ml of *thymolphthalein solution* as indicator, until a pure blue colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02483 g of $C_{11}H_{17}N_2NaO_3$.

Storage. Store protected from moisture.

Pentobarbitone Tablets

Pentobarbitone Sodium Tablets; Pentobarbital Sodium Tablets

Pentobarbitone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pentobarbitone sodium, $C_{11}H_{17}N_2NaO_3$.

Usual strength. 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Pentobarbitone Sodium with 10 ml of a 10 per cent w/v solution of *pyridine* and filter. Add to the filtrate 1 ml of *cupric sulphate* with *pyridine solution* and set aside for 10 minutes; a reddish violet precipitate is produced.

B. Shake a quantity of the powdered tablets containing 0.1 g of Pentobarbitone Sodium with 10 ml of *water* and filter. To the filtrate add 2 ml of *hydrochloric acid*; a white precipitate is produced (distinction from pentobarbitone).

C. The residue obtained in the Assay melts at 127° to 130°(2.4.21).

D. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

Tests

Isomer. Dissolve a quantity of the powdered tablets containing 0.3 g of Pentobarbitone Sodium in 5 ml of a 5 per cent w/v solution of *anhydrous sodium carbonate* and add 0.3 g of *4-nitrobenzyl bromide* dissolved in 10 ml of *ethanol (95 per cent)*. Heat under a reflux condenser for 30 minutes, cool to 25° scratch the side of the vessel with a glass rod if necessary to induce crystallisation and filter. Wash the residue with five quantities, each of 5 ml, of *water*. Transfer the residue as completely as possible to a small flask, add 25 ml of *ethanol (95 per cent)* and heat under a reflux condenser for 10 minutes; filter the hot solution. Cool to 25° and scratch the side of the flask with a glass rod to induce crystallisation. Filter, wash the residue with two quantities, each of 5 ml, of *water* and dry at 105° for 30 minutes. The dried residue melts at 136° to 148° (2.4.21).

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Pentobarbitone Sodium, dissolve as completely as possible in 10 ml of a 2 per cent w/v solution of *sodium hydroxide*, saturate with *sodium chloride*, acidify with *hydrochloric acid* and extract with successive quantities, each of 15 ml, of *ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 2 ml, of *water* and extract the combined washings with 10 ml of *ether*. Add the ether to the main ether layer, filter and wash the filter with *ether*. Evaporate the solvent and dry the residue to constant weight at 105°.

1 g of residue is equivalent to 1.097 g of $C_{11}H_{17}N_2NaO_3$.

Storage. Store protected from moisture.

Pepsin

Pepsin is obtained from the gastric mucosa of pigs, cattle or sheep. It contains gastric proteinases that are active in an acid medium, pH 1 to 5. It may contain a suitable diluent such as Lactose.

Pepsin has an activity equivalent to its ability to digest not less than 3000 times its weight of coagulated egg albumin when determined by the method given under Assay.

Category. Proteolytic enzyme.

Dose. 300 mg to 1 g.

Description. A white or light buff-coloured, crystalline or amorphous powder or translucent scales; odour, faint and meaty but not rancid; hygroscopic.

Identification

A. Place 1 ml of *congo red fibrin* on a filter paper and wash until a colourless filtrate is obtained with a solution prepared by diluting 30 ml of *1 M hydrochloric acid* to 1000 ml with *water* and adjusting the pH 1.5 to 1.7. Perforate the filter paper and wash the congo red fibrin through it with 20 ml of the same hydrochloric acid solution. Shake this suspension before use. Dissolve about 10 mg of the substance under examination in 2 ml of the hydrochloric acid solution and adjust the pH 1.5 to 1.7. Place 4 ml of the congo red fibrin suspension in each of two tubes. To one of the tubes add 1 ml of the solution of the substance under examination and to the other tube add 1 ml of *water* (control solution). Mix the contents of each tube and place in a water-bath at 25° with gentle shaking for 15 minutes; the control solution is colourless and the solution of the substance under examination is violet blue.

B. The proteolytic activity of a solution in *water* is destroyed at once by boiling. It is destroyed by warming for 10 minutes at 40° at a pH of 8.0.

Tests

Microbial contamination (2.2.9). 1 g is free from *Escherichia coli* and 10 g is free from *Salmonellae.*

Sulphated ash (2.3.18). Not more than 5.0 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately 0.25 g, triturate with 1.0 g of *sodium chloride*, add slowly acidified *water* prepared by diluting 65 ml of *1 M hydrochloric acid* to 1000 ml with *water*, continue the trituration, dilute to 1000.0 ml with the acidified *water* and shake for 15 minutes. Prepare coagulated egg albumin by boiling fresh hen-eggs in *water* for 15 minutes, cooling rapidly to room temperature by immersion in cold *water*, separating

the whites and rubbing through a no. 44 sieve. Reject the first portion that passes through the sieve and triturate 15.0 g of freshly prepared coagulated egg albumin with 50 ml of the acidified *water* ensuring that the particles of egg albumin are thoroughly disintegrated, add a further 50 ml of the acidified *water* and keep in a water-bath at 51° ± 1° for 15 minutes. Add 20.0 ml of the prepared solution of the substance under examination and digest at 51° ± 1° for 4 hours, shaking at intervals of 15 minutes. Centrifuge and decant off most of the clear supernatant liquid, wash the remainder into a 10-ml graduated cylinder and allow to stand for 30 minutes. The volume of the undissolved albumin is not more than 2 ml.

Storage. Store protected from moisture.

Peritoneal Dialysis Solutions

Intraperitoneal Dialysis Fluids

Peritoneal Dialysis Solutions are sterile preparations for intraperitoneal use containing electrolytes with a concentration close to the electrolytic composition of plasma. They contain dextrose in varying concentrations and/or other suitable osmotic agents. They do not contain antioxidants such as metabisulphite salts.

Peritoneal Dialysis Solutions contain not less than 97.5 per cent and not more than 102.5 per cent of the stated amount of sodium, Na, not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of potassium, K, calcium, Ca, magnesium, Mg, chloride, Cl, acetate, $C_2H_3O_2$, lactate, $C_3H_5O_3$, sodium bicarbonate, $NaHCO_3$, bicarbonate, HCO_3 and dextrose, $C_6H_{12}O_6$.

Usual strengths: Several formulations are used. The concentrations of the components per litre of solution are usually in the following range.

	Concentration in mmol/litre
Sodium	125–150
Potassium	0–4.5
Calcium	0–2.5
Magnesium	0.25–1.5
Acetate and/or Lactate and/or Bicarbonate	30–60
Chloride	90–120
Dextrose	25–250

When bicarbonate is present, the solution of sodium bicarbonate is supplied in a separate container or a separate compartment and is added to the electrolyte solution immediately before use.

Unless otherwise justified and authorised, antioxidants such as metabisulphite salts are not added to the solutions.

Description. Clear, colourless or faintly straw-coloured solutions.

Identification

A. To 5 ml of the solution under examination, add 2 ml of *dilute sodium hydroxide solution* and 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling; a copious red precipitate is formed.

B. 20 ml gives reactions of chlorides, sodium salts, potassium salts and calcium salts (2.3.1).

C. To 5 ml add 1 ml of *hydrochloric acid* in a test-tube fitted with a stopper and a bent tube, heat and collect a few ml of the distillate. The distillate gives reaction C of acetates (2.3.1).

D. To 0.1 ml of *titan yellow solution* add 10 ml of *water*, 2 ml of the solution under examination and 1 ml of *1 M sodium hydroxide*; a pink colour is produced if magnesium salts are present.

E. Lactates and bicarbonates are identified together with the Assay for lactate and bicarbonate.

Tests

Appearance of solution. The solution under examination is clear (2.4.1), and not more intensely coloured than reference solution YS4 (2.4.1).

pH (2.4.24). 4.5 to 6.5. If the solution contains bicarbonate, 6.5 to 8.0.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 250.0 ml with *water* and measure the absorbance of the resulting solution (2.4.7) at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25.

Aluminium. Adjust the pH of 400 ml of the solution under examination to pH 6.0 and add 10 ml of *acetate buffer pH 6.0*. Extract the resulting solution with successive quantities of 20, 20 and 10 ml of a 0.5 per cent w/v solution of *8-hydroxyquinoline* in *chloroform* and dilute the combined extracts to 50.0 ml with *chloroform*. Use as the blank a mixture of 10 ml of *acetate buffer pH 6.0* and 100 ml of *water* treated in the same manner and as the standard solution a mixture of 2.0 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer pH 6.0* and 90 ml of *water* treated in the same manner. Measure the fluorescence of the test solution (I_1), of the standard solution (I_2) and of the blank (I_3), (2.4.5), using an excitation wavelength of 392 nm and a secondary filter with a transmission band centred at 518 nm, or a monochromator set to transmit at this wavelength. The fluorescence of the test solution ($I_1 - I_3$) is not greater than that of the standard solution ($I_2 - I_3$).

Particulate contamination (2.5.9). Carry out the test using 50 ml of the solution under examination.

The preparation meets the requirements of the test if it contains particles within the maximum limits shown below.

Particle size in µm (Equal to or larger than)	Maximum number of particles per ml
10	25
25	3

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per ml.

Pyrogens (2.2.8). Solutions for which a validated test for bacterial endotoxins cannot be carried out, comply with the test for pyrogens, injecting 10 ml of the solution per kg of the rabbit's body weight.

Sterility (2.2.11). Complies with the test for sterility.

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using sodium solution FP, or sodium solution AAS respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using potassium solution FP or potassium solution AAS respectively, suitably diluted with *water* for the standard solutions.

For calcium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 422.7 nm and using calcium solution FP or calcium solution AAS respectively, suitably diluted with *water* for the standard solutions.

For magnesium — To 50.0 ml add 50 ml of *water* and 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.005 M disodium edetate* using 50 mg of *eriochrome black T mixture* as indicator.

1 ml of *0.005 M disodium edetate* is equivalent to 0.1215 mg of Mg.

For total chloride — Dilute an accurately measured volume containing about 60 mg of chloride to 50.0 ml with *water*. Add 25.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For acetate (if present) — Determine by liquid chromatography (2.4.14).

Test solution. Dilute an accurately measured volume of the preparation under examination quantitatively with *water* to obtain a solution containing about 1.0 mg of acetate per ml.

Reference solution. Dissolve an accurately weighed quantity of *sodium acetate* in *water* to obtain a solution having a known concentration of about 0.12 per cent w/v of *sodium acetate*.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with a strong cation-exchange resin consisting of sulphonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form (7 µm),
- mobile phase: filtered and degassed *0.1 M sulphuric acid*,
- flow rate. 0.8 ml per minute,
- column temperature. 60°,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution and record the chromatograms. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test and standard solutions and record the chromatograms. Measure the responses for the major peak and calculate the content of acetate in the preparation under examination.

For lactate (if present) — Determine by liquid chromatography (2.4.14).

Test solution. Use the preparation under examination.

Reference solution(a). Dissolve an accurately weighed quantity of *sodium lactate RS* in *water* to obtain a solution having a known concentration of about 2 mg per ml.

Reference solution (b). Prepare a solution in *water* containing about 3 mg of *anhydrous sodium acetate* and 3 mg of *sodium lactate RS* per ml.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles 3 to 10 µm,
- mobile phase: a filtered and degassed solution in *water* containing about 1 ml of *formic acid* and 1 ml of *dicyclohexylamine* per litre,
- flow rate. 1 ml per minute,

- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject separately reference solutions (a) and (b), and record the chromatograms. The test is not valid unless the resolution between the peaks due to acetate and lactate is not less than 2.0, the tailing factor for the analyte peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and reference solution (a), and record the chromatograms. Measure the responses for the major peak and calculate the content of lactate in the preparation under examination.

For sodium bicarbonate — Titrate with *0.1 M hydrochloric acid* a volume of the preparation under examination containing about 0.1 g of *sodium bicarbonate*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M hydrochloric acid* is equivalent to 8.40 mg of $NaHCO_3$.

For lactate and bicarbonate — Determine by liquid chromatography (2.4.14).

Test solution. Use the preparation under examination.

Reference solutions. Dissolve accurately weighed quantities of lactates and bicarbonates in order to obtain solutions having concentrations of about 90 per cent, 100 per cent and 110 per cent of the claim in 100 ml of *water*.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with a cation-exchange resin (9 µm),
- column temperature. 85°,
- mobile phase: filtered and degassed *0.005 M sulphuric acid*,
- flow rate. 0.6 ml per minute,
- differential refractometer detector,
- injection volume. 20 µl.

Inject separately the test solution and the reference solutions in duplicate, and record the chromatograms in the prescribed conditions. The peaks elute in the following order, lactates, then bicarbonates. Determine the concentration of lactates and bicarbonates in the test solution by interpolating the peak area for lactate and the peak height for bicarbonate from the linear regression curve obtained with the solutions prepared as reference solutions.

For dextrose — Transfer a volume of the preparation under examination containing about 25 mg of Dextrose to a 250-ml conical flask with a ground-glass neck and add 25.0 ml of *cupri-citric solution*. Add a few grains of pumice, fit a reflux condenser, heat so that boiling occurs within 2 minutes and boil for exactly 10 minutes. Cool and add 3 g of *potassium iodide* dissolved in 3 ml of *water*. Carefully add, in small amounts, 25 ml of a 25 per cent w/w solution of *sulphuric acid*.

Titrate with *0.1 M sodium thiosulphate* using *starch solution* as indicator. Carry out a blank titration using 25 ml of *water*.

Calculate the content of anhydrous dextrose, $C_6H_{12}O_6$, from the following Table.

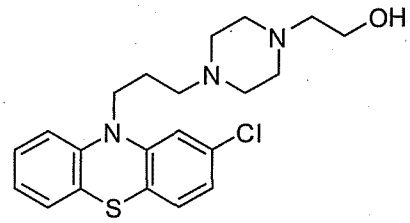
Volume of *0.1 M sodium thiosulphate* consumed (ml)	Anhydrous dextrose (mg)
8	19.8
9	22.4
10	25.0
11	27.6
12	30.3
13	33.0
14	35.7
15	38.5
16	41.3

Storage. Peritoneal Dialysis Solutions are supplied in rigid or semi-rigid plastic containers, in flexible plastic containers fitted with a special connecting device (these are generally filled to a volume below their nominal capacity and presented in closed protective envelopes) or in glass containers. Store at a temperature not exceeding 30°.

CAUTION — Exposure to temperatures below 4° may cause crystallisation and separation of solid particles rendering the preparation unsuitable for use.

Labelling. The label states (1) the formula of the solution for peritoneal dialysis, expressed in grams per litre and in millimoles per litre; (2) the total osmolar concentration in mOsmol per litre; (3) the nominal volume of the solution in the container; (4) that the solution is free from bacterial endotoxins, or where applicable, that it is apyrogenic; (5) that the solution is not to be used for intravenous infusion; (6) that any unused portion of the solution is to be discarded; (7) that the solution containing visible particles should not be used; (8) the storage conditions.

Perphenazine



$C_{21}H_{26}ClN_3OS$ Mol. Wt. 404.0

Perphenazine is 2-[4-[3-(2-chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol.

Perphenazine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{26}ClN_3OS$, calculated on the dried basis.

Category. Dopamine receptor antagonist; neuroleptic.

Description. A white or yellowish-white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *perphenazine RS* or with the reference spectrum of perphenazine.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*.

Mobile phase. A mixture of 2 volumes of *diethylamine* and 100 volumes of *light petroleum*, saturated with *phenoxyethanol* (add 6 volumes *phenoxyethanol* to 8 volumes of the above mixture until there is a persistent cloudiness after shaking, decant, and use the supernatant liquid, even if it is cloudy).

Test solution. Dissolve 20 mg of the substance under examination in 10 ml of *chloroform*.

Reference solution. A 0.2 per cent w/v solution of *perphenazine RS* in *chloroform*.

Impregnate the plate by placing it in a closed tank containing the necessary quantity of the impregnation mixture containing 2.5 per cent v/v of *phenoxyethanol* and 7.5 per cent v/v of *formamide* in *acetone* so that the plate dips about 5 mm beneath the surface of the liquid. When the impregnation mixture has risen at least 17 cm from the lower edge of the plate, remove the plate and use immediately. Carry out the chromatography in the same direction as the impregnation.

Apply to the plate 2 µl of each solution. Develop in the dark and allow the mobile phase to rise 15 cm. Expose the plate to ultraviolet light at 365 nm and examine after a few minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Dry the plate at 120° for 20 minutes, allow to cool and spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol (95 per cent)*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. When examined in the range 230 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima at 257 nm and 313 nm. The ratio of the absorbance measured at the maximum at 313 nm to that measured at the maximum at 257 nm is 0.120 to 0.128.

D. Melting range (2.4.21). 96° to 100°.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *methanol* is clear (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

NOTE—Prepare the solutions immediately before use.

Mobile phase. A mixture of 1 volume of *concentrated ammonia*, 14 volumes of *water* and 85 volumes of *butanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Reference solution. Dilute 0.5 ml of the test solution to 100 ml with *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 65° for 4 hours.

Assay. Weigh accurately about 0.15 g and dissolve in 25 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0202 g of $C_{21}H_{26}ClN_3OS$.

Calculate the content of $C_{21}H_{26}ClN_3OS$.

Storage. Store protected from light.

Perphenazine Tablets

Perphenazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of perphenazine, $C_{21}H_{26}ClN_3OS$.

Usual strengths. 2 mg; 4 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Perphenazine, add 10 ml of *water* and 2 ml of *1 M sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether layer with 5 ml of *water*, dry with *anhydrous sodium sulphate* and evaporate the *ether* to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6).

Compare the spectrum with that obtained with *perphenazine RS* or with the reference spectrum of perphenazine.

B. Extract a quantity of the powdered tablets containing 20 mg of Perphenazine with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dissolve the residue in 2 ml of *methanol*, pour into a 4.0 per cent w/v solution of *picric acid* in *methanol* at 50°, cool and allow to stand for 4 hours. After recrystallisation from *methanol*, the precipitate melts at about 248° (2.4.21) with decomposition.

C. To a quantity of the powdered tablets containing 5 mg of Perphenazine, add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. A red colour is produced.

Tests

Related substances. Comply with the test for Related substances in Phenothiazines (2.3.5) with the following modifications.

Use mobile phase (c) and applying to the plate 50 µl of each of the following freshly prepared solutions.

Test solution. Extract a quantity of the powdered tablets containing 20 mg of Perphenazine with 10 ml of *ethanol* (*95 per cent*) and filter.

Reference solution. Dilute 1 ml of the test solution to 200 ml with the *ethanol* (*95 per cent*).

Uniformity of content. *(For tablets containing 10 mg or less)*. Comply with the test stated under Tablets.

NOTE—Carry out the test protected from light.

Transfer one tablet to a 100-ml volumetric flask and add 5 ml of *water*, mix with the aid of ultrasound for 15 minutes or until the tablet has completely disintegrated, heat on a water-bath for 3 minutes, swirling continuously and cool. Add 50 ml of *ethanol* (*95 per cent*), mix with the aid of ultrasound for 2 minutes, shake for 5 minutes, dilute to 100 ml with *ethanol* (*95 per cent*) and filter through a glass microfibre filter paper. Dilute the filtrate with *ethanol* (*95 per cent*) to produce a solution containing 0.001 per cent w/v of Perphenazine. Record the second-derivative ultraviolet absorption spectra of the resulting solution in the range 210 to 290 nm (2.4.7). Measure the amplitude from the peak at 265 nm to the trough at 255 nm.

Calculate the content of $C_{21}H_{26}N_3OS$ in the tablet from the absorbance obtained by repeating the operation using *perphenazine RS* in place of the substance under examination.

Other tests. Comply with the tests stated under Tablets.

Assay. *For tablets containing more than 10 mg.*

NOTE—Carry out the test protected from light.

Shake 10 whole tablets with 50 ml of *water* and mix with the aid of ultrasound for 15 minutes or until the tablets have completely disintegrated, heat on a water-bath for 3 minutes, swirling continuously, cool, add 400 ml of *ethanol* (*95 per cent*), mix with the aid of ultrasound for 2 minutes, shake for 5 minutes, dilute to 500 ml with *ethanol* (*95 per cent*) and filter through a glass microfibre filter paper. Dilute the filtrate with *ethanol* (*95 per cent*) to produce a solution containing 0.001 per cent w/v of Perphenazine. Record second-derivative ultraviolet absorption spectra of the solutions in the range 210 to 290 nm (2.4.7). Measure the amplitude from the peak at 265 nm to the trough at 255 nm.

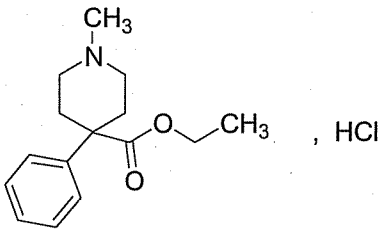
Calculate the content of $C_{21}H_{26}N_3OS$ in the tablet from the absorbance obtained by repeating the operation using *perphenazine RS* in place of the substance under examination.

For tablets containing 10 mg or less.

Use the average of the 10 individual results obtained in the test for Uniformity of content.

Pethidine Hydrochloride

Meperidine Hydrochloride



$C_{15}H_{21}NO_2,HCl$ Mol. Wt. 283.8

Pethidine Hydrochloride is ethyl 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride.

Pethidine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{21}NO_2,HCl$, calculated on the dried basis.

Category. Narcotic analgesic.

Dose. Orally, 50 to 150 mg every 4 hours; by subcutaneous or intramuscular injection, 25 to 100 mg every 4 hours; by slow intravenous injection, 25 to 50 mg every 4 hours.

Description. Colourless crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pethidine*

hydrochloride RS or with the reference spectrum of pethidine hydrochloride.

B. To 5 ml of a 1 per cent w/v solution add a few drops of *potassium mercuri-iodide solution*; a cream-coloured precipitate is produced.

C. Dissolve 5 mg in 0.5 ml of *water* and add 0.1 ml of *formaldehyde solution* and 2 ml of *sulphuric acid*; an orange-red colour is produced.

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of a 2.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is yellow. Add 0.3 ml of *0.01 M hydrochloric acid*; the solution is red.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water*.

Test solution (a). Dissolve about 0.1 g of the substance under examination in 25.0 ml of the solvent mixture.

Test solution (b). Dissolve about 0.125 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dilute 0.5 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (b). Dissolve 12.5 mg of *1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine* in 10.0 ml of the solvent mixture. Dilute 1.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (c). Dilute 5.0 ml of reference solution (b) and 1.0 ml of reference solution (c) to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.0 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS2),
- mobile phase: A. a mixture of equal volumes of 4.2 per cent w/v solution of *sodium perchlorate* and 1.2 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 2.0 with *triethylamine*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 15	80 →75	20 →25
15 – 31	75 →55	25 →45
31 – 40	55	45
40 – 41	55 →80	45 →20
41 – 50	80	20

Inject reference solution (c). The test is not valid unless the signal-to-noise ratio for the first peak is not less than 10 and peak-to-valley ratio where H_p is height above the baseline, and H_v is height above the baseline of the lowest point of the curve separating this peak from the peak due to impurity A is not less than 4.0. The relative retention time with reference to pethidine for pethidine impurity B is about 0.66 and for pethidine impurity A is about 0.68.

Inject test solution (a), (b), reference solution (a) and (c). In the chromatogram obtained with test solution (b), the area of the peak due to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (pethidine impurity B) is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (10 ppm). In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*, add 12 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02838 g of $C_{15}H_{21}NO_2,HCl$.

Storage. Store protected from light and moisture.

Pethidine Injection

Pethidine Hydrochloride Injection; Meperidine Hydrochloride Injection

Pethidine Injection is a sterile solution of Pethidine Hydrochloride in Water for Injections.

Pethidine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of pethidine hydrochloride, $C_{15}H_{21}NO_2,HCl$.

Usual strength. 50 mg per ml.

Identification

A. To a volume containing 50 mg of Pethidine Hydrochloride add sufficient *1 M sodium hydroxide* to make strongly alkaline to litmus paper and extract with two quantities, each of 10 ml, of *chloroform*. Wash the combined extracts with 5 ml of *water*, dry over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Remove the last traces of chloroform by drying the residual oil at 60° at a pressure not exceeding 0.7 kPa.

On the oily residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pethidine hydrochloride RS* or with the reference spectrum of pethidine.

B. To 0.5 ml add 0.1 ml of *formaldehyde solution* and 2 ml of *sulphuric acid*; an orange-red colour is produced.

C. Gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water*.

Test solution. Dilute a volume of the injection containing 0.1 g of Pethidine Hydrochloride with 25.0 ml of the solvent mixture.

Reference solution. Dilute 0.5 ml of the test solution to 100.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Kromasil C18),
- mobile phase: A. a mixture of equal volumes of 4.2 per cent w/v solution of *sodium perchlorate* and 1.2 per cent v/v solution of *orthophosphoric acid*, adjusted to pH 2.0 with *triethylamine*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 15	80 → 75	20 → 25
15 – 31	75 → 55	25 → 45
31 – 40	55	45
40 – 41	55 → 80	45 → 20
41 – 50	80	20

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing about 0.1 g of Pethidine Hydrochloride with 100.0 ml of the *water*. Dilute 3.0 ml of this solution to 25.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *pethidine hydrochloride RS*. Dilute 3.0 ml of this solution to 25.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS1),
- column temperature. 40°,
- mobile phase: a mixture of 110 volumes of *acetonitrile* and 90 volumes of a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*, add 10 ml *triethylamine*, adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoritical plates is not less than 8000.

Injet the test solution and the reference solution.

Calculate the content of $C_{15}H_{21}NO_2$,HCl in the injection.

Storage. Store protected from light.

Pethidine Tablets

Pethidine Hydrochloride Tablets; Meperidine Hydrochloride Tablets

Pethidine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pethidine hydrochloride, $C_{15}H_{21}NO_2$,HCl.

Usual strengths. 25 mg; 50 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Pethidine Hydrochloride with 20 ml of *chloroform*, filter, evaporate the filtrate to dryness and dry the residue at a pressure of 2 kPa.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pethidine hydrochloride RS* or with the reference spectrum of pethidine hydrochloride.

B. Shake a quantity of the powdered tablets containing 0.2 g of Pethidine Hydrochloride with 20 ml of *water* and filter. To 5 ml of the filtrate add 10 ml of picric acid solution. The crystals so obtained, after washing with *water* and drying, melt at about 190° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*.

Mobile phase. The upper layer obtained by shaking together 100 volumes of *light petroleum (50° to 70°)*, 8 volumes of *2-phenoxyethanol* and 1 volume of *diethylamine*.

Test solution. The upper layer obtained by shaking a quantity of the powdered tablets containing 0.1 g of Pethidine Hydrochloride with 5 ml of *water*, filtering, shaking the filtrate with 0.5 ml of *5 M sodium hydroxide* and 2 ml of *ether* and allowing the layers to separate.

Reference solution. Dilute 0.5 ml of the test solution to 50 ml with *ether*.

Impregnate the dry plate by placing it in a closed tank containing a mixture of 90 volumes of *acetone* and 10 volumes of *2-phenoxyethanol* so that the plate dips about 5 mm beneath the surface of the liquid, allowing the impregnating solvent to ascend at least 15 cm, removing the plate from the tank and drying in a current of air. Use immediately, with the flow of the mobile phase in the same direction as the impregnation.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air for 10 minutes, return the plate to the tank and repeat the development. Remove the plate, allow it to dry in air for 10 minutes and spray with a 0.2 per cent w/v solution of *2,7-dichlorofluorescein* in *methanol*. Allow to stand for 5 minutes and spray with *water* until the background is white to pale yellow. Examine in daylight. The chromatograms show red or orange spots. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Examine without delay in ultraviolet light at 365 nm. The chromatograms show spots with intense yellow fluorescence. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

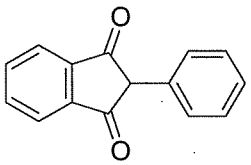
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Pethidine Hydrochloride in 40 ml of *water*, add 2 ml of *5 M sodium hydroxide* and extract immediately with successive quantities of 25, 10 and 10 ml of *chloroform*. Wash each extract with the same 15 ml of *water* and filter into a dry flask. Combine the extracts (which should be clear and free from droplets of *water*). Titrate with *0.05 M perchloric acid*, using 0.15 ml of *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.05 M perchloric acid* is equivalent to 0.01419 g of $C_{15}H_{21}NO_2,HCl$.

Storage. Store protected from light and moisture.

Phenindione



$C_{15}H_{10}O_2$ Mol. Wt. 222.2

Phenindione is 2-phenylindane-1,3-dione.

Phenindione contains not less than 98.0 per cent and not more than 100.5 per cent of $C_{15}H_{10}O_2$, calculated on the dried basis.

Category. Anticoagulant.

Dose. Initially, 200 to 300 mg; subsequently 25 to 100 mg daily, in accordance with the needs of the patient.

Description. Soft, white or creamy-white crystals; almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenindione RS*.

B. Dissolve 0.1 g in 30 ml of *ethanol (95 per cent)* with the aid of heat, cool and add sufficient *ethanol (95 per cent)* to produce 50 ml. Dilute 10 ml of this solution to 250 ml with *0.1 M sodium hydroxide* and further dilute 5 ml to 100 ml with *0.1 M sodium hydroxide*. When examined in the range 230 nm and 360 nm (2.4.7), the solution shows absorption maxima at

about 278 nm and at about 330 nm; absorbance at about 278 nm, about 0.55 and at about 330 nm, about 0.16.

C. To 1 g add 50 ml of *ethanol (95 per cent)* and 0.5 ml of *aniline*, heat gently under a reflux condenser for 3 hours, cool in ice and filter. The residue, after washing with 2 ml of *ethanol (95 per cent)* and recrystallisation from *chloroform*, melts at about 225° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A 0.02 per cent w/v solution of *butylated hydroxytoluene* in a mixture of 80 volumes of *toluene*, 20 volumes of *ethyl acetate* and 4 volumes of *glacial acetic acid.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *dichloromethane.*

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *dichloromethane.*

Reference solution (b). A 0.005 per cent w/v solution of the substance under examination in *dichloromethane.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 4 cm. Dry the plate in warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 105° for 2 hours.

Assay. Weigh accurately about 0.3 g, add 50 ml of *ethanol (95 per cent)* and warm until solution is effected. Cool to room temperature, add 10 ml of a 10 per cent v/v solution of *bromine* in *ethanol (95 per cent)* and allow to stand for 10 minutes, shaking occasionally. Add 1 g of *2-naphthol* and shake until the colour of the bromine is discharged. Remove any vapour of bromine in the flask with a current of air, add 50 ml of *water* and 10 ml of *dilute potassium iodide solution* and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution* as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01111 g of $C_{15}H_{10}O_2$.

Storage. Store protected from moisture.

Phenindione Tablets

Phenindione Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of phenindione, $C_{15}H_{10}O_2$.

Usual strength. 50 mg.

Identification

Shake a quantity of the powdered tablets containing 0.2 g of Phenindione with 50 ml of *chloroform*, filter and evaporate the filtrate to dryness. Recrystallise the residue from *ethanol (95 per cent).* The crystals complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenindione RS.*

B. Dissolve 0.1 g in 30 ml of *ethanol (95 per cent)* with the aid of heat, cool and add sufficient *ethanol (95 per cent)* to produce 50 ml. Dilute 10 ml of this solution to 250 ml with *0.1 M sodium hydroxide* and further dilute 5 ml to 100 ml with *0.1 M sodium hydroxide.* When examined in the range 230 nm to 360 nm (2.4.7), the solution shows absorption maxima at about 278 nm and 330 nm; absorbance at about 278. about 0.55 and at about 330 nm, about 0.16.

C. To 50 mg add 1 ml of *sulphuric acid*; a deep blue to violet solution is produced. On dilution with *water* the solution becomes colourless and a white precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A 0.02 per cent w/v solution of *butylated hydroxytoluene* in a mixture of 80 volumes of *toluene*, 20 volumes of *ethyl acetate* and 4 volumes of *glacial acetic acid.*

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Phenindione with 15 ml of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 5 ml of *dichloromethane.*

Reference solution (a). Dilute 1 volume of the test solution to 50 volumes with *dichloromethane.*

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with *dichloromethane.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 4 cm. Dry the plate in warm air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is

more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. *(For tablets containing 50 mg or less)* — Comply with the test stated under Tablets.

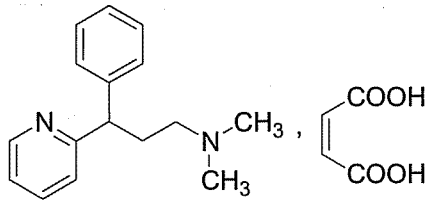
Place one tablet in 50 ml of *0.1 M sodium hydroxide*, dissolve completely by shaking gently, add a further 100 ml of *0.1 M sodium hydroxide* and shake for 1 hour. Dilute to 250.0 ml with *0.1 M sodium hydroxide*, filter and dilute a portion of the filtrate with sufficient *0.1 M sodium hydroxide* to produce a solution containing 4 µg of Phenindione per ml. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{15}H_{10}O_2$ taking 1310 as the specific absorbance at 278 nm.

Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 Tablets. Weigh accurately a quantity of the powder containing about 50 mg of Phenindione and shake with 150 ml of *0.1 M sodium hydroxide* for 1 hour, add sufficient *0.1 M sodium hydroxide* to produce 250.0 ml, filter and dilute 5.0 ml of the filtrate to 250.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{15}H_{10}O_2$ taking 1310 as the specific absorbance at 278 nm.

Storage. Store protected from moisture.

Pheniramine Maleate



$C_{16}H_{20}N_2,C_4H_4O_4$ Mol. Wt. 356.4

Pheniramine Maleate is (3*RS*)-*N*,*N*-dimethyl-3-phenyl-3-(pyridin-2-yl)propan-1-amine hydrogen maleate.

Pheniramine Maleate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{20}N_2,C_4H_4O_4$, calculated on the dried basis.

Category. Antihistaminic.

Dose. Orally, 25 to 50 mg daily, in divided doses; by intramuscular or slow intravenous injection, 22.5 to 45 mg daily, in divided doses.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pheniramine maleate RS* or with the reference spectrum of pheniramine maleate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M hydrochloric acid* shows an inflection at about 262 nm; absorbance at about 265 nm, about 0.42.

C. Dissolve 0.25 g in 5 ml of *water*, add 2 ml of *strong ammonia solution* and extract with three quantities, each of 5 ml, of *chloroform*. Evaporate the aqueous extract to dryness, add 0.2 ml of *1 M sulphuric acid* and 5 ml of *water*, extract with four quantities, each of 25 ml, of *ether* and evaporate the combined ether extracts to dryness in a current of warm air. To the residue add 50 mg of *resorcinol* and 1 ml of *sulphuric acid*, heat in a water-bath for 2 minutes, shake well, heat in a water-bath for a further 30 minutes and cool in ice. Carefully add 5 ml of *water*; a yellow colour is produced. To 2 ml of the solution add 3 ml of a 50 per cent w/v solution of *ammonium acetate*, previously cooled in ice; a pink colour is produced which persists for at least 10 minutes in the cooled solution.

Tests

pH (2.4.24). 4.5 to 5.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.004 per cent w/v solution of the substance under examination in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). Dissolve 1.0 g in 10 ml of *water* and add 2 ml of *acetic acid* and sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.4 g, dissolve in 20 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1- naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01782 g of $C_{16}H_{20}N_2,C_4H_4O_4$.

Storage. Store protected from light and moisture.

Pheniramine Injection

Pheniramine Maleate Injection

Pheniramine Injection is a sterile solution of Pheniramine Maleate in Water for Injections.

Pheniramine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pheniramine maleate, $C_{16}H_{20}N_2, C_4H_4O_4$.

Usual strength. 22.75 mg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

Test solution. Evaporate an appropriate volume of the injection to dryness in a current of nitrogen using the minimum amount of heat, dissolve the residue in sufficient *chloroform* to produce a solution containing 2.0 per cent w/v solution of Pheniramine Maleate and centrifuge.

Reference solution. A 2.0 per cent w/v solution of *pheniramine maleate RS* in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The two principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution. Spray the plate with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 5.5.

Related substances. Determine by the method described under the Identification test using as the reference solution a solution prepared by diluting 1 volume of the test solution to 500 volumes with *chloroform*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 0.11 g of Pheniramine Maleate add sufficient *water* to produce 50.0 ml and mix well. To 20.0 ml add sufficient *1 M sodium hydroxide* to make the solution just alkaline to *litmus paper*, add 2 ml in excess and extract with two quantities, each of 50 ml, of *ether*. Wash each ether extract in succession with 20, 20 and 5 ml of *0.1 M hydrochloric acid*, dilute the combined extracts to 100.0 ml with *0.1 M hydrochloric acid* and mix. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_{16}H_{20}N_2$, $C_4H_4O_4$ taking 210 as the specific absorbance at 265 nm.

Storage. Store protected from light.

Pheniramine Tablets

Pheniramine Maleate Tablets

Pheniramine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pheniramine maleate, $C_{16}H_{20}N_2, C_4H_4O_4$.

Usual strengths. 12.5 mg; 25 mg; 50 mg.

Identification

Boil a quantity of the powdered tablets containing about 0.5 g of Pheniramine Maleate with 150 ml of *acetone* under a reflux condenser for about 45 minutes. Filter and evaporate the filtrate to dryness on a water-bath. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pheniramine maleate RS* or with the reference spectrum of pheniramine maleate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M hydrochloric acid* shows an inflection at about 262 nm; absorbance at about 265 nm, about 0.42.

C. Dissolve 0.25 g in 5 ml of *water*, add 2 ml of *strong ammonia solution* and extract with three quantities, each of 5 ml, of *chloroform*. Evaporate the aqueous extract to dryness, add 0.2 ml of *1 M sulphuric acid* and 5 ml of *water*, extract with four quantities, each of 25 ml, of *ether* and evaporate the combined ether extracts to dryness in a current of warm air. To the residue add 50 mg of *resorcinol* and 1 ml of *sulphuric acid*, heat in a water-bath for 2 minutes, shake well, heat in a water-bath for a further 30 minutes and cool in ice. Carefully add 5 ml of *water*; a yellow colour is produced. To 2 ml of the solution add 3 ml of a 50 per cent w/v solution of *ammonium acetate*, previously cooled in ice; a pink colour is produced which persists for at least 10 minutes in the cooled solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 40 volumes of *chloroform* and 10 volumes of *diethylamine*.

Test solution. Shake a quantity of the powdered tablets containing 20 mg of Pheniramine Maleate with 10 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Reference solution (b). Dilute 1 volume of reference solution (a) to 20 volumes with *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

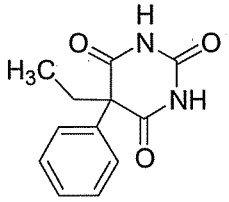
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 45 mg of Pheniramine Maleate, shake with 20 ml of *0.1 M hydrochloric acid*, centrifuge and transfer the supernatant liquid to a 100-ml volumetric flask. Repeat the extraction with three further quantities, each of 20 ml, of *0.1 M hydrochloric acid*. Combine the extracts and add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml. Mix and dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid*; measure the absorbance of the resulting solution at the maximum at about 265 nm (2.4.7), using *0.1 M hydrochloric acid* as the blank. Calculate the content of $C_{16}H_{20}N_2,C_4H_4O_4$ taking 210 as the specific absorbance at 265 nm.

Storage. Store protected from light and moisture.

Phenobarbitone

Phenobarbital



$C_{12}H_{12}N_2O_3$ Mol. Wt. 232.2

Phenobarbitone is 5-ethyl-5-phenylbarbituric acid.

Phenobarbitone contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{12}N_2O_3$, calculated on the dried basis.

Category. Sedative; anticonvulsant.

Dose. 60 to 300 mg at night.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests C, D and E may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenobarbitone RS* or with the reference spectrum of phenobarbitone.

B. Determine the melting point (2.4.21) of the substance under examination and of a mixture of equal quantities of the substance under examination and *phenobarbitone RS*. The difference between the melting points, which are about 175°, is not greater than 2°.

C. Complies with the test for identification of barbiturates (2.3.2).

D. Dissolve about 20 mg in 5 ml of *ethanol*, add a drop of *cobalt chloride solution* and a drop of *dilute ammonia solution*; a violet colour is produced.

E. Gives the reaction of non-nitrogen substituted barbiturates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in a mixture of 20 volumes of *2 M sodium hydroxide* and 30 volumes of *water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Acidity. Mix 1.0 g with 50 ml of *water*, boil for 2 minutes, allow to cool, filter and adjust the volume to 50 ml. To 10 ml of the

filtrate add 0.15 ml of *methyl red solution*; not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution from orange-yellow to pure yellow.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *concentrated ammonia*, 15 volumes of *ethanol (95 per cent)* and 80 volumes of *chloroform*.

Test solution. Dissolve about 1.0 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution. Dilute 0.5 ml of the test solution to 100 ml with *ethanol (95 per cent)*.

Apply 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution* (20 per cent w/v). Heat at 105° for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent w/w, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 0.1 g, dissolve in 5 ml of *pyridine*, add 0.25 ml of *thymolphthalein solution* and 10 ml of *silver nitrate-pyridine reagent* and titrate with *0.1 M ethanolic sodium hydroxide* until a pure blue colour is obtained. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.01161 g of $C_{12}H_{12}N_2O_3$.

Storage. Store protected from moisture.

Phenobarbitone Tablets

Phenobarbital Tablets

Phenobarbitone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of phenobarbitone, $C_{12}H_{12}N_2O_3$.

Usual strengths. 15 mg; 30 mg; 60 mg; 100 mg.

Identification

Extract a quantity of the powdered tablets containing about 0.5 g of Phenobarbitone with 50 ml of *ether*, filter through *anhydrous sodium sulphate* and evaporate the ether to dryness on a water-bath. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenobarbitone RS* or with the reference spectrum of phenobarbitone.

B. Dissolve 50 mg in 2 ml of a 0.2 per cent w/v solution of *cobaltous acetate* in *methanol*, warm, add 50 mg of powdered borax and heat to boiling; a bluish violet colour is produced.

Tests

Disintegration (2.5.1). 30 minutes

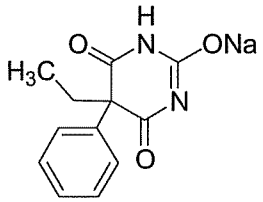
Other tests. Complies with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Phenobarbitone and extract in a continuous extraction apparatus (2.1.8) with *ether* until complete extraction is effected. Remove the ether and dry the residue, which is $C_{12}H_{12}N_2O_3$, to constant weight at 105°.

Storage. Store protected from moisture.

Phenobarbitone Sodium

Phenobarbital Sodium; Soluble Phenobarbitone; Soluble Phenobarbital



$C_{12}H_{11}N_2NaO_3$ Mol. Wt. 254.2

Phenobarbitone Sodium is sodium 5-ethyl-5-phenylbarbiturate.

Phenobarbitone Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{11}N_2NaO_3$, calculated on the dried basis.

Category. Sedative; anticonvulsant.

Dose. Orally, 60 to 300 mg at night; by intramuscular or intravenous injection, 50 to 200 mg, repeated after 6 hours if necessary; maximum 600 mg daily.

Description. A white powder or crystalline granules or flaky crystals; hygroscopic.

Identification

Test A may be omitted if tests B, C, D, E and F are carried out. Tests C, D and E may be omitted if tests A, B and F are carried out.

A. Dissolve 0.2 g in 20 ml of *ethanol (50 per cent)*, acidify with *dilute hydrochloric acid* and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of *water*, dry over *anhydrous sodium sulphate* and filter. Evaporate the filtrate to dryness and dry the residue at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenobarbitone RS* or with the reference spectrum of phenobarbitone.

B. Determine the melting point (2.4.21), of the residue obtained in test A and of a mixture of equal quantities of the residue and *phenobarbitone RS*. The difference between the melting points, which are about 175°, is not greater than 2°.

C. Complies with the test for identification of barbiturates (2.3.2), but using the following solutions.

Test solution. A 0.1 per cent w/v solution of the substance under examination in *ethanol (50 per cent)*.

Reference solution. A 0.09 per cent w/v solution of *phenobarbitone RS* in *ethanol (50 per cent)*.

D. 1 g dissolves completely in 20 ml of *ethanol (90 per cent)* (distinction from barbitone sodium).

E. Gives the reaction of non-nitrogen substituted barbiturates (2.3.1).

F. Ignite about 0.1 g; the residue gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *ethanol (50 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). Not more than 10.2, determined in a 10.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *concentrated ammonia*, 15 volumes of *ethanol (95 per cent)* and 80 volumes of *chloroform*.

Test solution. Dissolve about 1.0 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution. Dilute 0.5 ml of the test solution to 100 ml with *ethanol (95 per cent)*.

Apply 20 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray with *diphenylcarbazone mercuric reagent*. Allow the plate to dry in air and spray with freshly prepared *alcoholic potassium hydroxide solution* (20 per cent w/v). Heat at 105 ° for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution, is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 0.5 g by drying in an oven at 150° for 4 hours.

Assay. Weigh accurately about 0.15 g, dissolve in 2 ml of *water* and add 8 ml of *0.05 M sulphuric acid*. Heat to boiling and cool. Add 30 ml of *methanol* and shake until dissolution is complete. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). After the first inflection, stop the addition of the *sodium hydroxide*, add 10 ml of *pyridine*, mix and continue the titration until the second inflection is reached. The difference between the volumes represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02542 g of $C_{12}H_{11}N_2NaO_3$.

Storage. Store protected from moisture.

Phenobarbitone Injection

Phenobarbital Sodium Injection; Phenobarbitone Sodium Injection; Soluble Phenobarbitone Injection

Phenobarbitone Injection is a sterile solution of Phenobarbitone Sodium in a mixture of nine volumes of Propylene Glycol and one volume of Water for Injections.

Phenobarbitone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of phenobarbitone sodium, $C_{12}H_{11}N_2NaO_3$.

Usual strengths. 30 mg, 60 mg, 65 mg and 130 mg per ml.

Identification

To a volume containing 1 g of Phenobarbitone Sodium add 15 ml of *water* if necessary, make slightly acidic with *1 M sulphuric acid* and filter. The residue, after washing with *water* and drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenobarbitone RS* or with the reference spectrum of phenobarbitone.

B. Dissolve 50 mg in 2 ml of a 0.2 per cent w/v solution of *cobalt acetate* in *methanol*, warm, add 50 mg of powdered *borax* and heat to boiling; a bluish violet colour is produced.

Tests

pH (2.4.24). 10.0 to 11.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Weigh accurately about 2.0 g, add 30 ml of *water* and 3 g of *sodium carbonate*, stir to dissolve and titrate with *0.1 M silver nitrate* until a distinct turbidity is observed when viewed against a black background, the solution being stirred vigorously throughout the titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.02542 g of $C_{12}H_{11}N_2NaO_3$.

Determine the weight per ml of the injection (2.4.29) and calculate the percentage weight in volume of $C_{12}H_{11}N_2NaO_3$.

Storage. Store in single dose containers.

Labelling. The label states that the injection should not be used if the solution is discoloured or if it contains a precipitate.

Phenobarbitone Sodium Tablets

Phenobarbital Sodium Tablets; Soluble Phenobarbitone Tablets; Soluble Phenobarbital Tablets

Phenobarbitone Sodium Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of phenobarbitone sodium, $C_{12}H_{11}N_2NaO_3$.

Usual strengths. 30 mg; 60 mg.

Identification

A. Heat 0.1 g of the residue obtained in Assay on a water-bath with 15 ml of *ethanol (25 per cent)* until dissolved, filter while hot and allow to cool. Filter through a sintered-glass crucible, wash with a small quantity of *ethanol (25 per cent)* and dry at 105°. Heat in a sealed tube at 105° for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenobarbitone RS* or with the reference spectrum of phenobarbitone.

B. The residue obtained in test A melts at about 175° (2.4.21).

C. Dissolve 50 mg of the residue obtained in Assay in 2 ml of a 0.2 per cent w/v solution of *cobaltous acetate* in *methanol*, warm, add 50 mg of powdered *borax* and heat to boiling; a bluish violet colour is produced.

D. Triturate a quantity of the powdered tablets containing 0.2 g of Phenobarbitone Sodium with 5 ml of *water* and filter; the filtrate is alkaline to *litmus solution* and yields a white precipitate on the addition of *dilute hydrochloric acid*.

E. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

Tests

Other tests. Complies with the tests stated under Tablets.

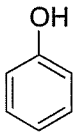
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Phenobarbitone Sodium, dissolve as completely as possible in 10 ml of a 2 per cent w/v solution of *sodium hydroxide*, saturate with *sodium chloride*, acidify with *hydrochloric acid* and extract with successive quantities, each of 15 ml, of *ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 2 ml, of *water* and extract the combined washings with 10 ml of *ether*. Add the ether to the main ether layer and dry the residue to constant weight at 105°.

1 g of the residue is equivalent to 1.095 g of $C_{12}H_{11}N_2NaO_3$.

Storage. Store protected from moisture.

Phenol

Carbolic acid



C_6H_6O Mol. Wt. 94.1

Phenol contains not less than 99.0 per cent and not more than 100.5 per cent of C_6H_6O.

Category. Antiseptic; antipruritic; pharmaceutical aid (antimicrobial preservative).

Description. Colourless or faintly pink or faintly yellowish crystals or crystalline masses; odour, characteristic; deliquescent.

Identification

A. Dissolve 0.5 g in 2 ml of *strong ammonia solution*. Dilute this solution to about 100 ml with *water* and to 2 ml of the resulting solution, add 0.05 ml of 3 per cent w/v solution of *sodium hypochlorite*; a blue colour develops which becomes progressively more intense.

B. Dissolve 1.0 g in sufficient *water* to produce 15 ml (solution A) and to 1 ml, add 10 ml of *water* and 0.1 ml of *ferric chloride*

solution; a violet colour is produced which disappears on the addition of 5 ml of *2-propanol*.

C. To 1 ml of solution A add 10 ml of *water* and 1 ml of *bromine solution*; a pale yellow precipitate is produced.

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

Acidity. To 2 ml of solution A add 0.05 ml of *methyl orange solution*; the solution is yellow.

Freezing point (2.4.11). Not less than 39.5°.

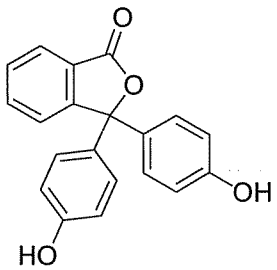
Non-volatile matter. Not more than 0.05 per cent, when 5.0 g is volatilised on a water-bath and dried to constant weight at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in sufficient *water* to produce 250.0 ml. Transfer 25.0 ml to a ground-glass-stoppered flask, add 50.0 ml of *0.05 M bromine* and 5 ml of *hydrochloric acid*, stopper, allow to stand for 30 minutes, swirling occasionally, and allow to stand for a further 15 minutes. Add 5 ml of a 20 per cent w/v solution of *potassium iodide*, shake and titrate with *0.1 M sodium thiosulphate* until a faint yellow colour remains. Add 0.5 ml of *starch solution* and 10 ml of *chloroform* and continue the titration with vigorous shaking. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of bromine required.

1 ml of *0.05 M bromine* is equivalent to 0.001569 g of C_6H_6O.

Storage. Store protected from light and moisture.

Phenolphthalein



$C_{20}H_{14}O_4$ Mol. Wt. 318.3

Phenolphthalein is 3,3-bis(4-hydroxyphenyl)phthalide.

Phenolphthalein contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{14}O_4$, calculated on the dried basis.

Category. Laxative.

Dose. 30 to 200 mg.

Description. A white or yellowish white, crystalline or amorphous powder; odourless or almost odourless.

Identification

A. Dissolves in dilute solutions of alkali hydroxides and in hot solutions of alkali carbonates forming a red solution which is decolorised by dilute acids.

B. Dissolve 25 mg in 100 ml of *ethanol (95 per cent)* (solution A). To 2.0 ml of solution A add 5.0 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *ethanol (95 per cent)* (solution A_1). To 10.0 ml of solution A add 5.0 ml of *1 M hydrochloric acid* and dilute to 50.0 ml with *ethanol (95 per cent)* (solution A_2). To 2.0 ml of solution A add 5.0 ml of *1 M sodium hydroxide* and dilute to 50.0 ml with *ethanol (95 per cent)* (solution B). Examined between 220 nm and 250 nm (2.4.7), solution A_1 shows an absorption maximum at 229 nm. The specific absorbance at the maximum at 229 nm is 922 to 1018. Examined between 250 nm and 300 nm, solution A_2 shows an absorption maximum at 276 nm. The specific absorbance at the maximum at 276 nm is 142 to 158. Examined between 230 nm and 270 nm, solution B shows an absorption maximum at 249 nm. The specific absorbance at the maximum at 249 nm is 744 to 822.

Tests

Solution A. To 2.0 g add 40 ml of *distilled water*, boil, cool and filter.

Appearance of solution. A 4 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Acidity or alkalinity. To 10 ml of solution A, add 0.15 ml of *bromothymol blue solution*. Add 0.05 ml of *0.01 M hydrochloric acid*, the solution is yellow. Add 0.1 ml of *0.01 M sodium hydroxide*, the solution is blue.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 50 volumes of *acetone* and 50 volumes of *dichloromethane*.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml *ethanol (95 per cent)*.

Reference solution (a). Dilute 1 ml of the test solution to 10 ml with *ethanol (95 per cent)*. Dilute 5 ml of this solution to 100 ml with *ethanol (95 per cent)*.

Reference solution (b). Dissolve 25 mg of *fluorene* in *ethanol (95 per cent)*, add 0.5 ml of the test solution and dilute to 10 ml with *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine at 254 nm

and re-examine after exposure to ammonia vapour. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (b) shows 2 clearly separated spots.

Chlorides (2.3.12). Dilute 10 ml of solution A to 15 ml with *water*. The solution complies with the limit test for chlorides (100 ppm).

Heavy metals (2.3.13). Heat 5 g with 50 ml of *dilute hydrochloric acid* on a water-bath for 5 min and filter. Evaporate the filtrate almost to dryness and dissolve the residue in 50 ml of *water*. 12 ml of this solution complies with the limit test for heavy metals, Method D (20 ppm). Use 10 ml of *lead standard solution (2 ppm Pb)* to prepare the standard.

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

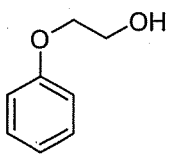
Assay. Dissolve 0.1 g in 5 ml of *dimethylformamide*. Add 5 ml of *sodium carbonate solution*, 10 ml of *sodium hydrogen carbonate solution*, 35 ml of *water* and 50.0 ml of *0.05 M iodine*. Add 10 ml of *dichloromethane* and 20 ml of *dilute sulphuric acid*. Titrate the excess of iodine with *0.1 M sodium thiosulphate*, using 0.3 ml of *starch solution* added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.05 M iodine* is equivalent to 0.003979 g of $C_{20}H_{14}O_4$.

Storage. Store protected from moisture.

Phenoxyethanol

2-Phenoxyethanol



$C_8H_{10}O_2$ Mol. Wt.138.2

Phenoxyethanol is 1-hydroxy-2-phenoxyethane.

Phenoxyethanol contains not less than 99.0 per cent and not more than 100.5 per cent of $C_8H_{10}O_2$, calculated on the dried basis.

Category. Pharmaceutical aid.

Description. A colourless, slightly viscous liquid.

Identification

Shake 2 ml of Phenoxyethanol with a mixture of 4.0 g of *potassium permanganate*, 5.4 g of *sodium carbonate* and 75 ml of *water* for 30 minutes. Add 25 g of *sodium chloride* and stir continuously for 60 minutes, filter and adjusted to pH 1.7 with *hydrochloric acid*. The precipitate, after recrystallisation from *water* complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenoxyethanol RS* or with the reference spectrum of phenoxyethanol.

B. When examined in the range 240 nm to 350 nm (2.4.7), a 0.008 per cent w/v solution in *water*, shows two absorption maxima at 269 nm and 275 nm and specific absorbance at 269 nm is 95 to 105 and at 275 nm is 75 to 85.

Tests

Refractive index (2.4.27). 1.537 to 1.539

Relative density (2.4.29). 1.105 to 1.110

Related substances. Determine by Gas chromatography (2.4.13).

Internal standard solution. Dissolve 1.25 g of *methyl laurate* in 25.0 ml of *dichloromethane*.

Test solution. Dissolve 5 g of the substance under examination in 10.0 ml of *dichloromethane* and add 1.0 ml of the internal standard solution.

Reference solution. Add 10.0 ml of the internal standard solution to 1.0 ml of the test solution and dilute to 100.0 ml with *dichloromethane*.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with silanised diatomaceous earth support (150 to 180 mesh) coated with 3 per cent w/w solution of polymethylphenyl-siloxane,
- temperature:
  column. 130°,
  inlet port and detector at 200°,
- flame ionization detector,
- flow rate. 30 ml per minute using nitrogen as a carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to phenoxyethanol and methyl laurate is not less than 12.

Inject 1 µl of the test solution and the reference solution. In the chromatogram obtained with the test solution, the ratio of the area due to peak corresponding to phenoxyethanol to the area of the peak due to the internal standard from the chromatogram obtained with the reference solution. from the

chromatogram obtained with test solution, the ratio of the area of the sum of any secondary peak and the peak due to the internal standard, is not more than 1.0 per cent.

Phenol. Not more than 0.1 per cent.

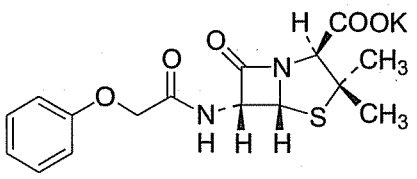
Shake about 1 g of *propoxyphenol* in 50 ml of *dichloromethane* with 1 ml of dilute *sodium hydroxide solution* and 10 ml of *water*. Wash the upper layer with 2 quantities, each of 20 ml of *dichloromethane* and dilute to 100.0 ml with *water*. The absorbance at the maxima at 287 nm (2.4.7) is not more than 0.27.

Assay. Weigh accurately 2 g, dissolve in 10.0 ml of freshly prepared *acetic anhydride solution,* heat in a water bath for 45 minutes. Cool, add 10.0 ml of *water*. further heat for 2 minutes, cool and add 10.0 ml of *butanol*, shake vigorously and titrate excess of *acetic acid* with *1 M sodium hydroxide* using 0.2 ml of *phenolphthalein* solution as indicator. Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1382 g of $C_8H_{10}O_2$.

Phenoxymethylpenicillin Potassium

Penicillin V Potassium



$C_{16}H_{17}KN_2O_5S$ Mol. Wt. 388.5

Phenoxymethylpenicillin Potassium is potassium (6*R*)-6-(2-phenoxyacetamido)penicillinate, produced by the growth of certain strains of *Penicillium notatum* or related organisms on a culture medium containing an appropriate precursor, or obtained by any other means.

Phenoxymethylpenicillin Potassium contains not less than 86.0 per cent of total penicillins $C_{16}H_{17}N_2O_5S$, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. For an adult, the equivalent of 250 to 500 mg of phenoxymethylpenicillin every 6 hours, at least 30 minutes before food. For a child every 6 hours, upto 1 year, 62.5 mg; 1 to 5 years, 125 mg; and 6 to 12 years, 250 mg.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenoxymethylpenicillin potassium RS.*

B. Gives reaction B of penicillins and cephalosporins (2.3.1).

C. Gives reaction A of potassium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 0.5 per cent w/v solution.

Specific optical rotation (2.4.22). +215.0° to +230.0°, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water.*

Light absorption (2.4.7). Absorbance of a 0.1 per cent w/v solution in *0.1 M sodium hydroxide* at the maximum at about 306 nm, not more than 0.33. Absorbance of a 0.02 per cent w/v solution in *0.1M sodium hydroxide* at the maximum at about 274 nm, not less than 0.50.

Phenoxyacetic acid. Not more than 0.5 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a suitable quantity of the substance under examination, dissolve in *phosphate buffer pH 6.6* and dilute to obtain a solution having a known concentration of about 20 mg per ml.

Reference solution. Weigh accurately a suitable quantity of phenoxyacetic acid, dissolve in the *phosphate buffer pH 6.6* and dilute to obtain a solution having a known concentration of about 0.1 mg per ml.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 1 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of phenoxyacetic acid.

Limit of *p*-hydroxyphenoxymethylpenicillin. Not more than 5.0 per cent.

Using the chromatograms obtained with the test solution in the Assay, calculate the content of *p*-hydroxyphenoxymethylpenicillin from the peak response of *p*-hydroxyphenoxymethylpenicillin and the sum of the peak responses of *p*-hydroxyphenoxymethylpenicillin and phenoxymethylpenicillin.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 125 mg of the substance under examination in the mobile phase and dilute to 50.0 ml with the mobile phase.

Reference solution (a). Dissolve an accurately weighed quantity of *phenoxymethylpenicillin potassium RS* in the mobile phase and dilute to obtain a solution having a known concentration of about 2.5 mg per ml.

Reference solution (b). A solution in the mobile phase containing 0.25 per cent w/v each of benzylpenicillin potassium and phenoxymethylpenicillin potassium.

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane chemically bonded to porous silica (3 to 10 µm),
- mobile phase: a mixture of 65 volumes of *water*, 35 volumes of *acetonitrile* and 5.75 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b).The relative retention times are about 0.8 for benzylpenicillin and 1.0 for phenoxymethylpenicillin. The column efficiency determined from the phenoxymethylpenicillin peak is not less than 1800 theoretical plates and the resolution between benzylpenicillin and phenoxymethylpenicillin is not less than 3.0.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution and reference solution (a). Record the chromatograms and measure the responses for the phenoxymethylpenicillin peak and any *p*-hydroxyphenoxymethylpenicillin peak with a retention time of about 0.4 relative to that of the main phenoxymethylpenicillin peak.

Calculate the content of $C_{16}H_{17}N_2O_5S$, from the sum of the peak responses of the *p*-hydroxyphenoxymethylpenicillin and phenoxymethylpenicillin peaks in the chromatograms obtained with the test solution and reference solution (a).

Storage. Store protected from moisture.

Phenoxymethylpenicillin Potassium Tablets

Phenoxymethylpenicillin Tablets; Penicillin V Potassium Tablets; Penicillin V Tablets

Phenoxymethylpenicillin Potassium Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of phenoxymethylpenicillin, $C_{16}H_{18}N_2O_5S$.

Usual strengths. The equivalent of 62.5 mg, 125 mg, 250 mg and 500 mg of phenoxymethylpenicillin.

Identification

A. Shake a quantity of the powdered tablets containing 80 mg of phenoxymethylpenicillin with *water*, dilute to 250 ml with *water* and filter. When examined between 230 and 360 nm (2.4.7), the filtrate shows absorption maxima at about 268 nm and 274 nm and a minimum at about 272 nm.

B. Shake a quantity of the powdered tablets containing 10 mg of phenoxymethylpenicillin with 10 ml of *water*, filter and add 0.5 ml of *neutral red solution*. Add sufficient *0.01 M sodium hydroxide* to produce a permanent orange colour and then add 1.0 ml of *penicillinase solution*; the solution changes rapidly to red.

C. Ignite 0.5 g of the powdered tablets, add 5 ml of *2 M hydrochloric acid*, boil, cool and filter. The filtrate gives reaction B of potassium salts (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 268 nm (2.4.7). At the same time measure the absorbance of a solution of known concentration of *phenoxymethylpenicillin potassium RS* at the maximum at about 268 nm. Calculate the content of $C_{16}H_{18}N_2O_5S$, in the medium.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{18}N_2O_5S$.

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and finely powder 20 tablets. Dissolve an accurately weighed quantity of the powder containing about 0.25 g of phenoxymethylpenicillin in the mobile phase by shaking for 5 minutes and dilute to 100.0 ml with the mobile phase. Filter through a 0.5 µm or finer filter and use the filtrate.

Reference solution (a). Dissolve an accurately weighed quantity of *phenoxymethylpenicillin potassium RS* in the mobile phase and dilute to obtain a solution having a known concentration of about 2.5 mg per ml.

Reference solution (b). A solution in the mobile phase containing 0.25 per cent w/v each of benzylpenicillin potassium and phenoxymethylpenicillin potassium.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane chemically bonded to porous silica,
- mobile phase: a mixture of 650 volumes of *water*, 350 volumes of *acetonitrile* and 5.75 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (b).The relative retention times are about 0.8 for benzylpenicillin and 1.0 for phenoxymethylpenicillin. The column efficiency determined from the phenoxymethylpenicillin peak is not less than 1800 theoretical plates and the resolution between the benzylpenicillin and phenoxymethylpenicillin peaks is not less than 3.0.

Inject reference solution (b). The relative standard deviation for replicate injections is not more than 1.0 per cent.

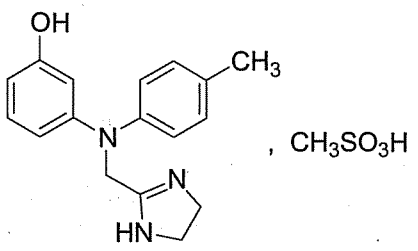
Inject the test solution and reference solution (a). Record the chromatograms and measure the responses for the phenoxymethylpenicillin peak and any *p*-hydroxyphenoxymethylpenicillin peak with a retention time of about 0.4 relative to that of the main phenoxymethylpenicillin peak.

Calculate the content of $C_{16}H_{17}N_2O_5S$, from the sum of the peak responses of the *p*-hydroxyphenoxymethylpenicillin and phenoxymethylpenicillin peaks in the chromatograms obtained with the test solution and reference solution (a)

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of phenoxymethylpenicillin.

Phentolamine Mesylate



$C_{17}H_{19}N_3O,CH_4O_3S$ Mol. Wt. 377.5

Phentolamine Mesylate is 3-[[4,5-dihydro-1*H*-imidazol-2-yl)methyl](4-methylphenyl)aminophenol methanesulphonate.

Phentolamine Mesylate contains not less than 99.0 per cent and not more than 100.5 per cent of $C_{17}H_{19}N_3O$, CH_4O_3S, calculated on the dried basis.

Category. Alpha-adrenoceptor antagonist.

Dose. By intravenous injection, 5 to 10 mg.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phentolamine mesylate RS* or with the reference spectrum of phentolamine mesylate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution shows an absorption maximum only at about 278 nm; absorbance at about 278 nm, about 0.5.

C. Dissolve 0.5 g in 5 ml of *ethanol (95 per cent)* and 5 ml of *0.1 M hydrochloric acid* and add 2 ml of a 0.5 per cent w/v solution of *ammonium metavanadate*; a light green precipitate is produced.

D. Mix 50 mg with 0.2 g of powdered *sodium hydroxide*, heat to fusion and continue the heating for a few seconds longer. Cool, add 0.5 ml of *water* and a slight excess of *2 M hydrochloric acid* and warm; *sulphur dioxide* is evolved, which turns moistened starch iodate paper blue.

Tests

Acidity or alkalinity. Dissolve 0.1 g in 10 ml of *carbon dioxide-free water* and add 0.1 ml of *methyl red solution*. The solution is not red and not more than 0.05 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 85 volumes of *2-butanone*, 15 volumes of *acetone* and 5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Reference solution. A 0.01 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the

chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g and dissolve in 100 ml of *anhydrous 2-propanol* with the aid of ultrasound if necessary. Titrate with *0.1 M tetrabutylammonium hydroxide* in *2-propanol*. Determine the end-point potentiometrically (2.4.25), using a glass electrode and a calomel electrode containing a saturated solution of *tetramethylammonium chloride* in *2-propanol*. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03775 g of $C_{17}H_{19}N_3O, CH_4O_3S$.

Storage. Store protected from light and moisture.

Phentolamine Injection

Phentolamine Mesylate Injection

Phentolamine Injection is a sterile solution of Phentolamine Mesylate in Water for Injections containing Dextrose.

Phentolamine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of phentolamine mesylate, $C_{17}H_{19}N_3O, CH_4O_3S$.

Usual strength. 10 mg per ml.

Identification

The residue obtained in the Assay melts at about 138° (2.4.21).

Tests

pH (2.4.24). 3.5 to 5.0.

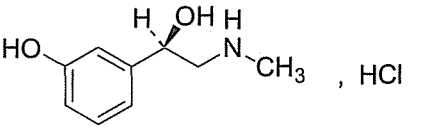
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume containing about 0.1 g of Phentolamine Mesylate to 40 ml with *water*, add 20 ml of a 20 per cent w/v solution of *trichloroacetic acid*, allow to stand for 3 hours, filter, wash the residue with two quantities, each of 5 ml, of *water* and dry to constant weight at 105°.

1 g of the residue is equivalent to 0.8487 g of $C_{17}H_{19}N_3O, CH_4O_3S$.

Storage. Store protected from light.

Phenylephrine Hydrochloride



$C_9H_{13}NO_2,HCl$ Mol. Wt. 203.7

Phenylephrine Hydrochloride is (*R*)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride.

Phenylephrine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_9H_{13}NO_2$, HCl, calculated on the dried basis.

Category. Sympathomimetic.

Dose. By subcutaneous or intramuscular injection, 2 to 5 mg; by slow intravenous injection, 100 to 500 µg; by intravenous infusion 5 to 20 mg in 500 ml, adjusted according to response.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenylephrine hydrochloride RS* or with the reference spectrum of phenylephrine hydrochloride.

B. Dissolve about 10 mg in 1 ml of *water* and add 0.05 ml of *cupric sulphate solution* and 1 ml of *5 M sodium hydroxide*; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

C. Dissolve 0.3 g in 3 ml of *water*, add 1 ml of *6 M ammonia* and initiate crystallisation by scratching the side of the tube with a glass rod. The melting range of the crystals, after washing with iced *water* and drying at 105° for 2 hours, is 171° to 176° (2.4.21).

D. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. Dissolve 2.0 g in 100 ml of *carbon dioxide-free water* prepared from *distilled water* (solution A). Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Specific optical rotation (2.4.22). –43.0° to –47.0°, determined in solution A.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 80 volumes of *2-propanol*, 15 volumes of *10 M ammonia* and 5 volumes of *chloroform.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol.*

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in *methanol.*

Reference solution (b). A 0.004 per cent w/v solution of the substance under examination in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in cold air, spray with *ninhydrin solution*, heat at 105° for 10 minutes and examine in daylight. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (500 ppm).

Phenones. To 10 ml of solution A add sufficient *0.01 M hydrochloric acid* to produce 50 ml. Absorbance of the resulting solution at the maximum at about 310 nm, not more than 0.20 (2.4.7).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in a mixture of 0.5 ml of *0.1 M hydrochloric acid* and 80 ml of *ethanol (95 per cent)* and titrate with *0.1 M ethanolic sodium hydroxide* determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02037 g of $C_9H_{13}NO_2$,HCl.

Storage. Store protected from light and moisture.

Phenylephrine Injection

Phenylephrine Hydrochloride Injection

Phenylephrine Injection is a sterile solution of Phenylephrine Hydrochloride in Water for Injections.

Phenylephrine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of phenylephrine hydrochloride, $C_9H_{13}NO_2$,HCl.

Usual strength. 10 mg per ml.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

B. To a volume containing 10 mg of Phenylephrine Hydrochloride add, if necessary, sufficient *water* to produce 1 ml and then add 0.05 ml of *cupric sulphate solution* and 1 ml of *5 M sodium hydroxide*; a violet colour is produced. Add 1 ml of *ether* and shake; the ether layer remains colourless.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 80 volumes of *2-propanol*, 15 volumes of *10 M ammonia* and 5 volumes of *chloroform.*

Test solution (a) Evaporate a volume of the injection containing 20 mg of Phenylephrine Hydrochloride to dryness and dissolve the residue in 1 ml of *methanol.*

Test solution (b). Dilute 1 volume of test solution (a) to 200 volumes with *methanol.*

Reference solution (a). Dilute 1 volume of test solution (b) to 2.5 volumes with *methanol.*

Reference solution (b). A 0.01 per cent w/v solution of *phenylephrine hydrochloride RS* in *methanol.*

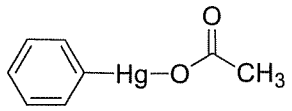
Apply to the plate 5 µl of each solution. After development, dry the plate in cold air, spray with *ninhydrin solution*, heat at 105° for 10 minutes and examine in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with test solution (b) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 50 mg of Phenylephrine Hydrochloride add sufficient *0.5 M sulphuric acid* to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *0.5 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 273 nm (2.4.7). Calculate the content of $C_9H_{13}NO_2$,HCl taking 90 as the specific absorbance at 273 nm.

Storage. Store protected from light.

Phenylmercuric Acetate



$C_8H_8HgO_2$ Mol. Wt. 336.7

Phenylmercuric Acetate is (acetato)phenylmercury.

Phenylmercuric Acetate contains not less than 98.0 per cent and not more than 100.5 per cent of $C_8H_8HgO_2$.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white to creamy white, crystalline powder, or small white prisms or leaflets.

Identification

A. To 100 mg add 0.5 ml of *nitric acid*, warm gently until a dark brown colour is produced and dilute with *water* to 10 ml; the characteristic odour of *nitrobenzene* is produced.

B. To 100 mg add 0.5 ml of *sulphuric acid* and 1 ml of *ethanol (95 per cent)* and warm; the characteristic odour of *ethyl acetate* is produced.

C. To 5 ml of a saturated solution in *water*, add a few drops of a freshly prepared 10 per cent w/v solution of *sodium sulphide*; a white precipitate is formed which darkens slowly on boiling and allowing to stand.

Tests

Mercuric salts and heavy metals. Heat about 100 mg with 15 ml of *water*, cool and filter. To the filtrate add a few drops of a freshly prepared 10 per cent w/v solution of *sodium sulphide*; the precipitate formed shows no immediate colour.

Polymercurated benzene compounds. Shake 2.0 g with 100 ml of *acetone*. Filter, wash the residue with successive portions of *acetone* until a total of 50 ml is used, dry the residue at 105° for 1 hour and weigh. The weight of the residue does not exceed 30 mg (1.5 per cent).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Assay. Weigh accurately about 0.4 g, transfer to a 100-ml flask, add 15 ml of *water*, 5 ml of *formic acid* and 1 g of *zinc dust* and reflux for 30 minutes. Cool, filter and wash the filter paper and the amalgam with *water* until the washings are no longer acidic to litmus. Dissolve the amalgam in 40 ml of *8 M nitric acid*. Heat on a *water*-bath for 3 minutes and add 0.5 g *urea* and sufficient *0.02 M potassium permanganate* to produce a permanent pink colour. Cool and add *hydrogen peroxide solution* to decolorise the solution. Add 1 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate*. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of ammonium thiocyanate required.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 0.01684 g of $C_8H_8HgO_2$.

Storage. Store protected from light and moisture.

Phenylmercuric Nitrate

Phenylmercuric Nitrate is a mixture of phenylmercuric nitrate, $C_6H_5HgNO_3$ and phenylmercuric hydroxide, C_6H_5HgOH.

Phenylmercuric Nitrate contains not less than 62.5 per cent and not more than 63.5 per cent of mercury, Hg, calculated on the dried basis.

Category. Antiseptic; pharmaceutical aid (antimicrobial preservative).

Description. A white or pale yellow powder.

Identification

A. To 0.1 g add 3 ml of *sulphuric acid*; the mixture becomes yellow and the characteristic odour of *nitrobenzene* is produced.

B. To 0.1 g add 45 ml of *water* and heat to boiling with shaking. Cool, filter and add sufficient *water* to produce 50 ml (solution A). To 1 ml of solution A add 1 ml of *2 M hydrochloric acid*; a white, flocculent precipitate is produced.

C. To 5 ml of solution A add 8 ml of *water* and 0.1 ml of a freshly prepared 10 per cent w/v solution of *sodium sulphide*; a white precipitate is formed which darkens slowly on boiling and allowing to stand.

D. To 5 ml of solution A add 1 ml of *2 M hydrochloric acid*, 2 ml of *dichloromethane* and 0.2 ml of *dithizone solution* and shake; the lower layer is orange-yellow.

E. Solution A gives reaction of nitrates (2.3.1).

Tests

Appearance of solution. Solution A is colourless (2.4.1).

Inorganic mercuric compounds. To a 10 ml of solution A add 2 ml of *potassium iodide solution* and 3 ml of *2 M hydrochloric acid* and filter; the filtrate is colourless. Wash the precipitate with 2 ml of *water*, combine the filtrate and washings and add 2 ml of *2 M sodium hydroxide* and sufficient *water* to produce 20 ml. 12 ml of the solution complies with the limit test for heavy metals, Method A (2.3.13). Use *lead standard solution (1 ppm Pb)* to prepare the standard (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

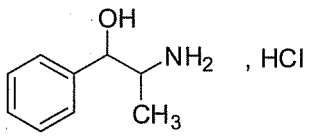
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 24 hours.

Assay. Weigh accurately about 0.2 g and dissolve in a mixture of 90 ml of *water* and 10 ml of *nitric acid*. Add 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate* until a persistent reddish yellow colour is obtained. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *ammonium thiocyanate* required.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 0.02006 g of Hg.

Storage. Store protected from light and moisture.

Phenylpropanolamine Hydrochloride



$C_9H_{13}NO,HCl$ Mol. Wt. 187.7

Phenylpropanolamine Hydrochloride is (1*RS*,2*SR*)-2-amino-1-phenylpropan-1-ol hydrochloride.

Phenylpropanolamine Hydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of $C_9H_{13}NO,HCl$, calculated on the dried basis.

Category. Adrenoreceptor antagonist.

Description. A white or almost white crystalline powder.

Identification

Tests A and E may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenylpropanolamine hydrochloride RS* or with the reference spectrum of phenylpropanolamine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. To a 1.0 per cent w/v solution of the substance under examination in the *water*, add 0.2 ml of *copper sulphate solution* and 0.3 ml of *dilute sodium hydroxide solution*. A violet colour develops. Add 2 ml of *ether* and shake. A violet precipitate is formed between the two layers.

D. Melting range (2.4.21). 194° to 197°.

E. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of 5.0 per cent w/v solution in *water*, add 0.1 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*. The solution turns yellow. Add 0.4 ml of *0.01 M hydrochloric acid*. The solution turns red.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 6 volumes of *13.5 M ammonia*, 24 volumes of *ethanol (95 per cent)* and 70 volumes of *butanol*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Test solution (b). Dilute 1.0 ml of test solution (a) to 10.0 ml with *ethanol (95 per cent)*.

Reference solution (a). Dissolve 20 mg of *phenylpropanolamine hydrochloride RS* in 10 ml of *ethanol (95 per cent)*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with *ethanol (95 per cent)*.

Reference solution (c). Dissolve 20 mg of *norpseudoephedrine hydrochloride RS* in *ethanol (95 per cent)*, add 1 ml of test solution (a) and dilute to 10 ml with *ethanol (95 per cent)*.

Reference solution (d). Dissolve 60 mg of *ammonium chloride* in 10 ml of the *methanol*.

Before use, spray the plate with a 2 per cent w/v solution of *disodium tetraborate*, using 8 ml for a plate 100 mm by 200 mm and dry in a stream of cold air for 30 minutes. Apply to the plate 10 µl of each solution as bands about 10 mm by 3 mm. Allow the mobile phase to rise 10 cm. Dry the plate in a current of warm air, allow to cool, spray with a 0.2 per cent w/v solution of *ninhydrin* in *ethanol (95 per cent)* and heat at 110° for 15 minutes. Any spot in the chromatogram obtained with test solution (a) other than the principal spot and the spot corresponding to ammonium chloride is not more intense than the spot in the chromatogram obtained with reference solution (b) (1.0 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Phenylpropanonamine. Absorbance of a 2.0 per cent w/v solution in *0.01 M hydrochloric acid* at the maximum at about 283 nm (2.4.7) is not more than 0.1.

Heavy metals (2.3.13). 12 ml of 5.0 per cent w/v solution in *water* complies with the limit test for heavy metals, Method D (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

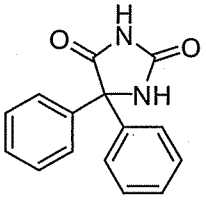
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g and dissolve in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)*. Titrate with *0.1 M sodium hydroxide*. Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01877 g of $C_9H_{14}ClNO$.

Phenytoin

Diphenylhydantoin



$C_{15}H_{12}N_2O_2$ Mol.Wt. 252.3

Phenytoin is 5,5-diphenylimidazolidine-2,4-dione.

Phenytoin contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{15}H_{12}N_2O_2$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenytoin RS* or with the reference spectrum of phenytoin.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. To about 10 mg, add 1 ml of *water* and 0.05 ml of *ammonia*. Heat until boiling begins. Add 0.05 ml of a 5 per cent w/v solution of *copper sulphate* in *dilute ammonia* and shake, a pink crystalline precipitate is formed.

Tests

Appearance of solution. Dissolve 1.0 g in a mixture of 5 ml of *1 M sodium hydroxide* and 20 ml of *water*. The solution is clear (2.4.1) and not more intensely coloured than reference solution BYS5 (2.4.1).

Acidity or alkalinity. To 1.0 g, add 45 ml of *water* and boil for 2 minutes. Allow to cool and filter. Wash the filter with *carbon dioxide-free water* and dilute the combined filtrate and washings to 50 ml with the same solvent. To 10 ml of the solution add 0.15 ml of *methyl red solution*; not more than 0.5 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red. To 10 ml of the solution add 0.15 ml of *bromothymol blue solution*; not more than 0.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to blue.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. Equal volumes of *acetone* and *methanol*.

Mobile phase. A mixture of 30 volumes of *dioxan* and 75 volumes of *hexane*.

Test solution (a). Dissolve 0.4 g of the substance under examination in 10 ml of the solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 20 ml with the solvent mixture.

Reference solution (a). Dissolve 20 mg of *phenytoin RS* in 10 ml of the solvent mixture.

Reference solution (b). Dissolve 8 mg of *benzophenone* in 100 ml of the solvent mixture.

Reference solution (c). Dissolve 8 mg of *benzil* in 100 ml of the solvent mixture.

Reference solution (d). Dilute 1 ml of test solution (a) to 100 ml with the solvent mixture.

Reference solution (e). Mix 1 ml of reference solution (b) and 1 ml of reference solution (c).

Before use, wash the plate with a mixture of 30 volumes of *dioxan* and 75 volumes of *hexane*. Allow the plate to dry in air. Apply to the plate 10 µl of each solution. Dry the plate in a current of cold air for 2 minutes. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine under ultraviolet light at 254 nm. In the chromatogram obtained with test solution (a) any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with the reference solution (b) (0.2 per cent); any spot corresponding to benzil is not more intense than the spot in the chromatogram obtained with reference solution (c) (0.2 per cent) and any spot, apart from the principal spot and any spot corresponding to benzophenone and benzil, is not more intense than the spot in the chromatogram obtained with reference solution (d) (1 per

cent). The test is not valid unless the chromatogram obtained with reference solution (e) shows two clearly separated spots.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method D (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

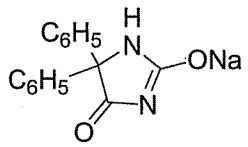
Assay. Weigh accurately about 0.2 g, dissolve in 50 ml of *dimethylformamide*. Titrate with *0.1 M sodium methoxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium methoxide* is equivalent to 0.02523 g of $C_{15}H_{12}N_2O_2$.

Storage. Store protected from light.

Phenytoin Sodium

Diphenylhydantoin Sodium



$C_{15}H_{11}N_2NaO_2$ Mol. Wt. 274.3

Phenytoin Sodium is 4-oxo-5,5-diphenyl-2-imidazolidin-2-olate

Phenytoin Sodium contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{15}H_{11}N_2NaO_2$, calculated on the anhydrous basis.

Category. Anticonvulsant; antiarrhythmic.

Dose. As anticonvulsant, orally, 150 to 300 mg daily, increasing gradually to 600 mg in accordance with the needs of patient. In status epileptieus, by slow intravenous injection, 10 to 15 mg per kg at a rate not exceeding 50 mg per minute as a loading dose; maintenance doses of 100 mg thereafter every 6 hours. In arrhythmias, by intravenous injection, 3.5 to 5 mg per kg at a rate not exceeding 50 mg per minute and repeated once if necessary.

Description. A white powder; odourless; somewhat hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenytoin sodium RS* or with the reference spectrum of phenytoin sodium.

B. Dissolve 0.25 g in 5 ml of *water* and acidify with *dilute hydrochloric acid*; a white precipitate is produced.

C. Dissolve 0.1 g in 10 ml of a 10 per cent w/v solution of *pyridine*, add 1 ml of *cupric sulphate with pyridine solution* and allow to stand for 10 minutes; a blue precipitate is produced.

D. Incinerate 0.1 g; the residue after neutralisation with *hydrochloric acid* and addition of 2 ml of *water* gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. Suspend 1.0 g in 5 ml of *water* and dilute to 20 ml with *0.1 M sodium hydroxide*; the solution is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Free phenytoin. Dissolve 0.3 g in 10 ml of a mixture of equal volumes of *pyridine* and *water* and add 0.5 ml of *dilute phenolphthalein solution* and 3 ml of *silver nitrate-pyridine reagent*. Not more than 1.0 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 45 volumes of *chloroform*, 45 volumes of *2-propanol* and 10 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.04 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *benzophenone* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 80° for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.18 g, suspend in 2 ml of *water*, add 8 ml of *0.05 M sulphuric acid* and heat gently for 1 minute. Add 30 ml of *methanol*, cool. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). After the first inflection, stop the addition of sodium hydroxide, add 5 ml of *silver nitrate*

solution in *pyridine*, mix and continue the titration until a second inflection is reached. Record the volume of *0.1 M sodium hydroxide* added between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.

Storage. Store protected from moisture.

Phenytoin Injection

Phenytoin Sodium Injection; Diphenylhydantoin Sodium injection

Phenytoin Injection is a sterile material consisting of Phenytoin Sodium with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injection or other suitable solvent, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Phenytoin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$.

Usual strengths. 100 mg; 250 mg.

Description. A white powder; odourless; somewhat hygroscopic.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenytoin sodium RS* or with the reference spectrum of phenytoin sodium.

B. Dissolve 0.25 g in 5 ml of *water* and acidify with *dilute hydrochloric acid*; a white precipitate is produced.

C. Dissolve 0.1 g in 10 ml of a 10 per cent w/v solution of *pyridine*, add 1 ml of *cupric sulphate with pyridine solution* and allow to stand for 10 minutes; a blue precipitate is produced.

D. Incinerate 0.1 g; the residue after neutralisation with *hydrochloric acid* and addition of 2 ml of *water* gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. Suspend 1.0 g in 5 ml of *water* and dilute to 20 ml with *0.1 M sodium hydroxide*; the solution is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Completeness of solution. The contents dissolve in the quantity of the solvent recommended on the label and give a clear solution.

pH (2.4.24). 10.0 to 12.0, determined in a 5.0 per cent w/v solution in the stated solvent.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 45 volumes of *chloroform*, 45 volumes of *2-propanol* and 10 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 0.04 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *benzophenone* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 80° for 5 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.18 g of the mixed contents of 10 containers, suspend in 2 ml of *water*, add 8 ml of *0.05 M sulphuric acid* and heat gently for 1 minute. Add 30 ml of *methanol*, cool. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). After the first inflection, stop the addition of *sodium hydroxide*, add 5 ml of *silver nitrate solution* in *pyridine*, mix and continue the titration until a second inflection is reached. Record the volume of *0.1 M sodium hydroxide* added between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states (1) the quantity of Phenytoin Sodium contained in it; (2) the directions for preparing the Injection.

Phenytoin Capsules

Phenytoin Sodium Capsules; Diphenylhydantoin Sodium Capsules

Phenytoin Capsules contain not less than 92.7 per cent and not more than 107.5 per cent of the stated amount of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$.

Usual strength. 10 mg.

Identification

A. Centrifuge the precipitated mixture obtained in test B, dissolve the residue in *methanol*, evaporate and dry at 105° for 30 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenytoin sodium RS* or with the reference spectrum of phenytoin sodium.

B. Shake a quantity of the content of capsules containing about 0.5 g of Phenytoin Sodium with 10 ml of *water* and filter. The filtrate yields a white precipitate on the addition of *2 M hydrochloric acid*.

C. To 0.1 g of the residue obtained in test A, add 0.5 ml of *1 M sodium hydroxide*, 10 ml of a 10 per cent w/v solution of *pyridine* and 1 ml of *copper sulphate-pyridine reagent* and allow to stand for 10 minutes. A blue precipitate is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *13.5 M ammonia*, 45 volumes of *chloroform* and 45 volumes of *propan-2-ol*.

Test solution. Shake a quantity of the content of capsules containing about 0.2 g of Phenytoin Sodium with 5 ml of *methanol*, warm on a water-bath with shaking and filter.

Reference solution (a). Dilute 1 ml of the test solution to 200 ml with *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *benzophenone* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry at 80° for 5 minutes and examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with the test solution corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.5 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Shake a quantity of the mixed contents of 20 capsules containing about 0.25 g of Phenytoin Sodium with 50 ml of *0.01 M sodium hydroxide*. Centrifuge, acidify 25 ml of the clear liquid with 10 ml of *0.1 M hydrochloric acid* and extract with successive quantities of 50, 40 and 25 ml of *ether*. Wash the combined extracts with 10 ml of *water*, evaporate to dryness and dry the residue at 105°. Dissolve in 50 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide* using 0.3 per cent w/v solution of *thymol blue* in *anhydrous pyridine* as indicator.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.

Phenytoin Tablets

Phenytoin Sodium Tablets; Diphenylhydantoin Sodium Tablets

Phenytoin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of phenytoin sodium, $C_{15}H_{11}N_2NaO_2$. The tablets are coated.

Usual strengths. 50 mg; 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Phenytoin Sodium with 20 ml of *water*, filter, acidify with *dilute hydrochloric acid* and extract with 10 ml of *chloroform*. Wash the chloroform extract with *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness and dry the residue at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *phenytoin sodium sodium RS* treated in the same manner or with the reference spectrum of phenytoin sodium.

B. Triturate a quantity of the powdered tablets containing 0.5 g of Phenytoin Sodium with 10 ml of *water* and filter. Acidify with *dilute hydrochloric acid*; a white precipitate is produced.

C. The powdered tablets, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, impart a yellow colour to the flame.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 75 volumes of *hexane* and 30 volumes of *dioxan.*

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Phenytoin Sodium with 5 ml of *methanol,* warm on a water-bath with shaking and filter.

Reference solution. A 0.01 per cent w/v solution of *benzophenone* in *methanol.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to benzophenone is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Phenytoin Sodium, shake with 40 ml of *0.01 M sodium hydroxide* for 5 minutes and add sufficient *0.01 M sodium hydroxide* to produce 50.0 ml. Centrifuge, acidify 25.0 ml of the clear liquid with 10 ml of *0.1 M hydrochloric acid* and extract successively with 50, 40, 25 and 25 ml of *ether.* Wash the combined extracts with 10 ml of *water,* evaporate to dryness and dry the residue at 105°. Dissolve in 50 ml of *anhydrous pyridine* and titrate with *0.1 M tetrabutylammonium hydroxide,* using 0.3 per cent w/v solution of *thymol blue* in *pyridine* as indicator and taking care to prevent absorption of carbon dioxide from the atmosphere. Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02743 g of $C_{15}H_{11}N_2NaO_2$.

Storage. Store protected from moisture.

Phenytoin Oral Suspension

Diphenylhydantoin Oral Suspension

Phenytoin Oral Suspension is a mixture of consisting of Phenytoin with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The suspension is constituted by dispersing the contents of the sealed container in the specific volume of Water just before use.

Phenytoin Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of phenytoin, $C_{15}H_{12}N_2O_2$.

When stored at the temperature and for the period stated on the label during which the constituted suspension may be expected to be satisfactory for use, it contains not less than 80.0 per cent of the stated amount of phenytoin, $C_{15}H_{12}N_2O_2$.

Usual strength. 25 mg per ml.

Storage. Store protected from moisture at a temperature not exceeding 30°.

The constituted suspension complies with the tests stated under Oral Liquids and with the following tests.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtain with *phenytoin RS* or with the reference spectrum of phenytoin.

Tests

pH (2.4.24). 4.5 to 5.5 determined on 1.0 g.

Benzil and benzophenone. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 30 volumes of *1, 4-dioxan* and 75 volumes of *hexane.*

Test solution. Disperse a quantity of suspension containing about 30 mg of Phenytoin in 5 ml of *water* add 2 ml of *2 M hydrochloric acid,* mix well and extract with five 20 ml quantities of *ether.* Combine the ether extracts, wash with three 10 ml quantities of *water,* evaporate to dryness and dissolve the residue in 1.5 ml of a mixture of 1 volume of *glacial acetic acid* and 9 volumes of *acetone.*

Reference solution (a). A 0.004 per cent w/v solution of *benzophenone* in *ethanol (95 per cent).*

Reference solution (b). A 0.004 per cent w/v solution of *benzil* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtain with reference solution (a) (0.2 per cent) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

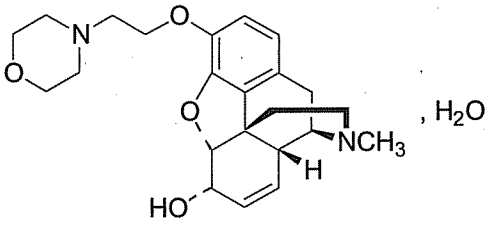
Assay. Disperse a quantity of the suspension containing about 0.2 g of Phenytoin, add 10 ml of *2 M hydrochloric acid* and 15 ml of *water,* extract with three 50 ml quantities of a mixture of 3 volumes of *chloroform* and 1 volume of *propan-2-ol* and evaporate the combined extract to dryness. Dry the residue at 105° for 1 hour, cool and dissolve in a mixture of 3 ml of *1 M sodium hydroxide* and 50 ml of *water* with the aid of gentle

heat. Cool and pass a current of *carbon dioxide* through the solution until precipitation of phenytoin is complete, filter and wash the residue with two 10 ml quantities of *water* and dry at 105° for 2 hours.

Determine the weight per ml (2.4.29) of the oral suspension.

Calculate the content of $C_{15}H_{12}N_2O_2$, weight in volume.

Pholcodine



$C_{23}H_{30}N_2O_4,H_2O$ Mol. Wt. 416.5

Pholcodine is 7,8-didehydro-4,5α-epoxy-17-methyl-3-[2-(morpholin-4-yl)ethoxy]morphinan-6α-ol monohydrate.

Pholcodine contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{23}H_{30}N_2O_4$, calculated on the dried basis.

Category. Cough suppressant.

Dose. Upto 40 mg daily, in divided doses.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pholcodine RS* or with the reference spectrum of pholcodine.

B. To 10 ml of a 0.1 per cent w/v solution add 75 ml of *water* and 10 ml of *1 M sodium hydroxide* and dilute to 100 ml with *water*. When examined in the range 230 nm and 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 284 nm; absorbance at about 284 nm, 0.36 to 0.39.

C. Dissolve 50 mg in 1 ml of *sulphuric acid* and add 0.05 ml of a 10 per cent w/v solution of *ammonium molybdate*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 ml of *2 M nitric acid*; the colour changes to brownish red.

Tests

Specific optical rotation (2.4.22). –94.0° to –98.0°, determined at 20° in a 2.0 per cent w/v solution in *ethanol (95 per cent)*.

Morphine. Dissolve 0.1 g in sufficient of *0.1 M hydrochloric acid* to produce 5 ml, add 2 ml of a 1 per cent w/v solution of *sodium nitrite*, allow to stand for 15 minutes and add 3 ml of *6 M ammonia*. The solution is not more intensely coloured than reference solution BS4 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *ethanol (95 per cent)*, 70 volumes of *toluene*, 65 volumes of *acetone* and 5 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of *chloroform*.

Reference solution (a). A 0.025 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.0125 per cent w/v solution of the substance under examination in *chloroform*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot of higher Rf value than the principal spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 3.9 to 4.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.18 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, warming gently. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically at the second inflection (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01993 g of $C_{23}H_{30}N_2O_4$.

Storage. Store protected from moisture.

Pholcodine Linctus

Pholcodine Linctus is a solution containing 0.1 per cent w/v solution of Pholcodine and 1 per cent w/v solution of Citric Acid Monohydrate in a suitable flavoured vehicle.

Pholcodine Linctus contains not less than 0.090 per cent and not more than 0.110 per cent w/v of pholcodine, $C_{23}H_{30}N_2O_4,H_2O$.

Category. Opioid cough suppressant.

Identification

To 20 ml add 20 ml of *water*, make alkaline to *litmus paper* with *5 M ammonia*, extract with two quantities, each of 20 ml, of *chloroform*, washing each extract with 5 ml of *water*, dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate to dryness. If necessary, add 0.1 ml of ether and scratch the sides of the vessel with a glass rod to induce crystallisation. The crystals, dried at a pressure not exceeding 2 kPa, comply with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pholcodine RS* or with the reference spectrum of pholcodine.

B. When examined in the range 230 nm and 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 284 nm.

C. To a portion of the crystals add 0.05 ml of *nitric acid* and mix; a yellow colour is produced.

D. Dissolve the remainder of the crystals in 1 ml of *sulphuric acid* and add 0.05 ml of *ammonium molybdate-sulphuric acid solution*; a pale blue colour is produced. Warm gently; the colour changes to deep blue. Add 0.05 ml of *2 M nitric acid*; the colour changes to brownish red.

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately about 50 g and add sufficient *5 M ammonia* to make the solution alkaline to *litmus paper*, extract with four quantities, each of 25 ml, of *chloroform*, washing each extract with the same 5 ml of *water*. Combine the extracts and evaporate until the volume is reduced to 15 ml. Titrate with *0.02 M perchloric acid,* using *quinaldine red solution* as indicator. Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.004165 g of $C_{23}H_{30}N_2O_4,H_2O$.

Determine the weight per ml of the linctus (2.4.29), and calculate the content of $C_{23}H_{30}N_2O_4,H_2O$, weight in volume.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of pholcodine.

Phosphoric Acid

Orthophosphoric Acid; Concentrated Phosphoric Acid

H_3PO_4 Mol. Wt. 98.0

Phosphoric acid contains not less than 84.0 per cent w/w and not more than 90.0 per cent w/w of H_3PO_4.

Category. Pharmaceutical aid (acidifying agent).

Description. A clear, colourless, syrupy liquid; corrosive. When kept at a low temperature it may solidify, producing a mass of colourless crystals which do not melt until the temperature reaches 28°.

Identification

A. Dilute with *water*; the solution is strongly acidic.

B. Dilute 10.0 g to 150 ml with *water* (solution A). Neutralise with *2 M sodium hydroxide*; the resulting solution gives the reactions of phosphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid*; the resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dilute 1.2 ml with 10 ml of *water*, neutralise with *dilute ammonia solution*, add sufficient *dilute acetic acid* to render the solution acidic and then dilute to 25 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method A (10 ppm).

Iron (2.3.14). 10 ml of solution A complies with the limit test for iron (60 ppm).

Chlorides (2.3.12). 3 ml of solution A complies with the limit test for chlorides (50 ppm).

Sulphates (2.3.17). 20 ml of solution A complies with the limit test for sulphates (100 ppm).

Alkali phosphates. To 1.7 g in a graduated cylinder add 6 ml of *ether* and 2 ml of *ethanol (95 per cent)*; no turbidity is produced.

Aluminium and calcium. To 1.7 g add 10 ml of *water* and 8 ml of *dilute ammonia solution*; the solution remains clear.

Hypophosphorus acid and phosphorous acid. To 5 ml of solution A add 2 ml of a 1.7 per cent w/v solution of *silver nitrate* and heat on a water-bath for 5 minutes; the appearance of the solution does not change.

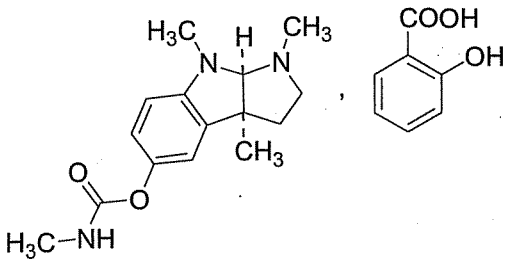
Assay. Weigh accurately about 1.0 g, add a solution of 10 g of *sodium chloride* in 30 ml of *water* and titrate with *1 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.04900 g of H_3PO_4.

Storage. Store protected from moisture, in glass containers.

Physostigmine Salicylate

Eserine Salicylate



$C_{15}H_{21}N_3O_2,C_7H_6O_3$ Mol. Wt. 413.5

Physostigmine Salicylate is (3*aS*,8*aR*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*]indol-5-yl methylcarbamate salicylate.

Physostigmine Salicylate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{15}H_{21}N_3O_2$, $C_7H_6O_3$, calculated on the dried basis.

Category. Anticholinesterase.

Dose. By subcutaneous or intramuscular injection, 600 µg to 1.2 mg.

Description. A colourless or faintly yellow crystals, turning red gradually under the action of air and light and rapidly in presence of moisture; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Test C may be omitted if tests A, B and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *physostigmine salicylate RS.*

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. To a 1 per cent w/v solution add *1 M sodium hydroxide*; a white precipitate which turns pink is produced. The precipitate dissolves in an excess of the reagent, producing a red solution.

D. A 0.9 per cent w/v solution in *carbon dioxide-free water* gives reaction A of salicylates (2.3.1).

Tests

Appearance of solution. Dissolve 0.9 g without heating in 95 ml of *carbon dioxide-free water* prepared from *distilled water*, and dilute to 100 ml with the same solvent (solution A). The solution, examined immediately after preparation, is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.1 to 5.9, determined in solution A immediately after preparation.

Specific optical rotation (2.4.22). –90.0° to –94.0°, determined in solution A immediately after preparation.

Eseridine. To 5 ml of solution A, examined immediately after preparation, add a few crystals of *potassium iodate* and a drop of *2 M hydrochloric acid* and 2 ml of *chloroform* and shake; the chloroform layer does not turn violet within 1 minute.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 100 volumes of *cyclohexane*, 23 volumes of *2-propanol* and 2 volumes of *strong ammonia solution.*

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of *ethanol (95 per cent).*

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of *ethanol (95 per cent).*

Reference solution (a). A 0.1 per cent w/v solution of *physostigmine salicylate RS* in *ethanol (95 per cent).*

Reference solution (b). A 0.01 per cent w/v solution of *physostigmine salicylate RS* in *ethanol (95 per cent).*

Apply to the plate 20 µl of each solution. After development, dry the plate in cold air, carry out a second chromatographic development in same direction, dry the plate in air and spray with freshly prepared *acetic potassium iodobismuthate solution* and then with *hydrogen peroxide solution (10 vol).* Examine the plate within 2 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (0.1 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on the residue obtained in the test for Loss on drying.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.35 g, dissolve in 50 ml of a mixture of equal volumes of *chloroform* and *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04135 g of $C_{15}H_{21}N_3O_2,C_7H_6O_3$.

Storage. Store protected from light and moisture.

Physostigmine Injection

Physostigmine Salicylate Injection; Eserine Salicylate Injection

Physostigmine Injection is a sterile solution of Physostigmine Salicylate in Water for Injections.

Physostigmine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of physostigmine salicylate, $C_{15}H_{21}N_3O_2, C_7H_6O_3$.

Usual strength. 600 µg per ml.

Identification

A. Warm a volume containing 3 mg of Physostigmine Salicylate with 0.3 ml of *5 M ammonia*; a yellowish red solution is produced which on evaporation gives a bluish residue.

B. The residue obtained in test A dissolves in *ethanol (95 per cent)* producing a blue solution which, on the addition of *6 M acetic acid*, appears blue by transmitted light and exhibits a red fluorescence which intensifies on dilution with *water*.

C. The residue obtained in test A dissolves in *sulphuric acid* producing a green solution which, on the gradual addition of *ethanol (95 per cent)*, changes to red but reverts to green when the ethanol is evaporated.

Tests

pH (2.4.24). 4.0 to 6.0.

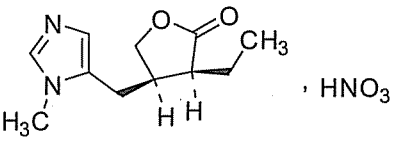
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Transfer an accurately measured volume containing 30 mg of Physostigmine Salicylate to a separator, add about 0.25 g of *sodium bicarbonate* and extract with six quantities, each of 15 ml, of *chloroform*. Filter the combined chloroform extracts through about 10 g of *anhydrous sodium sulphate*. Add 25 ml of *anhydrous glacial acetic acid* to the filtrate. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.01 M perchloric acid* is equivalent to 0.004135 g of $C_{15}H_{21}N_3O_2, C_7H_6O_3$.

Storage. Store protected from light, in single dose containers. The injection should not be used if it is more than slightly discoloured.

Pilocarpine Nitrate



$C_{11}H_{16}N_2O_2, HNO_3$ Mol. Wt. 271.3

Pilocarpine Nitrate is (3*S*,4*R*)-3-ethyl-4-[(1-methyl-1*H*-imidazol-5-yl)methyl]dihydrofuran-2(3*H*)-one nitrate.

Pilocarpine Nitrate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{11}H_{16}N_2O_2, HNO_3$, calculated on the dried basis.

Category. Cholinergic (ophthalmic) in glaucoma.

Description. Colourless crystals or a white, crystalline powder; odourless; sensitive to light.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pilocarpine nitrate RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve about 10 mg in 2 ml of *water*, add 2 drops of a 5 per cent w/v solution of *potassium dichromate*, 1 ml of *hydrogen peroxide solution (10 vol)* and 2 ml of *chloroform* and shake; the chloroform layer turns violet.

D. Gives reaction A of nitrates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 3.5 to 4.5, determined in a 5.0 per cent w/v solution prepared immediately before use in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). +79.5° to +83.0°, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*, prepared immediately before use.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 85 volumes of *chloroform*, 14 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Dissolve 0.3 g of the substance under examination in 10 ml of *water*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.1 per cent w/v solution of *pilocarpine nitrate RS* in *water*.

Reference solution (b). A 0.03 per cent w/v solution of *pilocarpine nitrate RS* in *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate at 105° for 10 minutes, cool and spray with *potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b).

Other alkaloids. To a 1 per cent w/v solution add *dilute ammonia solution*; no turbidity is produced. To a 1 per cent w/v solution add a few drops of *potassium dichromate solution*; no turbidity is produced.

Chlorides (2.3.12). 25 ml of a 10.0 per cent w/v solution complies with the limit test for chlorides (100 ppm).

Iron (2.3.14). 20 ml of a 10.0 per cent w/v solution complies with the limit test for iron (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 0.5 g.

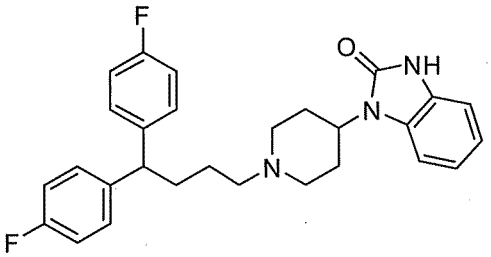
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02713 g of $C_{11}H_{16}N_2O_2, HNO_3$.

Storage. Store protected from light and moisture.

Pimozide



$C_{28}H_{29}F_2N_3O$ Mol.Wt. 461.6

Pimozide is 1-[1-[4,4-bis(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one.

Pimozide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{28}H_{29}F_2N_3O$, calculated on the dried basis.

Category. Antipsychotic.

Description. A white to almost white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pimozide RS* or with the reference spectrum of pimozide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 10 volumes of *acetone* and 90 volumes of *methanol*.

Test solution. Dissolve 30 mg of the substance under examination in 10 ml of the mobile phase.

Reference solution (a). A 0.3 per cent w/v solution of *pimozide RS* in the mobile phase.

Reference solution (b). A solution containing 0.3 per cent w/v each of *pimozide RS* and *benperidol RS* in the mobile phase.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in a current of warm air for 15 minutes and expose it to iodine vapour until the spots appear. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Mix about 5 mg of the substance under examination with 45 mg of *heavy magnesium oxide* and ignite in a crucible until an almost white residue is obtained. Allow to cool, add 1 ml of *water*, 0.05 ml of *phenolphthalein solution* and about 1 ml of *dilute hydrochloric acid* to render the solution colourless. Filter. To a freshly prepared mixture of 0.1 ml of *alizarin S solution* and 0.1 ml of *zirconyl nitrate solution*, add 1.0 ml of the filtrate. Mix allow to stand for 5 minutes and compare the colour of the solution with that of a blank prepared in the same manner. The test solution is yellow and the blank is red.

D. Melting point (2.4.21). 216° to 220°.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10 ml of *methanol.*

Reference solution (a). Dissolve 5 mg of *pimozide RS* and 2 mg of *mebendazole RS* in 100 ml of *methanol.*

Reference solution (b). Dilute 5.0 ml of the test solution to 100 ml with *methanol.* Dilute 1.0 ml of this solution to 10 ml with *methanol.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a solution containing 0.25 per cent w/v of *ammonium acetate* and 0.85 per cent w/v of *tetrabutylammonium hydrogen sulphate*,
  B. *acetonitrile,*
- flow rate. 2ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 10	80 → 70	20 → 30
10 – 15	70	30
15 – 20	80	20

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to mebendazole and pimozide is not less than 5.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any peak due to (1-(piperidin-4-yl)-1,3-dihydro-2H-benzimidazol-2-one) (pimozide impurity A), (1-[1-[(4RS)-4-(4-fluorophenyl)-4-phenylbutyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one) (pimozide impurity B), (1-[1-[(4RS)-4-(2-fluorophenyl)-4-(4-fluorophenyl)butyl]piperidin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one) (pimozide impurity C), (1-[1-[4,4-bis(4-fluorophenyl)butyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2H-benzimidazol-2-one) (pimozide impurity D), (1-[1-[4,4-bis(4-fluorophenyl)butyl]piperidin-4-yl 1-oxide]-1,3-dihydro-2H-benzimidazol-2-one) (pimozide impurity E), is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.3 g in 50 ml of a mixture of 1 volume of *anhydrous glacial acetic acid* and 7 volumes of *methyl ethyl ketone* and titrate with *0.1 M perchloric acid,* using 0.2 ml of *naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml *0.1 M perchloric acid* is equivalent to 0.04616 g of $C_{28}H_{29}F_2N_3O$.

Storage. Store protected from light.

Pimozide Tablets

Pimozide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pimozide, $C_{28}H_{29}F_2N_3O$.

Usual strengths. 1 mg; 2 mg; 4 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 20 mg of Pimozide with 20 ml of *dichloromethane* for 5 minutes, filter and evaporate the filtrate to dryness under reduced pressure. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pimozide RS* or with the reference spectrum of pimozide.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate, if necessary with the dissolution medium.

Reference solution. A 0.0002 per cent w/v solution of *pimozide RS* in dissolution medium.

Use chromatographic system as described under Related substances.

Calculate the content of pimozide, $C_{28}H_{29}F_2N_3O$.

D. Not less than 70 per cent of the stated amount of $C_{28}H_{29}F_2N_3O$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of powdered tablets containing about 40 mg of Pimozide with 20 ml of *methanol* for 30 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid.

Reference solution (a). Dilute 1 ml of the test solution to 200 ml with *methanol.*

Reference solution (b). A solution containing 0.005 per cent w/v of *pimozide RS* and 0.002 per cent w/v of *mebendazole RS* in *methanol.*

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (3 µm) (Such as Hypersil ODS),
- mobile phase: A. a solution containing 0.25 per cent w/v of *ammonium acetate* and 0.85 per cent w/v of *tetrabutylammonium hydrogen sulphate,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
10	70	30
15	70	30
16	80	20
30	80	20

Inject reference solution (b). The test is not valid unless the resolution between the peaks obtained with mebendazole and pimozide is not less than 5.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.75 per cent).

Uniformity of content *(For tablets containing 10 mg or less).* Comply with the test stated under Tablets.

Test solution. Shake one tablet with 7 ml of *methanol* for 30 minutes, dilute with *methanol* to produce a solution containing 0.01 per cent w/v of Pimozide, mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid.

Reference solution (a). A 0.01 per cent w/v solution of *pimozide RS* in *methanol.*

Reference solution (b). A solution containing 0.005 per cent w/v of *pimozide RS* and 0.002 per cent w/v of *mebendazole RS* in *methanol.*

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to pimozide and mebendazole is not less than 5.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{28}H_{29}F_2N_3O$ in each tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of powdered tablets containing about 20 mg of Pimozide with 35 ml of *methanol* for 30 minutes, dilute to 50 ml with *methanol,* mix with the aid of ultrasound for 10 minutes, centrifuge and filter the supernatant liquid.

Reference solution (a). A 0.04 per cent w/v solution of *pimozide RS* in *methanol.*

Reference solution (b). A solution containing 0.004 per cent w/v of *pimozide RS* and 0.002 per cent w/v of *mebendazole RS* in *methanol.*

Use chromatographic system as described under Related substances.

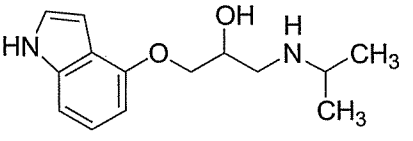
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to pimozide and mebendazole is not less than 5.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{28}H_{29}F_2N_3O$ in the tablets.

Storage. Store protected from light.

Pindolol



$C_{14}H_{20}N_2O_2$ Mol. wt. 248.3

Pindolol is (*RS*)-1-indol-4-yloxy-3-isopropylaminopropan-2-ol.

Pindolol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{20}N_2O_2$, calculated on the dried basis.

Category. Beta-adrenoceptor antagonist.

Dose. 10 to 30 mg daily.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pindolol RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in a 0.085 per cent v/v solution of *hydrochloric acid* in *methanol* shows absorption maxima at about 264 nm and at about 287 nm, and a shoulder at about 275 nm, absorbances at about 264 nm, 0.66 to 0.70 and at about 287 nm, 0.34 to 0.38.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *dilute acetic acid* is clear (2.4.1) and not more intensely coloured than reference solution BYS4 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

NOTE— Prepare the solutions immediately before use and protected from light.

Solvent mixture. 99 volumes of *methanol* and 1 volume of *anhydrous glacial acetic acid.*

Mobile phase. A freshly prepared mixture of 50 volumes of *ethyl acetate,* 50 volumes of *methanol* and 4 volumes of *strong ammonia solution.*

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of the solvent mixture.

Test solution (b). Dissolve 0.2 g of the substance under examination in 100 ml of the solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *pindolol RS* in the solvent mixture.

Reference solution (b). A 0.006 per cent w/v solution of *pindolol RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate immediately in a current of cold air and examine in ultraviolet at 254 nm without delay. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in 80 ml of *methanol.* Titrate with *0.1 M hydrochloric acid,* determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.02483 g of $C_{14}H_{20}N_2O_2$.

Storage. Store protected from light and moisture.

Pindolol Tablets

Pindolol Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pindolol, $C_{14}H_{20}N_2O_2$.

Usual strengths. 5 mg; 15 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Pindolol with 80 ml of *ether* for 30 minutes, filter and dry the extract with *anhydrous sodium sulphate.* Filter the extract, remove the *ether* using a rotary evaporator and dry the residue over *phosphorus pentoxide* at 110° at a pressure not exceeding 2 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pindolol RS.*

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 264 nm and 287 nm.

C. Shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 ml of a mixture of 99 volumes of *methanol* and 1 volume of *glacial acetic acid* for 45 minutes. Centrifuge and dilute 1 ml of the supernatant liquid to 50 ml with the *acetic acid-methanol* mixture. To 2 ml of this solution add 1 ml of *dimethylaminobenzaldehyde solution*; a violet-blue colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

NOTE— Prepare the solutions immediately before use and protected from light.

Solvent mixture. 99 volumes of *methanol* and 1 volume of *anhydrous glacial acetic acid.*

Mobile phase. A freshly prepared mixture of 50 volumes of *ethyl acetate*, 50 volumes of *methanol* and 4 volumes of *strong ammonia solution.*

Test solution. Shake a quantity of the powdered tablets containing 20 mg of Pindolol with 5 ml of the solvent mixture for 15 minutes, centrifuge and apply the supernatant liquid to the plate as the last solution.

Reference solution (a). Dilute 1 ml of the test solution to 10 ml with the solvent mixture and further dilute 7 ml of the solution to 100 ml with the solvent mixture.

Reference solution (b). Dilute 1 ml of the test solution to 10 ml with the solvent mixture and further dilute 3 ml of this solution to 100 ml with the solvent mixture.

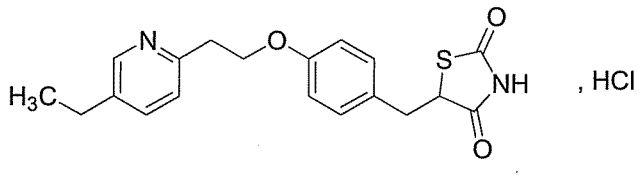
Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray immediately with *dimethylaminobenzaldehyde solution* and warm at 50° for 20 minutes. Any spot with R_f value of about 0.1 in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.7 per cent). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any spot remaining on the line of application.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 90 mg of Pindolol and shake with 100.0 ml of *methanol* for 45 minutes. Centrifuge and dilute 15.0 ml of the supernatant liquid to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 264 nm (2.4.7). Calculate the content of $C_{14}H_{20}N_2O_2$ taking 338 as the specific absorbance at 264 nm.

Storage. Store protected from light.

Pioglitazone Hydrochloride



$C_{19}H_{20}N_2O_3S,HCl$ Mol.Wt. 392.9

Pioglitazone Hydrochloride is (*RS*)-5-{4-[2-(5-ethyl-2-pyridinyl)ethoxy]benzyl}thiazolidine-2,4-dione hydrochloride.

Pioglitazone Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{20}N_2O_3S,HCl$, calculated on the dried basis.

Category. Antidiabetic.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pioglitazone hydrochloride RS* or with the reference spectrum of pioglitazone hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

Related Substances. Determine by liquid chromatography (2.4.14)

Test solution. Dissolve 30 mg of the substance under examination in 100.0 ml of *methanol.*

Reference solution (a). A 0.03 per cent w/v solution of *pioglitazone hydrochloride RS* in *methanol.*

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 25°,
- mobile phase: a mixture of 50 volumes of *0.01 M potassium dihydrogen phosphate* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by dying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14) as described under the test for Related substances.

Inject the test solution and reference solution (a).

Calculate the content of $C_{19}H_{20}N_2O_3S$,HCl.

Pioglitazone Tablets

Pioglitazone Hydrochloride Tablets

Pioglitazone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pioglitazone, $C_{19}H_{20}N_2O_3S$.

Usual strengths. 15 mg; 30 mg; 45 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of pioglitazone with 40 ml of *methanol,* filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pioglitazone hydrochloride RS* or with the reference spectrum of pioglitazone hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 270 nm (2.4.7). Calculate the content of $C_{19}H_{20}N_2O_3S$ in the medium from the absorbance obtained from a solution of known concentration *of pioglitazone hydrochloride RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{19}H_{20}N_2O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution: Weigh and powder 20 tablets. Transfer a quantity of the powder equivalent to 30 mg of pioglitazone and add about 150 ml of *methanol.* Mix with the aid of ultrasound at a temperature not exceeding 30° for 15 minutes and shake for 20 minutes. Dilute to 250 ml with *methanol* and mix. Filter through a membrane filter and discard the first 5 ml. Use the filtrate as the test solution.

Reference solution. A solution of *pioglitazone hydrochloride RS* in *methanol* containing 0.00012 per cent w/v of pioglitazone.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *0.01 M potassium dihydrogen phosphate* and 50 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (2.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 35 volumes of *water* and 65 volumes of *acetonitrile.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of pioglitazone, add about 20 ml of *water* and mix with the aid of ultrasound. Add about 150 ml of the solvent mixture and mix with the aid of ultrasound for 15 minutes. Dilute to 250.0 ml with the solvent mixture.

Reference solution. A 0.015 per cent w/v solution of *pioglitazone hydrochloride RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) ,
- mobile phase: a mixture of 35 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* and 1.15 g of *diammonium hydrogen phosphate* in 1000 ml of *water*, adding 1.0 ml of *triethylamine* and adjusting the pH to 5.0 with *orthophosphoric acid*, and 65 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,

– injection volume. 20 µl.

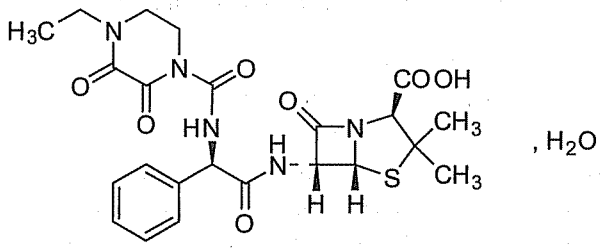
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{20}N_2O_3S$ in the tablets.

Labelling. The label states the strength in terms of the equivalent amount of pioglitazone.

Piperacillin



$C_{23}H_{27}N_5O_7S,H_2O$ Mol.Wt. 535.6

Piperacillin is (6*R*)-6-[*R*-2-(4-ethyl-2,3-dioxopiperazine-1-carboxamido)-2-phenylacetamido]penicillanic acid monohydrate.

Piperacillin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{23}H_{27}N_5O_7S$, calculated on the anhydrous basis.

Category. Penicillin antibacterial.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piperacillin RS or* with the reference spectrum of piperacillin.

Tests

Appearance of solution. A 10 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than the reference solution BS8 (2.4.1).

Specific optical rotation (2.4.22). + 165° to + 175°, determined in a 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of 3.12 per cent w/v solution of *sodium dihydrogen phosphate.*

Test solution (a). Dissolve 25 mg of the substance under examination in 50 ml of the solvent mixture.

Test solution (b). Dissolve 40 mg of the substance under examination in 20 ml of the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *piperacillin RS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 25 ml with the solvent mixture.

Reference solution (c). Dissolve 10 mg of *piperacillin RS* and 10 mg of *anhydrous ampicillin RS* (*piperacillin impurity A RS*) in 50 ml solvent mixture.

Reference solution (d). Dilute 1.0 ml of reference solution (a) to 100 ml with the solvent mixture. Dilute 1.0 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system

– a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
– mobile phase: A. a mixture of 576 volumes of *water,* 200 volumes of 3.12 per cent w/v solution of *sodium dihydrogen phosphate* and 24 volumes of an 8.0 per cent w/v solution of *tetrabutylammonium hydroxide,* adjusted to pH 5.5 with *dilute phosphoric acid* or *dilute sodium hydroxide solution*; add 200 volumes of *acetonitrile,*

B. a mixture 126 volumes of *water,* 200 volumes of a 3.12 per cent w/v solution of *sodium dihydrogen phosphate* and 24 volumes of an 8.0 per cent w/v solution of *tetrabutylammonium hydroxide,* adjusted to pH 5.5 with *dilute phosphoric acid* or *dilute sodium hydroxide solution*; add 650 volumes of *acetonitrile,*.

– a linear gradient programme using the conditions given below,
– flow rate. 1 ml per minute,
– spectrophotometer set at 220 nm,
– injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
$0 - t_g$	88	12
$t_g - (t_g + 30)$	88→0	12→100
$(t_g + 30) - (t_g - 45)$	0→88	100→12

where, t_g = retention time of piperacillin determined with reference solution (b)

Inject reference solution (b), (c) and (d). The test is not valid unless the resolution between the peaks due to piperacillin impurity A and piperacillin in the chromatogram obtained with

reference solution (c) is not less than 10; signal-to-noise ratio is not less than 3 for the principal peak in the chromatogram obtained with reference solution (d); mass distribution ratio is (2:3) for the peak due to piperacillin in the chromatogram obtained with reference solution (c). The area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

***N,N*-Dimethylaniline** (2.3.21). Not more than 20 ppm.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water. (2.3.43). 2.0 per cent to 4.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14), as described in the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject test solution (a) and reference solution (a).

Calculate the content of $C_{23}H_{27}N_5O_7S$.

Storage. Store protected from light and moisture.

Piperacillin Intravenous Infusion

Piperacillin Intravenous Infusion is a sterile solution of Piperacillin Sodium in Water for Injections. It is prepared by dissolving Piperacillin for Intravenous Infusion in the requisite amount of Water for Injections before use.

The intravenous infusion complies with the requirements stated under Parenteral Preparations (Injections).

Piperacillin for Intravenous Infusion

Piperacillin for Intravenous Infusion is a sterile material consisting of Piperacillin Sodium or Piperacillin Sodium prepared by the interaction of piperacillin with *sodium bicarbonate*, with or without excipients. It is filled in a sealed container.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parental Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Piperacillin for Intravenous Infusion contains not less than 94.0 per cent and not more than 115.0 per cent of the stated amount of piperacillin, $C_{23}H_{27}N_5O_7S$.

Usual strengths. 1 g per vial; 2 g per vial; 4 g per vial.

The contents of the sealed container comply with the requirements stated under Parental Preparations (Powder for Injections) and with the following requirements.

Identification

A. Dissolve a quantity of the powder containing about 0.25 g of anhydrous piperacillin in 20 ml of *water*, add 0.5 ml of *dilute hydrochloric acid,* mix well, allow to stand until a precipitate appears and filter. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piperacillin RS* or with the reference spectrum of piperacillin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

C. Gives the reaction of sodium (2.3.1).

Tests

pH (2.4.24). 4.8 to 6.8, determined in a 10.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powder containing about 39 mg of anhydrous piperacillin in 100 ml of the mobile phase.

Reference solution (a). A 0.0008 per cent w/v solution of *piperacillin RS* in the mobile phase.

Reference solution (b). A 0.0004 per cent w/v solution of *piperacillin RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 to 10 µm), (Such as Beckman Ultrasphere ODS),
- mobile phase: a mixture of 0.3 volume of *0.4 M tetrabutylammonium hydroxide*,, 10 volumes of *0.2 M sodium dihydrogen orthophosphate monohydrate*, 44.7 volumes of *water* and 45 volumes of *methanol,* cool and adjust to pH 5.5 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with the test solution the area of the peak with a relative retention time of about 0.6 is not more than 3.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent).

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Bacterial endotoxins (2.2.3). Not more than 2.5 Endotoxin Units per ml of a solution prepared in the following manner. Dissolve a quantity in *water BET* to obtain a solution containing 50 mg of piperacillin per ml. Carry out the test using Maximum Valid dilution of this solution calculated from the declared sensitivity of the lysate used in the test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powder containing about 39 mg of anhydrous piperacillin in 100 ml of the mobile phase.

Reference solution (a). Dissolve 40 mg of *piperacillin RS* in *methanol* and dilute to 100 ml with the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v of *anhydrous ampicillin RS* and 0.02 per cent w/v of *piperacillin RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Beckman Ultrasphere ODS),
- mobile phase: a mixture of 0.3 volume of *0.4 M tetrabutylammonium hydroxide*, 10 volumes of *0.2 M sodium dihydrogen orthophosphate monohydrate*, 44.7 volumes of *water* and 45 volumes of *methanol*, cool and adjust to pH 5.5 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject reference solution (b). The resolution between the peaks due to anhydrous ampicillin and piperacillin is not less than 16.

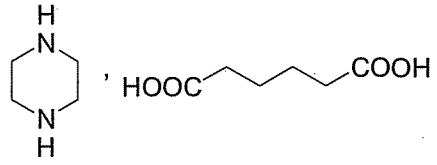
Inject the test solution and the reference solution (a).

Calculate the content of $C_{23}H_{27}N_5O_7S$.

Storage. Store protected from moisture.

Labelling. The label of the sealed container states the quantity of active ingredient contained in it in terms of the equivalent amount of anhydrous piperacillin.

Piperazine Adipate



$C_4H_{10}N_2, C_6H_{10}O_4$ Mol. Wt. 232.3

Piperazine Adipate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_{10}N_2$, $C_6H_{10}O_4$, calculated on the anhydrous basis.

Category. Anthelmintic.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piperazine adipate RS* or with the reference spectrum of piperazine adipate.

B. In the test for Related substances, examine the plate after spraying with both the *ninhydrin* and *iodine* solutions. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.1 g in 5 ml of *water*, add 0.5 g of *sodium bicarbonate*, 0.5 ml of a freshly prepared 5.0 per cent w/v solution of *potassium ferricyanide* and 0.1 ml of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes; a reddish colour slowly develops.

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 5.0 to 6.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 60 volumes of *strong ammonia solution* and 40 volumes of *ethanol*.

Mobile phase. A freshly prepared mixture of 80 volumes of *acetone* and 20 volumes of *strong ammonia solution*.

Test solution (a). Dissolve 1 g of the substance under examination in 10 ml of the solvent mixture.

Test solution (b). Dissolve 1 g of the substance under examination in 100 ml of the solvent mixture.

Reference solution (a). A 1 per cent w/v solution of *piperazine adipate RS* in the solvent mixture.

Reference solution (b). A 0.025 per cent w/v solution of *ethylenediamine* in the solvent mixture.

Reference solution (c). A 0.025 per cent w/v solution of *triethylenediamine* in the solvent mixture.

Reference solution (d). A solution containing 0.025 per cent w/v of *triethylenediamine* and 1 per cent w/v of the substance under examination in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105°, spray with a 0.3 per cent w/v solution of *ninhydrin* in a mixture of 3 volumes of *anhydrous acetic acid* and 100 volumes of *1-butanol* and then with a 0.15 per cent w/v solution of *ninhydrin* in *ethanol* and dry at 105° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with *0.05 M iodine* and allow to stand for about 10 minutes. Any spot corresponding to *triethylenediamine* in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the chromatogram obtained with reference solution (d) shows two separated spots. Ignore any spot remaining on the line of application.

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of *water*, 0.5 ml of *0.1 M hydrochloric acid* and add sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.2 g, dissolve in 10 ml of *anhydrous glacial acetic acid* with gentle heating and dilute to 70 ml with the same solvent. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *1-naphtholbenzein solution* as indicator and titrating until the colour of the solution changes from brownish yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01161 g of $C_4H_{10}N_2$, $C_6H_{10}O_4$.

Storage. Store protected from moisture.

Piperazine Adipate Tablets

Piperazine Adipate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of piperazine hydrate, $C_4H_{10}N_2$, $6H_2O$.

Usual strength. The equivalent of 300 mg of piperazine hydrate. (144 mg of Piperazine Adipate is approximately equivalent to 120 mg of Piperazine Hydrate).

Identification

A. Extract a quantity of the powdered tablets containing 1 g of Piperazine Hydrate with 20 ml of *water* and filter. Dilute 1 ml of the filtrate to 5 ml with *water*, add 0.5 g of *sodium bicarbonate*, 0.5 ml of a freshly prepared 5.0 per cent w/v solution of *potassium ferricyanide* and 0.1 ml of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes; a reddish colour slowly develops.

B. To 10 ml of the filtrate obtained in test A add 5 ml of *hydrochloric acid* and extract with three quantities, each of 10 ml, of *ether*, evaporate the combined *ether* extracts to dryness; the residue, after washing with a small volume of *water* and drying at 105°, melts at about 152° (2.4.21).

Tests

Other tests. Comply with the tests stated under Tablets.

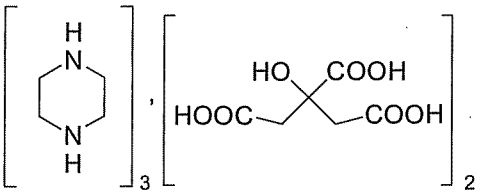
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Piperazine Hydrate, shake with 10 ml of *water* for 1 hour, filter and wash the residue with two quantities, each of 10 ml, of *water*. To the combined extract and washings add 5 ml of *1 M sulphuric acid* and 50 ml of *picric acid solution*, bring to boil, allow to stand for 1 hour and filter through a sintered-glass crucible (porosity No. 4) and wash the residue with successive quantities, each of 10 ml, of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash with five quantities, each of 10 ml, of *ethanol* and dry to constant weight at 105°.

1 g of the residue is equivalent to 0.3567 g of $C_4H_{10}N_2$, $6H_2O$.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of piperazine hydrate.

Piperazine Citrate



$(C_4H_{10}N_2)_3,2C_6H_8O_7$ Mol. Wt. 642.7 (anhydrous)

Piperazine Citrate is a salt of piperazine with citric acid containing a variable amount of water of crystallisation.

Piperazine Citrate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_4H_{10}N_2)_3$, $2C_6H_8O_7$, calculated on the anhydrous basis.

Category. Anthelmintic.

Dose. For an adult, in the treatment of threadworm infestation, equivalent of 1 to 2 g of Piperazine Hydrate daily, in divided doses for 7 days; in the treatment of roundworm infestation, equivalent of 4.5 g of Piperazine Hydrate as a single dose. For a child, in the treatment of threadworm infestation, the

equivalent of 40 mg of Piperazine Hydrate per kg of body weight daily, in divided doses for 7 days; in the treatment of roundworm infestation, as a single dose, the equivalent of 120 mg of Piperazine Hydrate per kg of body weight, up to a maximum dose of 4 g.

(150 mg of Piperazine Citrate or 132 mg of anhydrous piperazine citrate is approximately equivalent to 120 mg of Piperazine Hydrate).

Description. A white, granular powder; almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piperazine citrate RS* or with the reference spectrum of piperazine citrate.

B. In the test for Related substances, examine the plate after spraying with both the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. A 10 per cent w/v solution gives the reactions of citrates (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 5.0 to 6.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*.

Solvent mixture. 60 volumes of *strong ammonia solution* and 40 volumes of *ethanol.*

Mobile phase. A freshly prepared mixture of 80 volumes of *acetone* and 20 volumes of *strong ammonia solution.*

Test solution (a). Dissolve 1 g of the substance under examination in 10 ml of the solvent mixture.

Test solution (b). Dissolve 1 g of the substance under examination in 100 ml of the solvent mixture.

Reference solution (a). A 1.0 per cent w/v solution of *piperazine citrate RS* in the solvent mixture.

Reference solution (b). A 0.025 per cent w/v solution of *ethylenediamine* in the solvent mixture.

Reference solution (c). A 0.025 per cent w/v solution of *triethylenediamine* in the solvent mixture.

Reference solution (d). A solution containing 0.025 per cent w/v of *triethylenediamine* and 1 per cent w/v of the substance under examination in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105°, spray with a 0.3 per cent w/v solution of *ninhydrin* in a mixture of 3 volumes of *anhydrous acetic acid* and 100 volumes of *1-butanol* and then with a 0.15 per cent w/v solution of *ninhydrin* in *ethanol* and dry at 105° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with *0.05 M iodine* and allow to stand for about 10 minutes. Any spot corresponding to *triethylenediamine* in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the chromatogram obtained with reference solution (d) shows two separated spots. Ignore any spot remaining on the line of application.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.4.19). 10.0 to 14.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.2 g, dissolve in 10 ml of *anhydrous glacial acetic acid* with gentle heating and dilute to 70 ml with the same solvent. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *1-naphtholbenzein solution* as indicator and titrating until the colour of the solution changes from brownish yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01071 g of $(C_4H_{10}N_2)_3, 2C_6H_8O_7$.

Storage. Store protected from light and moisture.

Piperazine Citrate Syrup

Piperazine Citrate Oral Solution; Piperazine Citrate Elixir

Piperazine Citrate Syrup is a solution of Piperazine Citrate in a suitable flavoured Vehicle.

Piperazine Citrate Syrup contains the equivalent of not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of piperazine hydrate, $C_4H_{10}N_2, 6H_2O$.

Usual strength. The equivalent of 750 mg of piperazine hydrate in 5 ml.

(125 mg of Piperazine Citrate or 110 mg of anhydrous piperazine citrate is approximately equivalent to 100 mg of Piperazine Hydrate).

Identification

A. To 1 ml add 5 ml of *2 M hydrochloric acid* and, with stirring, 1 ml of a freshly prepared 50 per cent w/v solution of *sodium nitrite*, cool in ice for 15 minutes, induce crystallisation, wash

the crystalline precipitate with *water* and dry at 105°. The crystals melt at about 159° (2.4.21).

B. Warm 10 ml with *activated charcoal* and filter. Boil a portion of the filtrate with an excess of *mercuric sulphate solution*, filter, boil the filtrate and add 0.25 ml of *dilute potassium permanganate solution*; the permanganate solution is decolorised and a white precipitate is produced.

C. Acidify a portion of the filtrate obtained in test B with *1 M sulphuric acid*, add 0.25 ml of *dilute potassium permanganate solution*, warm until the colour is discharged and add an excess of *bromine water*; a white precipitate is produced either immediately or on cooling.

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity containing about 0.2 g of Piperazine Hydrate, add 3.5 ml of *0.5 M sulphuric acid* and 10 ml of *water*, add 100 ml of *picric acid solution*, heat on a water-bath for 15 minutes, allow to stand for 1 hour and filter through a sintered-glass crucible (porosity No. 4). Wash the residue with successive quantities, each of 10 ml, of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash the residue with five quantities, each of 10 ml, of *ethanol* and dry to constant weight at 105°.

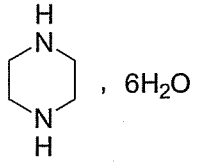
1 g of the residue is equivalent to 0.3567 g of $C_4H_{10}N_2, 6H_2O$.

Determine the weight per ml of the syrup (2.4.29), and calculate the content of $C_4H_{10}N_2, 6H_2O$, weight in volume.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of piperazine hydrate.

Piperazine Hydrate



$C_4H_{10}N_2, 6H_2O$ Mol. Wt. 194.2

Piperazine Hydrate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_4H_{10}N_2, 6H_2O$.

Category. Anthelmintic.

Dose. For an adult, in the treatment of threadworm infestation, 1 to 2 g daily, in divided doses for 7 days; in the treatment of roundworm infestation, 4 g as a single dose. For a child, in the treatment of threadworm infestation, 40 mg per kg of body weight daily, in divided doses for 7 days. In the treatment of roundworm infestation, as a single dose, 120 mg per kg of body weight, up to a maximum dose of 4 g.

Description. Colourless, glassy deliquescent crystals.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piperazine hydrate RS*.

B. In the test for Related substances, examine the plate after spraying with both the ninhydrin solutions. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Dissolve 0.2 g in 5 ml of *dilute hydrochloric acid*, add 0.5 g of *sodium nitrite* and heat to boiling. Cool in ice for 15 minutes, scratching the walls of the container with a glass rod and filter. The crystals, after washing with 10 ml of ice-cold *water* and drying at 105°, melt at about 159° (2.4.21).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 10.5 to 12.0, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 60 volumes of *strong ammonia solution* and 40 volumes of *ethanol*.

Mobile phase. A freshly prepared mixture of 80 volumes of *acetone* and 20 volumes of *strong ammonia solution*.

Test solution (a). Dissolve 1 g of the substance under examination in 10 ml of the solvent mixture.

Test solution (b). Dissolve 1 g of the substance under examination in 100 ml of the solvent mixture.

Reference solution (a). A 1 per cent w/v solution of *piperazine hydrate RS* in the solvent mixture.

Reference solution (b). A 0.025 per cent w/v solution of *ethylenediamine* in the solvent mixture.

Reference solution (c). A 0.025 per cent w/v solution of *triethylenediamine* in the solvent mixture.

Reference solution (d). A solution containing 0.025 per cent w/v of *triethylenediamine* and 1 per cent w/v of the substance under examination in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105°, spray with a 0.3 per cent w/v solution of *ninhydrin* in a mixture of 3 volumes of *anhydrous acetic acid* and 100 volumes of *1-butanol* and then with a 0.15 per cent w/v solution of *ninhydrin* in *ethanol* and dry at 105° for 10 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b). Spray the plate with *0.05 M iodine* and allow to stand for about 10 minutes. Any spot corresponding to triethylenediamine in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless the chromatogram obtained with reference solution (d) shows two separated spots. Ignore any spot remaining on the line of application.

Heavy metals (2.3.13).1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.2 g, dissolve in 10 ml of *anhydrous glacial acetic acid* with gentle heating and dilute to 70 ml with the same solvent. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *1-naphtholbenzein solution* as indicator and titrating until the colour of the solution changes from brownish yellow to green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.009705 g of $C_4H_{10}N_2, 6H_2O$.

Storage. Store protected from light and moisture.

Piperazine Phosphate

$C_4H_{10}N_2, H_3PO_4, H_2O$ Mol. Wt. 202.2

Piperazine Phosphate contains not less than 98.5 per cent and not more than 100.5 per cent of $C_4H_{10}N_2, H_3PO_4$, calculated on the anhydrous basis.

Category. Anthelmintic.

Dose. For an adult, in the treatment of threadworm infestation, the equivalent of 1 to 2 g of Piperazine Hydrate daily, in divided doses for 7 days; in the treatment of roundworm infestation, the equivalent of 4.5 g of Piperazine Hydrate as a single dose. For a child, in the treatment of threadworm infestation, the equivalent of 40 mg of Piperazine Hydrate per kg of body weight daily, in divided doses for 7 days; in the treatment of roundworm infestation, as a single dose, the equivalent of 120 mg of Piperazine Hydrate per kg of body weight, up to a maximum dose of 4 g.

(125 mg of piperazine phosphate is approximately equivalent to 120 mg of Piperazine Hydrate).

Description. A white, crystalline powder; odourless or almost odourless.

Identification

A. Dissolve 0.2 g in 5 ml of *dilute hydrochloric acid*, add 0.5 g of *sodium nitrite* and heat to boiling. Cool in ice for 15 minutes, scratching the walls of the container with a glass rod and filter. The crystals, after washing with 10 ml of ice-cold *water* and drying at 105°, melt at about 159° (2.4.21).

B. Dissolve 0.1 g in 5 ml of *water*, add 0.5 g of *sodium bicarbonate*, 0.5 ml of a freshly prepared 5.0 per cent w/v solution of *potassium ferricyanide* and 0.1 ml of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes; a reddish colour slowly develops.

C. Gives the reactions of phosphates (2.3.1).

Tests

pH (2.4.24). 6.0 to 6.5, determined in a 1.0 per cent w/v solution.

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of *2 M acetic acid* and add sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Water (2.3.43). 8.0 to 9.5 per cent, determined on 0.25 g.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 3.5 ml of *0.5 M sulphuric acid* and 10 ml of *water*. Add 100 ml of *picric acid solution*, heat on a water-bath for 15 minutes and allow to stand for 1 hour. Filter through a sintered-glass crucible (porosity No. 4) and wash the residue with successive quantities, each of 10 ml, of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash the residue with five quantities, each of 10 ml, of *ethanol* and dry to constant weight at 105°.

1 g of the residue is equivalent to 0.3382 g of $C_4H_{10}N_2, H_3PO_4$.

Storage. Store protected from moisture.

Piperazine Phosphate Tablets

If the tablets are intended to be chewed before swallowing they may contain suitable flavouring agents.

Piperazine Phosphate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of piperazine phosphate, $C_4H_{10}N_2, H_3PO_4, H_2O$.

Usual strengths. The equivalent of 260 mg; 520 mg Piperazine Hydrate (125 mg of piperazine phosphate is approximately equivalent to 120 mg of Piperazine Hydrate).

Identification

Extract a quantity of the powdered tablets containing 1 g of Piperazine Phosphate with 20 ml of *water* and filter. The filtrate complies with the following tests.

A. To 4 ml of the filtrate, add 1 ml of *hydrochloric acid*, 0.5 g of *sodium nitrite* and heat to boiling. Cool in ice for 15 minutes, scratching the walls of the container with a glass rod and filter. The crystals, after washing with 10 ml of ice-cold *water* and drying at 105°, melt at about 159° (2.4.21).

B. Dilute 1 ml of the filtrate to 5 ml with *water*, add 0.5 g of *sodium bicarbonate*, 0.5 ml of a freshly prepared 5.0 per cent w/v solution of *potassium ferricyanide* and 0.1 ml of *mercury*, shake vigorously for 1 minute and allow to stand for 20 minutes; a reddish colour slowly develops.

C. Gives the reactions of phosphates (2.3.1).

Tests

Disintegration. The test does not apply to Piperazine Phosphate Tablets intended to be chewed before swallowing.

Other tests. Comply with the tests stated under Tablets.

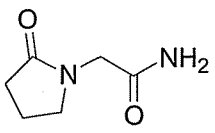
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Piperazine Phosphate, shake with 10 ml of *water* for 1 hour, filter and wash the residue with two quantities, each of 10 ml, of *water*. To the combined extract and washings add 5 ml of *1 M sulphuric acid* and 50 ml of *picric acid solution*, boil, allow the mixture to stand for several hours and filter through a sintered glass crucible (porosity No. 4). Wash the residue with successive quantities, each of 10 ml, of a mixture of equal volumes of a saturated solution of *picric acid* and *water* until the washings are free from sulphate. Wash the residue with five quantities, each of 10 ml, of *ethanol (95 per cent)* and dry to constant weight at 105°.

1 g of the residue is equivalent to 0.3714 g of $C_4H_{10}N_2$, H_3PO_4, H_2O.

Storage. Store protected from moisture.

Labelling. The label states, where applicable, that the tablets are to be chewed before swallowing.

Piracetam



$C_6H_{10}N_2O_2$ Mol.Wt.142.2

Piracetam is 2-(2-oxopyrrolidin-1-yl)acetamide.

Piracetam contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_{10}N_2O_2$, calculated on the dried basis.

Description. A white or almost white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piracetam RS* or with the reference spectrum of piracetam.

Tests

Appearance of solution. A 20.0 per cent w/v solution in *water* is clear (2.4.1) and colourless (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 10 volumes of *acetonitrile* and 90 volumes of *water*.

Test solution (a). Dissolve 50.0 mg of the substance under examination in 100.0 ml of the solvent mixture.

Test solution (b). Dilute 10.0 ml of test solution (a) to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve 5 mg of the substance under examination and 10 µl of 2-pyrrolidone in sufficient solvent mixture to produce 100.0 ml.

Reference solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (c). Dissolve 50.0 mg of *piracetam RS* in 100.0 ml of the solvent mixture. Dilute 10.0 ml of this solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with end capped octadecylsilane bonded to silica (5 µm),
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of 0.1 per cent w/v solution of *dipotassium hydrogen phosphate*, adjusted to pH 6.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to 2-pyrrolidone and piracetam is not less than 3.0 and the symmetry factor is not more than 2.0 for the peak due to piracetam.

Inject test solution (a) and reference solution (b). Run the chromatogram for 8 times the retention time of piracetam. In the chromatogram obtained with the test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.1 per cent) and the sum of areas of all the secondary

peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy Metals (2.3.13). 2.0 g complies with limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14) as described in the test for Related substances.

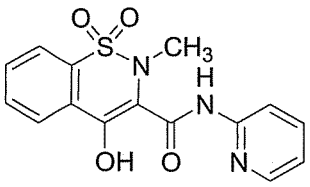
Inject reference solution (c). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject test solution (b) and reference solution (c).

Calculate the content of $C_6H_{10}N_2O_2$.

Storage. Store protected from light and moisture.

Piroxicam



$C_{15}H_{13}N_3O_4S$ Mol. Wt. 331.4

Piroxicam is 4-hydroxy-2-methyl-*N*-(2-pyridyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide.

Piroxicam contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{15}H_{13}N_3O_4S$, calculated on the anhydrous basis.

Category. Analgesic; antiinflammatory; antipyretic.

Dose. 10 to 20 mg daily.

Description. An off-white to light tan or light yellow powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *piroxicam RS* or with the reference spectrum of piroxicam.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M methanolic hydrochloric acid* shows absorption maxima at about 242 nm and 334 nm and a minimum at about 270 nm; absorbance at about 334 nm, about 0.87.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 95 volumes of *toluene* and 5 volumes of *acetic acid*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of a mixture of equal volumes of *chloroform* and *methanol*.

Reference solution. A 0.1 per cent w/v solution of *piroxicam RS* in a mixture of equal volumes of *chloroform* and *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 75 mg of the substance under examination in 50.0 ml of *acetonitrile*.

Reference solution. Dilute 1.0 ml of the test solution to 10.0 ml with *acetonitrile*. Dilute 1.0 ml of this solution to 50.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of a 0.7 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the symmetry factor of the peak due to piroxicam impurity B is not more than 5.0. The relative retention time with reference to piroxicam for piroxicam impurity B is about 0.85.

Inject the test solution and the reference solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the sum of all the secondary peaks is not

more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Heavy metals (2.3.13). 0.4 g complies with the limit test for heavy metals, Method B (50 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 0.5 per cent , determined on 2.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. A 0.005 per cent w/v solution of the substance under examination in *0.1 M methanolic hydrochloric acid.*

Reference solution. A 0.005 per cent w/v solution of *piroxicam RS* in *0.1 M methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *methanol* and 55 volumes of a buffer solution prepared by diluting a mixture of 7.72 g of *anhydrous citric acid* in 400 ml of *water* and 5.35 g of *sodium phosphate* in 100 ml of *water* to 1000 ml with *water,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{15}H_{13}N_3O_4S$.

Storage. Store protected from light and moisture.

Piroxicam Capsules

Piroxicam Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of piroxicam, $C_{15}H_{13}N_3O_4S$.

Usual strengths. 10 mg; 20 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 95 volumes of *toluene* and 5 volumes of *acetic acid.*

Test solution. Dissolve a portion of the contents of the capsules in sufficient of a mixture of equal volumes of *chloroform* and *methanol* to obtain a solution containing about 0.1 per cent w/v of Piroxicam. Shake for 10 minutes, filter and use the filtrate.

Reference solution. A 0.1 per cent w/v solution of *piroxicam RS* in a mixture of equal volumes of *chloroform* and *methanol.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2,

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 100 rpm and 45 minutes.

Withdraw 10 ml of the medium and filter. Measure the absorbance of the filtrate (2.4.7), suitably diluted if necessary, at the maximum at about 242 nm. Calculate the content of $C_{15}H_{13}N_3O_4S$, in the medium taking 352 as the specific absorbance at 242 nm.

D. Not less than 75 per cent of the stated amount of $C_{15}H_{13}N_3O_4S$.

Uniformity of content. *(For capsules containing 10 mg or less)*— Comply with the test stated under Capsules.

Test solution. Dissolve the contents of a capsule in 100.0 ml of *0.1 M methanolic hydrochloric acid* and filter. Dilute further if necessary.

Determine by liquid chromatography (2.4.14) using the chromatographic system and the reference solution described under Assay.

Calculate the content of $C_{15}H_{13}N_3O_4S$ in the capsule.

Water (2.3.43). Not more than 8.0 per cent, determined on 0.25 g.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve an accurately weighed quantity of the mixed contents of the capsules containing about 50 mg of Piroxicam in 100.0 ml of *0.1 M methanolic hydrochloric acid.* Further dilute 1.0 ml of the solution to 10.0 ml with the same solvent.

Reference solution. A 0.005 per cent w/v solution of *piroxicam RS* in *0.1 M methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 45 volumes of *methanol* and 55 volumes of a buffer solution prepared by diluting a mixture of 7.72 g of *anhydrous citric acid* in 400 ml of *water* and 5.35 g of *sodium phosphate* in 100 ml of *water* to 1000 ml with *water*;
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{15}H_{13}N_3O_4S$ in the capsules.

Storage. Store protected from light and moisture.

Plaster of Paris

Dried Calcium Sulphate; Exsiccated Calcium Sulphate

$CaSO_4, ½H_2O$ Mol. Wt. 145.1

Plaster of Paris is prepared by heating powdered gypsum, $CaSO_4,½H_2O$, at about 150° in a controlled manner such that it is substantially converted into the hemihydrate, $CaSO_4,½H_2O$, with minimum production of the anhydrous phases of calcium sulphate. It may contain suitable accelerators or decelerators.

Category. Surgical aid.

Description. A white or almost white powder; odourless or almost odourless; hygroscopic.

Identification

Gives the reactions of calcium salts and of sulphates (2.3.1).

Tests

pH (2.4.24). 6.5 to 9.0, determined in a 20.0 per cent w/v slurry in *water*.

Acid insoluble matter. Dissolve 0.5 g in 30 ml of a mixture of 1 volume of *hydrochloric acid* and 2 volumes of *water* and evaporate to dryness in a dish on a water-bath. Heat for 2 hours at 120° and again add 20 ml of the acid mixture. Warm for a few minutes and filter. Wash the residue with warm *water* until free from chlorides, dry, ignite and weigh. The residue weighs not more than 5 mg (1.0 per cent).

Setting properties. 20 g mixed with 10 ml of *water* at 15° to 20° in a cylindrical mould about 2.4 cm in diameter sets in not less than 4 minutes and not more than 6 minutes. The mass thus formed, after standing for 3 hours, possesses sufficient hardness to resist pressure of the fingers at the edges, which retain their sharpness of outline and do not crumble under pressure.

Loss on ignition (2.4.20). 4.5 to 8.0 per cent, determined by igniting to constant weight at red heat.

Storage. Store protected from moisture.

Poloxamers

$(CH_2CH_2O)_a\,(CH(CH_3)CH_2OH)_b\,(CH_2CH_2O)_c\,H$

b is at least 15 and $(CH_2CH_2O)_{a+c}$ is varied from 20 to 90% by weight

Mol. Wt. ranges from 1000 to >16000

Poloxamers are polyethylenepropylpropyleneglycols.

Synthetic block copolymer of ethylene oxide and propylene oxide represented by the following general formula:

Poloxamer Type	Ethylene oxide units (a)	Propylene oxide units (b)	Content of oxyethylene (per cent)	Average relative molecular mass
124	10-15	18-23	44.8-48.6	2090-2360
188	75-85	25-30	79.9-83.7	7680-9510
237	60-68	35-40	70.5-74.3	6840-8830
338	137-146	42-47	81.4-84.9	12700-17400
407	95-105	54.60	71.5-74.9	9840-14600

A suitable antioxidant may be added.

Description. Poloxamer 124 is a colourless or almost colourless liquid and poloxamers 188, 237, 338, 407 are a white or almost white, waxy powder, microbeads or flakes.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *poloxamer RS* or with the reference spectrum of poloxamer.

B. Average relative molecular mass.

C. Oxypropylene: oxyethylene ratio.

Tests

Melting point (2.4.21). About 50° for poloxamers 188, 237, 338 and 407.

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 5.0 to 7.5, determined in solution A.

Ethylene oxide, propylene oxide and dioxan. Determine by gas chromatography (2.4.13).

Ethylene oxide stock solution. Disperse about 0.5 g of *ethylene oxide RS* with 50.0 ml of *dimethyl sulphoxide.*

Ethylene oxide solution. Dilute 1.0 ml of the ethylene oxide stock solution to 250.0 ml with *dimethyl sulphoxide.*

Propylene oxide stock solution. Introduce about 7 ml of *dichloromethane* into a volumetric flask, add 0.5 g of *propylene oxide* and dilute to 10.0 ml with *dichloromethane.* Dilute 0.5 ml of this solution to 50.0 ml with *dimethyl sulphoxide.*

Propylene oxide solution. Dilute 1.0 ml of the propylene oxide stock solution to 50.0 ml with *dimethyl sulphoxide.*

Dioxan solution. Introduce 0.1 g of *dioxan* into a flask and dilute to 50.0 ml with *dimethyl sulphoxide.* Dilute 2.5 ml of this solution to 100.0 ml with *dimethyl sulphoxide.*

Mixture solution. Dilute a mixture of 6.0 ml of the ethylene oxide solution, 6.0 ml of the propylene oxide solution and 2.5 ml of the dioxan solution to 25.0 ml with *dimethyl sulphoxide.*

Test solution. To 1.0 g of the substance under examination in a head-space vial, add 4.0 ml of *dimethyl sulphoxide* and close the vial immediately.

Reference solution. To 1.0 g of the substance under examination in a head-space vial, add 2.0 ml of *dimethyl sulphoxide* and 2.0 ml of the mixture solution. Close the vial immediately.

Chromatographic system
- a fused silica column 50 m x 0.32 mm, packed with poly(dimethyl)(diphenyl)siloxane (film thickness 5 µm),
- temperature :
  column 70° to 240°,
  inlet port and detector at 250°,
- a flame ionisation detector,
- flow rate. 1.4 ml per minute using nitrogen as the carrier gas,

Head-space conditions
- equilibration temperature 110°,
- equilibration time 30 minutes,
- transfer line temperature 140°,
- pressurisation time 1 minute,
- injection time 0.05 minute.

Inject 1 µl of the gaseous phase of the test solution and the reference solution. The relative retention time with reference to ethylene oxide is about 6 minutes, for propylene oxide is about 1.3, for dichloromethane is about 1.6, for dioxan is about 3.0, for dimethyl sulphoxide is about 3.7.

The ethylene oxide is not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (1 ppm); propylene oxide is not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (5 ppm) and dioxan is not more than 0.5 times the area of the corresponding peak in the chromatogram obtained with the reference solution (10 ppm).

Average relative molecular mass. Weigh 15 g of the substance under examination into a 250-ml ground glass stoppered flask, add 25.0 ml of *phthalic anhydride solution* and a few glass beads and swirl to dissolve. Boil gently under a reflux condenser for 60 minutes, cool and add 2 quantities, each of 10 ml, of *pyridine*, through the condenser. Add 10 ml of *water*, mix and allow to stand for 10 minutes. Add 40.0 ml of *0.5 M sodium hydroxide* and 0.5 ml of a 1.0 per cent w/v solution of *phenolphthalein* in *pyridine*. Titrate with *0.5 M sodium hydroxide* to a light pink endpoint that persists for 15 second and record the volume of sodium hydroxide used (S). Prepare a blank. Record the volume of sodium hydroxide used (B). Calculate the average relative molecular mass using the following expression:

Oxypropylene:oxyethylene ratio. Nuclear magnetic resonance spectrometry (2.4.34).

Use a 10.0 per cent w/v solution of the substance under examination in *deuterated chloroform.* Record the average area of the doublet appearing at about 1.08 ppm due to the methyl groups of the oxypropylene units (A_1) and the average area of the composite band from 3.2 ppm to 3.8 ppm due to CH_2O groups of both the oxyethylene and oxypropylene units and the CHO groups of the oxypropylene units (A_2) with reference to the internal standard. Calculate the percentage of oxyethylene, by weight, in the sample under examination using the following expression:

Total ash (2.3.19). Not more than 0.4 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Storage. Store protected from moisture.

Labelling. The label states the type of poloxamer.

Polyethylene Glycol 1500

Macrogol 1500

Polyethylene Glycol 1500 is a mixture of the polycondensation products of ethylene oxide and water obtained under controlled conditions. It is represented by the formula $HOCH_2[CH_2OCH_2]_nCH_2OH$, where n is between 28 and 36.

Category. Pharmaceutical aid (ointment base).

Description. A white or creamy white, soft, wax-like solid; odour, faint and characteristic.

Tests

Appearance of solution (2.4.1). A 25.0 per cent w/v solution is not more intensely coloured than reference solution BYS6.

pH (2.4.24). 4.0 to 7.0, determined in a 5.0 per cent w/v solution.

Freezing point (2.4.11). 42° to 46°.

Hydroxyl value (2.3.27). 70 to 86, determined on 5.0 g.

Viscosity (2.4.28). 25 mm^2s^{-1} to 32 mm^2s^{-1}, determined at 100° by Method A using a U-tube viscometer (size D).

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g in 5 ml of a 1.0 per cent w/v solution of *hydrochloric acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from moisture.

Polyethylene Glycol 4000

Macrogol 4000

Polyethylene Glycol 4000 is a mixture of the polycondensation products of ethylene oxide and water obtained under controlled conditions. It is represented by the formula $HOCH_2[CH_2OCH_2]_nCH_2OH$, where n is between 69 and 84.

Category. Pharmaceutical aid (ointment base).

Description. A creamy white, hard, wax-like solid, powder or flakes; odour, faint and characteristic.

Tests

Appearance of solution (2.4.1). A 20.0 per cent w/v solution is not more intensely coloured than reference solution BYS6.

pH (2.4.24). 4.5 to 7.5, determined in a 5.0 per cent w/v solution.

Freezing point (2.4.11). 53° to 56°.

Hydroxyl value (2.3.27). 30 to 36, determined on 20.0 g.

Viscosity (2.4.28). 76 mm^2s^{-1} to 110 mm^2s^{-1}, determined at 100° by Method A using a U-tube viscometer (size E).

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g in 5 ml of a 1.0 per cent w/v solution of *hydrochloric acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from moisture.

Polyethylene Glycol 6000

Macrogol 6000

Polyethylene Glycol 6000 is a mixture of the polycondensation products of ethylene oxide and water obtained under controlled conditions. It is represented by the formula $HOCH_2[CH_2OCH_2]nCH_2OH$, where n is between 112 and 158.

Category. Pharmaceutical aid (ointment base and tablet excipient).

Description. A white to creamy white, wax-like solid or flakes; odour, faint and characteristic.

Tests

Appearance of solution (2.4.1). A 15.0 per cent w/v solution is not more intensely coloured than reference solution BYS6.

pH (2.4.24). 4.5 to 7.5, determined in a 5.0 per cent w/v solution.

Freezing point (2.4.11). 56° to 60°.

Viscosity (2.4.28). 250 mm^2s^{-1} to 390 mm^2s^{-1}, determined at 100° by Method A using a U-tube viscometer (size F).

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). Dissolve 4.0 g in 5 ml of a 1.0 per cent w/v solution of *hydrochloric acid* and sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (5 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Storage. Store protected from moisture.

Polyoxyl 35 Castor Oil

Polyoxyl 35 Castor Oil contains mainly the tri-ricinoleate ester of ethoxylated glycerol, with smaller amounts of polyethylene glycol ricinoleate and the corresponding free glycols. It results from the reaction of glycerol ricinoleate with about 35 moles of ethylene oxide.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polyoxyl 35 castor oil RS* or with the reference spectrum of polyoxyl 35 castor oil.

B. Dissolve about 0.1 g in 10 ml of *ethanolic potassium hydroxide solution*, boil for about 3 minutes and evaporate to dryness. The residue dissolve with 5 ml of *water*, yielding a clear solution. Add a few drops of *glacial acetic acid*; a white precipitate is formed.

C. To a 5 .0 per cent w/v solution, add *bromine solution*, dropwise, the *bromine* is decolorized.

Tests

Weight per ml (2.4.29). 1.05 to 1.06.

Viscosity (2.4.28). 600 to 850 centipoises at 25°, a capillary viscometer being used.

Acid value (2.3.23). Not more than 2.0.

Hydroxyl value (2.3.27). 65 to 80.

Saponification value (2.3.37). 60 to 75.

Iodine value (2.3.28). 25 to 35.

Heavy metals (2.3.13). Weigh about 2 g of the substance under examination in a crucible and add sufficient *sulphuric acid* to wet the substance, carefully ignite at a low temperature until thoroughly charred. Add to the carbonized mass 2 ml of *nitric acid* and 5 drops of *sulphuric acid*, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 ml of *dilute hydrochloric acid*, cover, digest on a steam-bath for 15 minutes, and slowly evaporate on a steam-bath to dryness. Moisten the residue with 1 drop of *hydrochloric acid*, add 10 ml of hot *water*, and digest for 2 minutes. Add *dilute ammonium hydroxide* dropwise until the solution is just alkaline to litmus paper, dilute with *water* to 25 ml, and adjust at pH 3.0 with *dilute acetic acid*, filter. Rinse the crucible and the filter with 10 ml of *water*, combine the filtrate and rinsing in a 50-ml Nessler cylinder, dilute with *water* to 40 ml, and mix.

To each of the cylinder containing the standard solution and the test solution, add 2 ml of pH 3.5 *acetate buffer*, then add 1.2 ml of *thioacetamide- glycerine* base , dilute with *water* to 50 ml, mix, allow to stand for 2 minutes, and view downward over a white surface: the colour of the solution obtained from the test solution is not more intense than that of the solution obtained from the standard solution, produced by treating 2 ml of *lead standard solution* (1 ppm) in a similar manner.

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 1 g.

Storage. Store protected from moisture.

Polyoxyl 40 Hydrogenated Castor Oil

Polyoxyl 40 Hydrogenated Castor Oil contains mainly the tri-hydroxystearate ester of ethoxylated glycerol, with smaller amounts of polyethylene glycol tri-hydroxystearate and of the corresponding free glycols. It results from the reaction of glycerol tri-hydroxystearate with about 40 to 45 moles of ethylene oxide.

Category. Pharmaceutical aid.

Identification

A. Dissolve 0.1 g in 1 ml of *water*, add 9 ml of 5.0 per cent w/v solution of *sodium chloride* and heat on a water-bath, the solution becomes turbid at a temperature between 70° and 85°.

B. Dissolve about 0.1 g in 10 ml of *ethanolic potassium hydroxide solution*, boil for about 3 minutes, and evaporate to dryness. Dissolve the residue with 5 ml of *water*, yielding a clear solution. Add a few drops of *glacial acetic acid*; a white precipitate is formed.

Tests

Congealing temperature (2.4.10). 16° to 26°.

Acid value (2.3.23). Not more than 2.0.

Hydroxyl value (2.3.27). 60 to 80.

Saponification value (2.3.37). 45 to 69.

Iodine value (2.3.28). Not more than 2.0.

Heavy metals (2.3.13). Weigh 2 g of the substance under examination in a crucible and add sufficient *sulphuric acid* to wet the substance, carefully ignite at a low temperature until thoroughly charred. Add to the carbonized mass 2 ml of *nitric acid* and 5 drops of *sulphuric acid*, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 ml of *dilute hydrochloric acid*, cover,

digest on a steam bath for 15 minutes, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of *hydrochloric acid*, add 10 ml of hot *water*, and digest for 2 minutes. Add *dilute ammonium hydroxide* dropwise until the solution is just alkaline to litmus paper, dilute with *water* to 25 ml, and adjust with *dilute acetic acid* to a pH between 3.0 and 4.0, using short-range pH indicator paper as an external indicator. Filter if necessary, rinse the crucible and the filter with 10 ml of *water*, combine the filtrate and rinsing in a 50-ml Nessler cylinder, dilute with *water* to 40 ml, and mix.

To each of the cylinder containing the standard solution and the test solution , add 2 ml of *acetate buffer* pH 3.5, then add 1.2 ml of *thioacetamide- glycerine* base , dilute with *water* to 50 ml, mix, allow to stand for 2 minutes, and view downward over a white surface: the colour of the solution obtained from the test solution is not more intense than that of the solution obtained from the standard solution, produced by treating 2 ml of *lead standard solution* (1 ppm) in a similar manner .

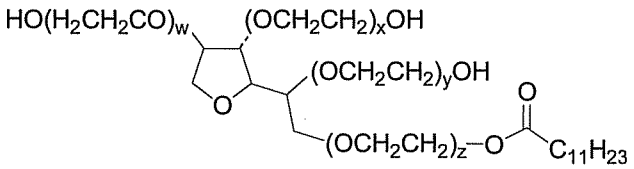
Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 1 g.

Storage. Store protected from moisture.

Polysorbate 20

Polyoxyethylene 20 Sorbitan Monolaurate



w + x + y + z = 20

Polyoxyethylene 20 Sorbitan Monolaurate

Polysorbate 20 is a mixture of partial lauric esters of D-glucitol and its anhydrides copolymerised with approximately 20 moles of ethylene oxide for each mole of D-glucitol and its anhydrides. The lauric acid used for the esterification may contain other fatty acids.

Category. Pharmaceutical aid (non-ionic surfactant).

Description. A clear or slightly opalescent, oily, yellowish or brownish yellow liquid; odour, faint and characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polysorbate RS* or with the reference spectrum of polysorbate.

B. Dissolve 0.1 g in 5 ml of *chloroform*, add 0.1 g of *potassium thiocyanate* and 0.1 g of *cobalt nitrate* and stir with a glass rod; a blue colour is produced.

Tests

pH (2.4.24). 5.0 to 7.0, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Acid value (2.3.23). Not more than 2.0, determined on 5.0 g.

Hydroxyl value (2.3.27). 96 to 108, determined on 2.0 g.

Saponification value (2.3.37). 40 to 50, using 15 ml of *0.5 M ethanolic potassium hydroxide* and diluting with 50 ml of *water* before carrying out the titration.

Iodine value (2.3.28). Not more than 5.0, determined by the iodine bromide method.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Reducing impurities. Dissolve 2.0 g in 25 ml of hot *water*, add 25 ml of *1 M sulphuric acid* and 0.1 ml of *ferroin solution* and titrate with *0.01 M ceric ammonium sulphate* shaking continuously until the colour changes from red to greenish blue persists for 30 seconds. Carry out a blank titration. Not more than 2.0 ml of *0.01 M ceric ammonium sulphate* is required.

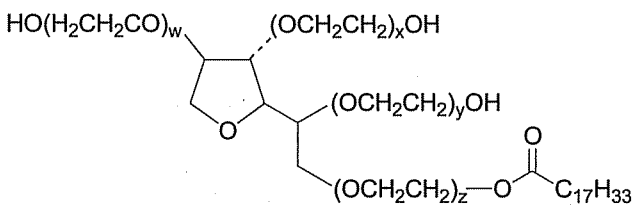
Sulphated ash. Not more than 0.2 per cent, determined by the following method. Weigh accurately about 2.0 g in a silica or platinum crucible, add 0.5 ml of *sulphuric acid* and heat on a water-bath for 2 hours. Carefully ignite at a low temperature until thoroughly charred. Add to the carbonised mass 2 ml of *nitric acid* and 0.25 ml of *sulphuric acid*, cautiously heat until white fumes are evolved and then ignite at 600° until the carbon is completely burnt off. Allow to cool, weigh and repeat the operation for periods of 15 minutes until two successive weighings do not differ by more than 0.5 mg.

Water (2.3.43). Not more than 3.0 per cent , determined on 1.0 g.

Storage. Store protected from light and moisture.

Polysorbate 80

Polyoxyethylene 80 Sorbitan Monooleate



w+x+y+z = 20

Polysorbate 80 is a mixture of partial oleic esters of D-glucitol and its anhydrides copolymerised with approximately 20 moles of ethylene oxide for each mole of D-glucitol and its anhydrides.

Category. Pharmaceutical aid (non-ionic surfactant).

Description. A clear or almost clear, oily, yellowish or brownish yellow liquid; odour, faint and characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *polysorbate RS* or with the reference spectrum of polysorbate.

B. Dissolve 0.1 g in 5 ml of *chloroform*, add 0.1 g of *potassium thiocyanate* and 0.1 g of *cobalt nitrate* and stir with a glass rod; a blue colour is produced.

C. To 2 ml of a 5 per cent w/v solution add 0.5 ml of *bromine water*; the bromine is decolourised.

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 5.0 per cent w/v solution in *carbon dioxide-free water*.

Acid value (2.3.23). Not more than 2.0, determined on 5 g.

Hydroxyl value (2.3.27). 65 to 80, determined on 2.0 g.

Saponification value (2.3.37). 45 to 55, using 15 ml of *0.5 M ethanolic potassium hydroxide* and diluting with 50 ml of *water* before carrying out the titration.

Iodine value (2.3.28). 18 to 24, determined by the iodine bromide method.

Reducing impurities. Dissolve 2.0 g in 25 ml of hot *water*, add 25 ml of *1 M sulphuric acid* and 0.1 ml of *ferroin solution* and titrate with *0.01 M ceric ammonium sulphate* shaking continuously until the colour changes from red to greenish blue persists for 30 seconds. Carry out a blank titration. Not more than 5.0 ml of *0.01 M ceric ammonium sulphate* is required.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash. Not more than 0.2 per cent, determined by the following method. Weigh accurately about 2.0 g in a silica or platinum crucible, add 0.5 ml of *sulphuric acid* and heat on a water-bath for 2 hours. Carefully ignite at a low temperature until thoroughly charred. Add to the carbonised mass 2 ml of *nitric acid* and 0.25 ml of *sulphuric acid*, cautiously heat until white fumes are evolved and then ignite at 600° until the carbon is completely burnt off. Allow to cool, weigh and repeat the operation for periods of 15 minutes until two successive weighings do not differ by more than 0.5 mg.

Water (2.3.43). Not more than 3.0 per cent, determined on 1.0 g.

Storage. Store protected from light and moisture.

Potassium Chloride

KCl Mol. Wt. 74.6

Potassium Chloride contains not less than 99.0 per cent and not more than 100.5 per cent of KCl, calculated on the dried basis.

Category. Electrolyte replenisher.

Dose. Prophylactic; 2 to 4 g (approximately 25 to 50 mmol) daily; therapeutic, in established potassium depletion, 10 to 15 g (approximately 135 to 200 mmol) daily.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Dissolve 10 g in 100 ml of *carbon dioxide-free water* prepared from *distilled water* (solution A). The solution gives the reactions of potassium salts and of chlorides (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. 5.0 g dissolved in 50 ml of *carbon dioxide-free water* requires not more than 0.5 ml of *0.01 M sodium hydroxide* or *0.01 M hydrochloric acid* for neutralisation to *bromothymol blue solution*.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Barium. To 5 ml of solution A add 5 ml of *water* and 1 ml of *1 M sulphuric acid*; the solution, after not less than 15 minutes, is not more opalescent than a mixture of 5 ml of solution A and 6 ml of *water*.

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of *water* to which are added 2 ml of *dilute acetic acid* and 13 ml of *water*. The solution complies with the limit test for heavy metals, Method A (10 ppm).

Calcium and magnesium. Dissolve 1 g in 20 ml of *water*, add 1 ml of *dilute ammonia solution* and 1 ml of *sodium phosphate solution*; the solution remains clear.

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Bromides. Dilute 1.0 ml of solution A to 50.0 ml with *water*. To 5.0 ml of the solution add 2.0 ml of *phenol red reagent* and 1.0 ml of a 0.01 per cent solution of *chloramine T* and mix immediately. After exactly 2 minutes, add 0.15 ml of *0.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water*. The absorbance of the solution measured at about 590 nm (2.4.7),

using *water* as the blank, is not more than that of a standard prepared at the same time and in the same manner, using 5.0 ml of a 0.0003 per cent w/v solution of *potassium bromide* (0.1 per cent).

Iodides. Moisten 5 g by adding dropwise, a solution freshly prepared by mixing 25 ml of *iodide-free starch solution*, 2 ml of *0.5 M sulphuric acid*, 0.15 ml of *sodium nitrite solution* and 25 ml of *water* and examine the mixture in daylight; the substance shows no blue colour after 5 minutes.

Sulphates (2.3.17). 0.5 g complies with the limit test for sulphates (300 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 50 ml of *water* and titrate with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007455 g of KCl.

Potassium Chloride intended for use in the manufacture of dialysis solutions complies with the following additional requirement.

Aluminium. Dissolve 4.0 g in 100 ml of *water* and add 10 ml of *acetate buffer pH 6.0*. Extract with successive quantities of 20, 20 and 10 ml of a 0.5 per cent w/v solution of *8-hydroxyquinoline* in *chloroform* and dilute the combined extracts to 50 ml with *chloroform*. Use as the standard solution a mixture of 2 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer pH 6.0* and 98 ml of *water* treated in the same manner and as the blank solution a mixture of 10 ml of *acetate buffer pH 6.0* and 100 ml of *water* treated in the same manner. Measure the fluorescence of the test and standard solutions (2.4.5), using an excitation wavelength of about 392 nm and emission wavelength of about 518 nm and setting the instrument to zero with the blank solution in each case. The fluorescence of the test is not greater than that of the standard solution (1 ppm).

Potassium chloride intended for use in the manufacture of Parenteral Preparations or for the preparation of haemodialysis solution complies with the following additional requirement.

Sodium. Not more than 0.1 per cent, determined by atomic absorption spectrophotometry (2.4.2), using a 1.0 per cent w/v solution and measuring at 589 nm. Use *sodium solution AAS*, suitably diluted with *water*, for the standard solutions.

Storage. Store protected from moisture.

Labelling. The label states whether or not the material is suitable for use in the manufacture of Parenteral Preparations or for the preparation of haemodialysis or dialysis solutions.

Potassium Chloride and Dextrose Injection

Potassium Chloride in Dextrose Injection; Potassium Chloride and Dextrose Intravenous Infusion; Potassium Chloride and Glucose Intravenous Infusion

Potassium Chloride and Dextrose Injection is a sterile solution of Potassium Chloride and Dextrose in Water for Injections.

Potassium Chloride and Dextrose Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of potassium chloride, KCl, and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Usual strengths: Injections containing the following amounts of potassium chloride, KCl, and dextrose, $C_6H_{12}O_6$.

per cent w/v of Potassium Chloride (KCl)	per cent w/v of Dextrose ($C_6H_{12}O_6$)
0.075	5
0.15	5
0.225	5
0.30	5

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction B of potassium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm, absorbance at about 284 nm (2.4.7); not more than 0.25.

Heavy metals (2.3.13). Evaporate a volume containing 4.0 g of Dextrose to 10 ml and add 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For potassium chloride* — Titrate an accurately measured volume containing 0.1 g of Potassium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.007455 g of KCl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers.

Labelling. The label states (1) the strength as the percentages w/v of Potassium Chloride and Dextrose; (2) the total osmolar concentration in mOsmol per litre; (3) where the contents are less than 100 ml, or where the label states that the Injection is not for direct use but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per ml; (4) the content of potassium in millimoles; (5) that the injection containing visible particles in the solution should not be used.

Potassium Chloride, Sodium Chloride and Dextrose Injection

Potassium Chloride in Dextrose and Sodium Chloride Injection; Potassium Chloride, Sodium Chloride and Dextrose Intravenous Infusion; Potassium Chloride, Sodium Chloride and Glucose Intravenous Infusion

Potassium Chloride, Sodium Chloride and Dextrose Injection is a sterile solution of Potassium Chloride, Sodium Chloride and Dextrose in Water for Injections.

Potassium Chloride, Sodium Chloride and Dextrose Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amounts of sodium, Na, potassium, K, and chloride, Cl, and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Usual strengths: Injections containing the following amounts of potassium chloride, KCl, sodium chloride, NaCl, and dextrose, $C_6H_{12}O_6$.

per cent w/v of Potassium Chloride (KCl)	*per cent w/v of Sodium Chloride (NaCl)*	*per cent w/v of Dextrose ($C_6H_{12}O_6$)*
0.075	0.250	5
0.075	0.330	5
0.075	0.450	5
0.150	0.225	5
0.150	0.225	10
0.150	0.330	5
0.150	0.450	5
0.150	0.900	5
0.225	0.225	5
0.225	0.225	10
0.225	0.330	5
0.225	0.450	5
0.300	0.225	5
0.300	0.330	5
0.300	0.450	5
0.300	0.900	5

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction A of potassium salts, reaction B of sodium salts and reaction A of chlorides (2.3.1)

Tests

pH (2.4.24). 3.5 to 6.5, determined on a portion diluted with *water*, if necessary, to a concentration of not more than 5.0 per cent of dextrose.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm, absorbance at about 284 nm (2.4.7); not more than 0.25.

Heavy metals (2.3.13). Evaporate a volume containing 4.0 g of Dextrose to 10 ml and add 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute appropriately with *water* and determine by Method A for flame photometry (2.4.4), or by

Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute appropriately with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total chlorides — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of *silver nitrate* with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

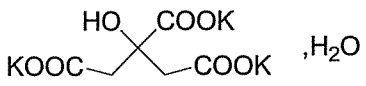
1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers.

Labelling. The label states (1) the strength as the percentages w/v of Potassium Chloride, Sodium Chloride and Dextrose; (2) the total osmolar concentration in mOsmol per litre; (3) where the contents are less than 100 ml, or where the label states that the Injection is not for direct use but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per ml; (4) the content of potassium, sodium and chloride in millimoles; (5) that the injection containing visible particles in the solution should not be used.

Potassium Citrate



$C_6H_5K_3O_7,H_2O$ Mol. Wt. 324.4

Potassium citrate is tripotassium 2-hydroxypropane-1,2,3-tricarboxylate monohydrate.

Potassium Citrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_5K_3O_7$, calculated on the anhydrous basis.

Category. Systemic alkaliniser.

Dose. 4 to 10 g.

Description. White, granular crystals or a crystalline powder; odourless; hygroscopic.

Identification

A. Dissolve 10 g in 100 ml of *carbon dioxide-free water* prepared from *distilled water* (solution A). 0.5 ml of solution A gives the reactions of potassium salts (2.3.1).

B. To 1 ml of solution A add 4 ml of *water*; the solution gives the reactions of citrates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.1 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* is required to change the colour of the solution.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 15 ml of *stannated hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sodium. Not more than 0.3 per cent, determined by atomic absorption spectrophotometry (2.4.2), Method II, measuring at 589 nm. Prepare the test solution by addition of 1 ml of *2 M hydrochloric acid* to 10.0 ml of solution A and diluting to 100.0 ml with *distilled water*. Use *sodium solution AAS*, suitably diluted with *distilled water*, for the standard solutions.

Chlorides (2.3.12). Dilute 25.0 ml of solution A to 35 ml with *water*. The resulting solution complies with the limit test for chlorides (100 ppm).

Oxalate. Dissolve 0.5 g in 4 ml of *water*, add 3 ml of *hydrochloric acid* and 1 g of *granulated zinc* and heat on a water-bath for 1 minute. Allow to stand for 2 minutes, decant into 0.25 ml of a 1 per cent w/v solution of *phenylhydrazine hydrochloride*, heat to boiling, cool rapidly, add an equal volume of *hydrochloric acid* and 0.25 ml of *potassium ferricyanide solution*, shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that obtained by treating 4.0 ml of a 0.005 per cent w/v solution of *oxalic acid* at the same time and in the same manner (300 ppm, calculated as anhydrous oxalic acid).

Sulphates (2.3.17). To 10.0 ml of solution A add 2 ml of *7 M hydrochloric acid* and dilute to 15 ml with *distilled water*; the solution complies with the limit test for sulphates (150 ppm).

Readily carbonisable substances. Heat 0.20 g, in powder, with 10 ml of *sulphuric acid (96 per cent w/w)* in a water-bath at 90° ± 1° for 60 minutes and cool rapidly. The solution is not more intensely coloured than reference solution YS2 or GYS2 (2.4.1).

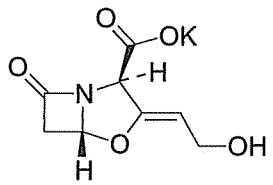
Water (2.3.43). 4.0 to 7.0 per cent, determined on 0.5 g, titration being done after stirring for 15 minutes subsequent to addition of the substance under examination.

Assay. Weigh accurately about 0.2 g, dissolve in 20 ml of *anhydrous glacial acetic* acid, heat to about 50°, allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *1-naphtholbenzein solution* as indicator and titrating until a green colour is obtained. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01021 g of $C_6H_5K_3O_7$.

Storage. Store protected from moisture.

Potassium Clavulanate



$C_8H_8KNO_5$ Mol. Wt. 237.3

Potassium Clavulanate is potassium (*Z*)-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate.

Potassium Clavulanate contains not less than 96.5 per cent and not more than 102.0 per cent of potassium clavulanate, $C_8H_8KNO_5$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white to off white, crystalline hygroscopic powder.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

B. Gives reaction A of potassium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 8.0, determined in 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 0.25 g of the substance under examination in mobile phase A and dilute to 25 ml with mobile phase A.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with mobile phase A.

Reference solution (b). A solution containing 0.01 per cent w/v each of *lithium clavulanate RS* and *amoxycillin trihydrate RS* in mobile phase A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen phosphate* adjusted to pH 4.0 with *phosphoric acid* and filtered through a 0.5 µm filter,
  B. a mixture of equal volumes of mobile phase A and *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 4	100	0
4 – 15	100 →50	0 50
15 – 18	50	50
18 – 24	50→100	50→ 0
24 – 39	100	0

Inject reference solution (b). The resolution between clavulanate (first peak) and amoxycillin (second peak) is not less than 13.

Inject the test solution and reference solution (b). The area of any individual impurity peak obtained is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). The sum of all impurity peaks obtained is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2.0 per cent). Ignore any peaks with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Water (2.3.43). Not more than 1.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE —Prepare the solutions immediately before use.

Test solution. Dissolve 50.0 mg of the substance under examination in a 0.41 per cent w/v solution of *sodium acetate* previously adjusted to pH 6.0 with *glacial acetic acid*, and dilute to 50.0 ml with the same solution.

Reference solution (a). A 0.1 per cent w/v solution of *lithium clavulanate RS* in a 0.41 per cent w/v solution of *sodium acetate* previously adjusted to pH 6.0 with *glacial acetic acid.*

Reference solution (b). A solution containing 0.1 per cent w/v each of *lithium clavulanate RS* and *amoxycillin trihydrate RS* in a 0.41 per cent w/v solution of *sodium acetate* previously adjusted to pH 6.0 with *glacial acetic acid.*

Chromatographic system
- a stainless steel column 30 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *methanol* and 95 volumes of a 1.5 per cent w/v solution of *sodium dihydrogen phosphate* previously adjusted to pH 4.0 with *dilute phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject reference solution (b). The resolution between clavulanate (first peak) and amoxycillin (second peak) is not less than 3.5.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_8H_8KNO_5$.

1 mg of clavulanate ($C_8H_9NO_5$) is equivalent to 1.191 mg of $C_8H_8KNO_5$.

Potassium Clavulanate intended for use in the manufacture of Parenteral Preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.03 Endotoxin Unit per mg of potassium clavulanate.

Potassium Clavulanate intended for use in the manufacture of Parenteral Preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture.

Labelling. The label states whether it is intended for use in the manufacture of parenteral preparations.

Potassium Clavulanate Diluted

$C_8H_8KNO_5$ Mol. Wt. 237.3

Potassium Clavulanate Diluted is a dry mixture of Potassium Clavulanate and Microcrystalline Cellulose, or Silica, colloidal anhydrous or Silica, colloidal hydrated.

Potassium Clavulanate Diluted contains not less than 91.2 per cent and not more than 107.1 per cent of the stated amount of potassium clavulanate, $C_8H_8KNO_5$.

Category. Antibacterial.

Description. A white or almost white powder, hygroscopic.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

B. Gives reaction A of potassium salts (2.3.1).

C. Depending on the diluent used, carry out one of the relevant identification tests given below.

(i) On a watch-glass place a quantity of the substance under examination corresponding to 20 mg of cellulose and disperse in 4 ml of *iodinated zinc chloride solution.* The powder becomes violet-blue in colour.

(ii) It gives the reaction of silicates (2.3.1).

Tests

pH (2.4.24) 4.8 to 8.0, determined on a quantity of the substance under examination containing 0.2 g of potassium clavulanate dissolved in 20 ml of *carbon dioxide-free water.*

Light absorption. When examined in the range 230 nm to 360 nm (2.4.7), a 0.1 per cent w/v solution in *0.1 M phosphate buffer solution pH 7.0* shows an absorption maximum 0.40 measured immediately at 278 nm.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the substance under examination containing 0.25 g of potassium clavulanate in 5 ml of mobile phase A, dilute to 25.0 ml with mobile phase A and filter.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase A.

Reference solution (b). Dissolve 10 mg of *amoxycillin trihydrate RS* in 1 ml of the test solution and dilute to 100 ml with mobile phase A.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.78 per cent w/v solution of *sodium dihydrogen phosphate* with the pH adjusted to 4.0 with *dilute phosphoric acid,*

  B. a mixture of equal volumes of mobile phase A and *methanol,*

- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Comment
0	100	0	Isocratic
4	100	0	begin linear gradient
15	50	50	
18	50	50	end chromatogram, return to 100A
24	100	0	end equilibration, begin next chromatogram

Inject reference solution (b). The resolution between the clavulanate (first peak) and amoxycillin (second peak) is not less than 13.

Inject alternatively the test solution and reference solution (a). Any individual impurity is not more than 1.0 per cent and the sum of all impurities found is not more than 2.0 per cent.

Water (2.3.43). Not more than 2.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the substance under examination containing about 50.0 mg of potassium clavulanate in a 0.4 per cent w/v solution of *sodium acetate* with the pH previously adjusted to 6.0 with *glacial acetic acid*, dilute to 50.0 ml with the same solution and filter.

Reference solution (a). Dissolve 50.0 mg of *lithium clavulanate RS* in a 0.4 per cent w/v solution of *sodium acetate* with the pH previously adjusted to 6.0 with *glacial acetic acid* and dilute to 50.0 ml with the same solution.

Reference solution (b). Dissolve 10 mg of *amoxycillin trihydrate RS* in 10 ml of reference solution (a).

Chromatographic system
- a stainless steel column 30 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 5 volumes of *methanol* and 95 volumes of a 1.5 per cent w/v solution of *sodium dihydrogen phosphate* with the pH previously adjusted to 4.0 with *dilute phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between the clavulanate peak and amoxycillin peak is not less than 3.5.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_8H_8KNO_5$.

1 mg of clavulanate ($C_8H_9NO_5$) is equivalent to 1.191 mg of $C_8H_8KNO_5$.

Storage. Store protected from moisture.

Labelling. The label states the percentage contents of potassium clavulanate and the diluent used to prepare the mixture.

Potassium Iodide

KI Mol. Wt. 166.0

Potassium Iodide contains not less than 99.0 per cent and not more than 100.5 per cent of KI, calculated on the dried basis.

Category. Antithyroid; antifungal; expectorant.

Dose. In preoperative treatment of thyrotoxicosis, 30 to 60 mg; as expectorant, 250 to 500 mg.

Description. Colourless crystals or a white powder; odourless.

Identification

Dissolve 10 g in 100 ml of *carbon dioxide-free water* (solution A). The solution gives the reactions of potassium salts, and of iodides (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Alkalinity. To 10 ml of solution A add 0.2 ml of *0.01 M sulphuric acid*; no colour is produced on addition of a drop of *phenolphthalein solution*.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Barium. Dissolve 0.5 g in 10 ml of *water* and add 1 ml of *dilute sulphuric acid*; no turbidity develops within one minute.

Cyanide. Warm 5 ml of Solution A, add one drop of *ferrous sulphate solution* and 0.5 ml of *sodium hydroxide solution*

and acidify with *hydrochloric acid*; no blue colour is produced.

Iodate. Dissolve 0.5 g in 10 ml of *carbon dioxide-free water* and add 0.15 ml of *dilute sulphuric acid* and a drop of *iodide-free starch solution*; no blue colour is produced within 2 minutes.

Sulphates (2.3.17). 1.0 g dissolved in 15 ml of *water* complies with the limit test for sulphates (150 ppm).

Thiosulphate. Dissolve 1 g in 10 ml of *water*, add 0.1 ml of *starch solution* and 0.1 ml of *0.005 M iodine*; a blue colour is produced.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g of the powdered substance by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.35 g, dissolve in about 10 ml of *water*, add 35 ml of *hydrochloric acid* and 5 ml of *chloroform*. Titrate with *0.05 M potassium iodate* until the purple colour of iodine disappears from the chloroform. Add the last portion of the iodate solution dropwise and agitate vigorously and continuously. Allow to stand for 5 minutes. If any colour develops in the chloroform layer continue the titration until the chloroform is decolorised.

1 ml of *0.05 M potassium iodate* is equivalent to 0.0166 g of KI.

Storage. Store protected from light and moisture.

Potassium Permanganate

$KMnO_4$ Mol. Wt. 158.0

Potassium Permanganate contains not less than 99.0 per cent and not more than 100.5 per cent of $KMnO_4$.

Category. Antiseptic.

Description. A dark purple or brownish black, granular powder or dark purple or almost black slender, prismatic crystals, having a metallic lustre; odourless. It decomposes on contact with certain organic substances.

Identification

A. A solution in *water* acidified with *sulphuric acid* and heated to 70° is decolorised by *hydrogen peroxide solution (20 vol)*.

B. Heated to redness, it decrepitates, evolves oxygen and leaves a black residue which with *water* forms *potassium hydroxide solution*; the resulting solution when neutralised with *dilute hydrochloric acid* gives the reactions of potassium salts (2.3.1).

Tests

Appearance of solution. Dissolve 1.5 g in 50 ml of distilled *water*, heat on a water-bath and add gradually 6 ml of *ethanol (95 per cent)*, cool, dilute to 60 ml with distilled *water* and filter. The filtrate (solution A) is colourless (2.4.1).

Chlorides (2.3.12). 40 ml of solution A complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 10 ml of solution A complies with the limit test for sulphates (600 ppm).

Water-insoluble matter. Dissolve 0.5 g in 50 ml of *water*, heat to boiling, filter through a tared, sintered-glass filter (porosity No. 4) and wash with *water* until the filtrate is colourless. The weight of the residue, dried at 105° to constant weight, is not more than 5 mg (1.0 per cent).

Assay. Weigh accurately about 0.3 g, dissolve in sufficient *water* to produce 100.0 ml. To 20.0 ml add 20 ml of *water*, 1 g of *potassium iodide* and 10 ml of *2 M hydrochloric acid* and titrate the liberated iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.003160 g of $KMnO_4$.

Storage. Store protected from moisture.

CAUTION — Great care should be taken in handling potassium permanganate as dangerous explosions are liable to occur if it is brought into contact with organic or other readily oxidisable substances, either in solution or in the dry condition.

Potassium Sorbate

$CH_3CH=CHCH=CHCOOK$

$C_6H_7KO_2$ Mol. Wt.150.2

Potassium Sorbate is potassium 2,4-hexadienoate.

Potassium Sorbate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_7KO_2$, calculated on the dried basis.

Category. Food preservativ.

Description. A white or almost white powder or granules.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *potassium*

sorbate RS or with the reference spectrum of potassium sorbate.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.02 per cent w/v solution in *water*, dilute 1.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid* shows an absorption maximum at about 264 nm and the specific absorbance at 264 nm is 1650 to 1900.

C. Dissolve 0.2 g in 2 ml of *water* and add 2 ml of *dilute acetic acid*, filter. The solution gives reaction B of potassium (2.3.1).

Tests

Appearance of solution. A 5 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1) and not more intensely coloured than reference solution YS5 (2.4.1).

Acidity or alkalinity. To 20 ml of solution A, add 0.1 ml of *phenolphthalein solution*. Titrate with *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid*. Not more than 0.25 ml is required to change the colour of the indicator.

Aldehydes. Not more than 0.15 per cent, determined by dissolving 1.0 g in a mixture of 50 ml of *2-propanol* and 30 ml of *water*, adjust to pH 4.0 with *1 M hydrochloric acid* and dilute to 100 ml with *water*. To 10 ml of the solution add 1 ml of *decolorised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained in a solution prepared simultaneously by adding by 1 ml of decolorised *fuchsin solution* to a mixture of 1.5 ml of *acetaldehyde standard solution (100 ppm C_2H_4O)*, 4 ml of *2-propanol* and 4.5 ml of *water*.

Heavy metals (2.3.13). Weigh in a silica crucible 2 g of the substance under examination, mix with 0.5 g of *magnesium oxide*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained. After 30 minutes of ignition if mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800° for about 1 hour. Dissolve the residue in 5 ml of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 0.1 ml *of phenolphthalein solution* and then *concentrated ammonia* until a pink colour is obtained. Cool, add *glacial acetic acid* until the solution is decolourised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water*, 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (10 ppm). Use lead standard solution (10 ppm pb) , diluting 1 ml standard solution to 10 ml with a mixture of equal volumes of *hydrochloric acid* and *water*.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

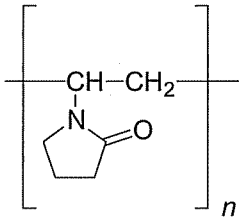
Assay. Weigh accurately about 0.12 g, dissolve in 20 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *crystal violet solution* as indicator until the colour changes from violet to bluish-green. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01502 g of $C_6H_7KO_2$.

Storage. Store protected from light.

Povidone

Polyvinylpyrrolidone; Polyvidone



$(C_6H_9NO)_n$ Mol. Wt. $(111.2)_n$

Povidone is poly(2-oxopyrrolidin-1-ylethylene) and consists of linear polymers of 1-vinylpyrrolidin-2-one. The different types of Povidone are characterised by their viscosity in solution, expressed as K-value, in the range 10 to 95.

Povidone with a nominal K-value of 15 or less has a K-value of not less than 85.0 per cent and not more than 115.0 per cent of the declared value. The K-value of povidone with a nominal K-value of more than 15, or a nominal K-value range with an average of more than 15, is not less than 90.0 per cent and not more than 107.0 per cent of the declared value or of the average of the declared range. It contains not less than 11.5 per cent and not more than 12.8 per cent of nitrogen, N, calculated on the anhydrous basis.

Category. Pharmaceutical aid (tablet binder, coating agent, dispersing and suspending agent).

Description. A white or yellowish white powder or flakes; odourless or almost odourless; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *povidone RS*.

B. Add 2.5 g in small portions to a suitable volume of *carbon dioxide-free water*, stirring with a magnetic stirrer, and dilute to 25 ml with the same solvent (solution A). To 0.4 ml of solution A add 10 ml of *water*, 5 ml of *2 M hydrochloric acid* and 2 ml

of *potassium dichromate solution*; an orange-yellow precipitate is produced.

C. To 1 ml of solution A add 0.2 ml of *dimethylamino-benzaldehyde reagent* and 0.1 ml of *sulphuric acid*; a pink colour is produced.

D. To 0.1 ml of solution A add 5 ml of *water* and 0.2 ml of *0.05 M iodine*; a red colour is produced.

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BS6 or BYS6 (2.4.1).

Heavy metals. Mix 2.0 g with 0.5 g of *magnesium oxide* in a silica crucible. Ignite to dull red heat until a homogeneous white or greyish white mass is produced. Heat at 800° for about 1 hour, dissolve the residue using two quantities, each of 5 ml, of *5 M hydrochloric acid*, add 0.1 ml of *phenolphthalein solution* and *strong ammonia solution* until a pink colour is produced. Cool, add *glacial acetic acid* until the solution is decolorised and add a further 0.5 ml. Filter if necessary and dilute the solution to 20 ml with *water*. To 12 ml of the resulting solution add 2 ml of *acetate buffer pH 3.5*, mix, add 1.2 ml of *thioacetamide reagent*, mix immediately and allow to stand for 2 minutes. Any brown colour produced is not more intense than that obtained by treating in the same manner a mixture of 2 ml of the test solution obtained above and 10 ml of the 20 ml of solution obtained by repeating the procedure using 2 ml of *lead standard solution (10 ppm Pb)* in place of the substance under examination, adding 0.5 g of *magnesium oxide* in a silica crucible and continuing as described above beginning at the words "Ignite to dull red heat...." (10 ppm).

Aldehydes. Boil 20.0 g in 180 ml of a 25 per cent v/v solution of *sulphuric acid* in a round-glass-stoppered flask under a reflux condenser for 45 minutes and allow to cool. Fit a distillation head, distil and collect 60 ml of the distillate in 20 ml of a 7.0 per cent w/v solution of *hydroxylamine hydrochloride*, previously adjusted to pH 3.1 using *1 M sodium hydroxide*, and cooled in ice. Titrate with *0.1 M sodium hydroxide* to pH 3.1. Carry out a blank titration. Not more than 9.1 ml of *0.1 M sodium hydroxide* is required (0.2 per cent, calculated as acetaldehyde, C_2H_4O).

1-vinylpyrrolidin-2-one. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.25 g of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *1-vinylpyrrolidin-2-one* in *methanol.* Dilute 1.0 ml of this solution to 100.0 ml with *methanol.* Further dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *1-vinylpyrrolidin-2-one* and 0.5 g of *vinyl acetate* in 100.0 ml of *methanol.* Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 10 volumes of *acetonitrile* and 90 volumes of *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 50 µl.

Inject reference solution (a) and (b). The test is not valid unless the resolution between the peaks due to 1-vinylpyrrolidin-2-one and vinyl acetate is not less than 2.0 in the chromatogram obtained with reference solution (b) and the relative standard deviation for replicate injections is not more than 2.0 in the chromatogram obtained with reference solution (a).

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of the peak due to 1-vinylpyrrolidin-2-one is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 5.0 per cent determined on 0.5 g.

K-value. For Povidone with a stated K-value of 18 or less, prepare a 5.0 per cent w/v solution. For Povidone with a declared K-value of more than 18, prepare a 1.0 per cent w/v solution. Allow the solution to stand for 1 hour and carry out Method B for the determination of viscosity (2.4.28), at 25° ± 0.2° using a size no. 1 viscometer with a minimum flow time of 100 seconds. Calculate the K-value (K_o) from the expression

$$K_o = \frac{1.5\log z - 1}{0.15 + 0.003c} + \frac{\sqrt{300c\log z + (c + 1.5c\log z)^2}}{0.15c + 0.003c^2}$$

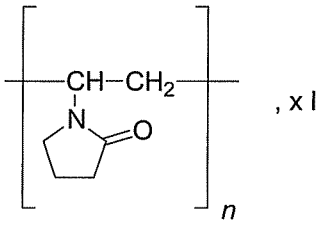
where c is the percentage concentration w/v of the substance under examination, calculated on the anhydrous basis, and z is the viscosity of the solution relative to that of *water*.

Nitrogen (2.3.30). Follow Method C, using 0.3 g, accurately weighed and 11 ml of *nitrogen-free sulphuric acid.* For complete destruction of organic matter repeat the addition of *hydrogen peroxide (10 vol)* (usually 3 to 6 times) until a clear, light-green solution is obtained, then heat for a further 4 hours.

Storage. Store protected from moisture.

Labelling. The label states the viscosity in terms of a K-value or K-range.

Povidone-Iodine



Povidone-Iodine is a complex produced by interaction between iodine and poly(2-oxopyrrolidin-1-ylethylene).

Povidone-Iodine contains not less than 9.0 per cent and not more than 12.0 per cent of available iodine, I, calculated on the dried basis.

Category. Topical anti-infective; antiseptic.

Description. A yellowish brown amorphous powder; odour, slight and characteristic of iodine.

Identification

A. Add 0.05 ml of a 10 per cent w/v solution to a mixture of 1 ml of *starch solution* and 9 ml of *water*; a deep blue colour is produced.

B. Spread 1 ml of a 10 per cent w/v solution over an area of about 20 cm x 20 cm on a glass plate and allow it to dry in air at room temperature and in an atmosphere of low humidity overnight; a brown, dry, non-smearing film is formed which dissolves readily in *water*.

Tests

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Nitrogen (2.3.30). 9.5 to 11.5 per cent, calculated on the dried basis, determined on about 0.3 g, by Method A.

Iodide. Not more than 6.6 per cent, calculated on the dried basis, determined by the following method. Weigh accurately about 0.5 g and dissolve in 100 ml of *water*. Add sufficient *sodium bisulphite solution* until the colour of iodine is discharged. Add 25.0 ml of *0.1 M silver nitrate* and 10 ml of *nitric acid* and mix. Titrate with *0.1 M ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Repeat the procedure without the substance under examination. The difference between the titrations represents the amount of silver nitrate required. 1 ml of *0.1 M silver nitrate* is equivalent to 0.01269 g of I. From the percentage of total iodine, calculated on the dried basis, subtract the percentage of available iodine determined in the Assay and obtain the percentage of iodide.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 3 g, transfer to a beaker and add 200 ml of *water*. Cover the beaker and stir with a mechanical stirrer at room temperature for not more than 1 hour to dissolve as completely as possible. Titrate immediately thereafter with *0.1 M sodium thiosulphate* using 3 ml of *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01269 g of I.

Storage. Store protected from light.

Povidone-Iodine Solution

Povidone-Iodine Solution is a solution of Povidone-Iodine in Purified Water. It may contain a small amount of Ethanol.

Povidone-Iodine Solution contains not less than 85.0 per cent and not more than 120.0 per cent of the stated amount of iodine, I.

Usual strengths. 5 per cent w/v; 7.5 per cent w/v; 10 per cent w/v.

Description. A deep brown liquid; odour, characteristic of iodine.

Identification

A. Dilute 1 ml to 20 ml with *water* and add 1 ml of the resulting solution to a mixture of 1 ml of *starch solution* and 9 ml of *water*; a deep blue colour is produced.

B. Transfer 10 ml to a small flask and cover the mouth of the flask with a filter paper soaked in *starch solution*; no blue colour is produced on the paper within 60 seconds.

C. Dilute 20 ml to 100 ml with *water*. To 10 ml add dropwise *0.1 M sodium thiosulphate* until the colour is just discharged. To 5 ml of the resulting solution add 5 ml of *2 M hydrochloric acid* and 2 ml of *potassium dichromate solution*; a red precipitate is produced.

Tests

pH (2.4.24). 3.0 to 6.5.

Ethanol (if present) (2.3.45). 90.0 to 110.0 per cent of the stated amount of C_2H_5OH, determined by Method A after decolorising the solution with just sufficient quantity of a 10 per cent w/v solution of *sodium thiosulphate* and treating with a few drops of *dilute sodium hydroxide solution*.

Assay. To an accurately measured volume containing about 50 mg of iodine add sufficient *water* to produce not less than 30 ml and titrate immediately with *0.02 M sodium thiosulphate*,

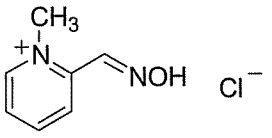
using 3 ml of *starch solution*, added towards the end of the titration, as indicator. Carry out a blank titration.

1 ml of *0.02 M sodium thiosulphate* is equivalent to 0.002538 g of I.

Storage. Store protected from light.

Labelling. The label states the quantities of iodine and ethanol (if present) as percentages w/v.

Pralidoxime Chloride



$C_7H_9ClN_2O$ Mol. Wt. 172.6

Pralidoxime Chloride is 2-hydroxyiminomethyl-1-methylpyridinium chloride.

Pralidoxime Chloride contains not less than 97.0 per cent and not more than 103.0 per cent of $C_7H_9ClN_2O$, calculated on the dried basis.

Category. Antidote for cholinesterase inhibitors.

Dose. By intravenous injection, 1 to 2 g as a 5 per cent w/v solution, administered over not less than 5 minutes period; by intramuscular injection, 1 g initially, followed by 1 to 2 further doses if necessary, upto a maximum of 12 g in 24 hours.

Description. A white to pale yellow, crystalline powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pralidoxime chloride RS* or with the reference spectrum of pralidoxime chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 242 nm and 292 nm and in *0.1 M sodium hydroxide*, a maximum at about 332 nm.

C. To 0.1 ml of a 20 per cent w/v solution add 1 ml of a 0.6 per cent w/v solution of *ferric chloride*; an amber-brown colour is produced.

D. Gives the reactions of chlorides (2.3.1).

Tests

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Chloride content. 20.2 to 20.8 per cent, calculated on the dried basis, determined by the following method. Weigh accurately about 0.3 g, dissolve in about 150 ml of *water*, add 20 ml of *glacial acetic acid* and 10 drops of *(4-tert-octylphenoxy) nonaethoxyethanol* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of Cl.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.5 g, dissolve in sufficient *water* to produce 250.0 ml and mix. Dilute 5.0 ml of this solution to 100.0 ml with *water*. Transfer 5.0 ml to a 50-ml volumetric flask, dilute to about 40 ml with *water*, add 5.0 ml of *1 M sodium hydroxide* and dilute to volume with *water*. Within 10 minutes of the addition of the alkali, measure the absorbance of the solution at the maximum at about 332 nm (2.4.7), using a solution of 5.0 ml of *1 M sodium hydroxide* diluted with *water* to 50.0 ml as the blank.

Calculate the content of $C_7H_9ClN_2O$ from the absorbance obtained by carrying out the assay simultaneously using about 0.5 g, accurately weighed, of *pralidoxime chloride RS*.

Storage. Store protected from moisture.

Pralidoxime Chloride Injection

Pralidoxime Chloride Injection is a sterile material consisting of Pralidoxime Chloride with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Pralidoxime Chloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pralidoxime chloride, $C_7H_9ClN_2O$.

Usual strength. 1 g.

Description. A white to pale yellow powder.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pralidoxime chloride RS* or with the reference spectrum of pralidoxime chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 242 nm and 292 nm and in *0.1 M sodium hydroxide*, a maximum at about 332 nm.

C. To 0.1 ml of a 20 per cent w/v solution add 1 ml of a 0.6 per cent w/v solution of *ferric chloride*; an amber-brown colour is produced.

D. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 4.5, determined in a 5.0 per cent w/v solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

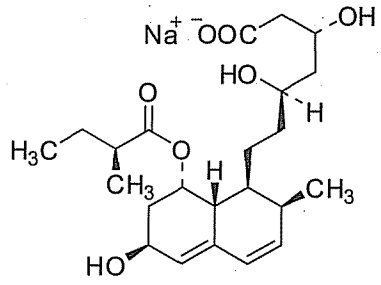
Bacterial endotoxins (2.2.3). Not more than 0.1 Endotoxin Unit per mg of pralidoxime chloride.

Assay. Weigh accurately about 0.5 g, dissolve in sufficient *water* to produce 250.0 ml and mix. Dilute 5.0 ml of this solution to 100.0 ml with *water*. Transfer 5.0 ml to a 50-ml volumetric flask, dilute to about 40 ml with *water*, add 5.0 ml of *1 M sodium hydroxide* and dilute to volume with *water*. Within 10 minutes of the addition of the alkali, measure the absorbance of the solution at the maximum at about 332 nm (2.4.7), using a solution of 5.0 ml of *1 M sodium hydroxide* diluted with *water* to 50.0 ml as the blank.

Calculate the content of $C_7H_9ClN_2O$ from the absorbance obtained by carrying out the assay simultaneously using about 0.5 g, accurately weighed, of *pralidoxime chloride RS*.

Labelling. The label states the period within which the constituted injection should be used.

Pravastatin Sodium



$C_{23}H_{35}NaO_7$ Mol. Wt. 446.5

Pravastatin Sodium is sodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoate

Pravastatin Sodium contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{23}H_{35}NaO_7$, calculated on the anhydrous and ethanol free basis.

Category. Antihyperlipidaemic.

Description. A white to yellowish white powder or crystalline powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pravastatin sodium RS* or with the reference spectrum of pravastatin sodium.

B. 1 ml of a 5 per cent w/v solution in *carbon dioxide-free water* (Solution A) gives reaction A of sodium (2.3.1).

Tests

Appearance of solution. Dilute 2.0 ml of solution A to 10 ml with *water*. The solution is clear (2.4.1) and not more intensely coloured than the reference solution BYS6 (2.4.1).

pH (2.4.24). 7.2 to 9.0, determined in solution A.

Specific optical rotation (2.4.22). +153° to +159 °, determined in a 0.5 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 9 volumes of *methanol* and 11 volumes of *water*.

Test solution (a). Dissolve 100 mg of the substance under examination in 100 ml of the solvent mixture.

Test solution (b). Dilute 10 ml of test solution (a) to 100 ml with the solvent mixture.

Reference solution (a). Dissolve the contents of a vial of *pravastatin impurity A RS* in 1 ml of test solution (b).

Reference solution (b). Dilute 2 ml of test solution (a) to 100 ml with the solvent mixture. Dilute 1 ml of this solution to 10 ml with the solvent mixture.

Reference solution (c). Dissolve 12.4 mg of *pravastatin 1,1,3,3- tetramethylbutylamine RS* in 100 ml of the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: a mixture of 1 volume of *glacial acetic acid,* 1 volume of *triethylamine,* 450 volumes of *methanol* and 550 volumes of *water,*
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to pravastatin impurity A and pravastatin is not less than 7.0.

The relative retention times with reference to pravastatin for (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-8-[[(2*S*,3*R*)-3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl] heptanoic acid (3″-hydroxypravastatin) (pravastatin impurity B), (3*R*,5*R*)-3,5-dihydroxy-7-[(1S,2S,6S,8S,8a*R*)-6-hydroxy-8-[[(2S,3S)-3-hydroxy-2-methylbutanoyl]oxy]-2-methyl-1,2,6,7,8,8a-hexahydronapthalen-1-yl] heptanoic acid (3″-(*S*)-hydroxypravastatin) (pravastatin impurity E), (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid (6'-epipravastatin) (pravastatin impurity A), (1*S*,3*S*,7*S*,8*S*,8a*R*)-3-hydroxy-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate (pravastatin lactone) (pravastatin impurity D) and (3*R*,5*R*) -3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylpentanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl] heptanoic acid) (pravastatin impurity C) and (3*R*, 5*R*)-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6,8-dihydroxy-2-methyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (pravastatin impurity F) are about 0.2, 0.3, 0.6, 1.9 and 2.1 respectively.

Inject test solution (a) and reference solution (b). Run the chromatogram 2.5 times the retention time of the principal peak of pravastatin. In the chromatogram obtained with test solution (a), the area of peak due to pravastatin impurity A is not more than 1.5 times the area of the principal peak obtained with reference solution (b) (0.3 per cent), the area of peaks due to pravastatin impurities B, C, D and E is not more than the area of the principal peak obtained with reference solution (b) (0.2 per cent), the area of peak due to pravastatin impurity F is not more than 0.75 times the area of the principal peak obtained with reference solution (b) (0.15 per cent) the area of other secondary peak is not more than 0.5 times the area of the principal peak obtained with reference solution (b) (0.1 per cent) and sum of the areas of all the secondary peaks is not more than 3 times the area of the principal peak obtained with reference solution (b) (0.6 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 2.0 g in a mixture of 15 volumes of *water* and 85 volumes of *methanol* and dilute to 20 ml with the same solvent mixture and use 12 ml of the solution. The resulting solution complies with the limit test for heavy metals, Method B (20 ppm). Prepare the reference solution using *lead standard solution (2 ppm Pb)* obtained by diluting *lead standard solution (100 ppm Pb)* with a mixture of 15 volumes of *water* and 85 volumes of *methanol.*

Ethanol (2.3.45). Not more than 3.0 per cent v/v (determined by method I).

Water (2.3.43). Not more than 4.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14), as described in the test for Related substances with the following modification.

Inject the test solution (b) and reference solution (c).

Calculate the content of $C_{23}H_{35}NaO_7$ from the declared content of pravastatin in *pravastatin 1,1,3,3-tetramethylbutylamine RS.*

1 mg of pravastatin is equivalent to 1.052 mg of pravastatin sodium.

Storage. Store protected from light and moisture.

Pravastatin Tablets

Pravastatin Sodium Tablets

Pravastatin Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pravastatin sodium, $C_{23}H_{35}NaO_7$.

Usual strengths. 10 mg; 20 mg; 40 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 10 mg of Pravastatin Sodium with 80 ml of *water* for 10 minutes, dilute to 100 ml with *water*, filter. Dilute 1 ml to 10 ml with *water*. When examined in the range 220 to 350 nm (2.4.7), exhibits a maximum only at 238 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solution A. Dissolve 4.1 g of *anhydrous sodium acetate* in 400 ml of *water*, adjust the pH to 5.6 with *glacial acetic acid* and add sufficient *water* to produce 500 ml.

Solution B. Mix 20 volumes of *methanol* with 80 volumes of solution A.

Test solution. Add 20 ml of solution A to a quantity of the powdered tablets containing about 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes, centrifuge. Dilute 1 ml of the clear supernatant liquid to 5 ml with solution B.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with solution B.

Reference solution (b). Dilute 1 ml of reference solution (a) to 5 ml with solution B.

Reference solution (c). Dilute 1 ml of reference solution (b) to 4 ml with solution B.

Reference solution (d). Dissolve 2 mg of *(3R,5R)-3,5-dihydroxy-7-[(1S,2S,6R,8S,8aR)-6-hydroxy-2-methyl-8-[[(2S)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]heptanoic acid RS (pravastatin impurity A RS)* in the minimum volume of *methanol* and dilute to 20 ml with the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (such as Ultrasphere ODS),
- mobile phase: a mixture of 1 volume of *glacial acetic acid* , 1 volume of *triethylamine*, 450 volumes of *methanol* and 550 volumes of *water*,
- flow rate.1.3 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to pravastatin impurity A and pravastatin is not less than 7.0.

Inject the test solution, reference solution (a), (b) and (c). In the chromatogram obtained with the test solution the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (2 per cent), the area of one such peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1 per cent), not more than a further one such peak has an area more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (3 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Add 20 ml of solution A to a quantity of the powdered tablets containing about 10 mg of Pravastatin Sodium, mix using a vortex mixer and then with the aid of ultrasound for 15 minutes, centrifuge. Dilute 1 ml of the clear supernatant liquid to 5 ml with solution B.

Reference solution (a). Dissolve 12.4 mg of *pravastatin 1,1,3,3,-tetramethylbutylamine RS* in 20 ml of solution A. Dilute 1 ml of this solution to 5 ml with solution B.

Reference solution (b). Dissolve 2 mg of *pravastatin impurity A RS* in the minimum volume of *methanol* and dilute to 20 ml with the test solution.

Use the chromatographic system, solution A and B as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to pravastatin impurity A and pravastatin is not less than 7.0.

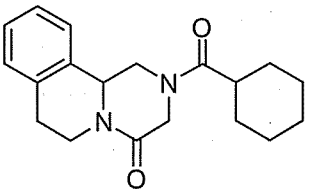
Inject the test solution and reference solution (a).

Calculate the content of $C_{23}H_{35}NaO_7$ in the tablets using the declared content of pravastatin in *pravastatin 1,1,3,3-tetramethylbutylamine RS*.

1 mg of $C_{23}H_{36}O_7$ is equivalent to 1.052 mg of $C_{23}H_{35}NaO_7$.

Storage. Store protected from light and moisture.

Praziquantel



$C_{19}H_{24}N_2O_2$ Mol.Wt. 312.4

Praziquantel is (11b*RS*)-2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4*H*-pyrazino[2,1-*a*]isoquinolin-4-one.

Praziquantel contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{19}H_{24}N_2O_2$, calculated on the dried basis.

Category. Antihelminthic.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *praziquantel RS* or with the reference spectrum of praziquantel.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 40 mg of the substance under examination in 10 ml of the mobile phase.

Test solution (b). Dilute 1.0 ml of test solution (a) to 20 ml with the mobile phase.

Reference solution (a). Dissolve 40 mg of *praziquantel RS* in 10 ml of the mobile phase. Dilute 1.0 ml to 20 ml with the mobile phase.

Reference solution (b). Dissolve 5.0 mg of *(11bRS)-2-benzoyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one RS (praziquantel impurity A RS)* in 25 ml of reference solution (a). Dilute 2.0 ml of this solution to 20 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of test solution (a) to 20 ml with the mobile phase. Dilute 5.0 ml of this solution to 50 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate.1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solutions (b). The test is not valid unless the resolution between the peaks corresponding to praziquantel impurity A and praziquantel is not less than 3.0.

Inject test solution (a), reference solution (b) and (c). Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent). The area of one such peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent). The sum of the areas of all other secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 50° over *diphosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution (b) and reference solution (a).

Calculate the content of $C_{19}H_{24}N_2O_2$.

Storage. Store protected from light.

Praziquantel Tablets

Praziquantel Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of Praziquantel, $C_{19}H_{24}N_2O_2$.

Usual strength. 600 mg.

Identificaton

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *praziquantel RS* or with the reference spectrum of praziquantel.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gelG*.

Mobile phase. Ethyl acetate.

Test solution. Shake a quantity of the powdered tablets containing 30 mg of praziquantel in 5 ml of *methanol* for 5 minutes, centrifuge and use the clear supernatant liquid.

Reference solution. A 0.6 per cent w/v solution of *praziquantel RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine under ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No.1,
Medium. 900 ml of *0.1 M hydrochloric acid* containing 2.0 mg per ml of *sodium lauryl sulphate*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate at the maximum at about 263 nm (2.4.7). Calculate the content of $C_{19}H_{24}N_2O_2$ in the medium from the absorbance obtained from a solution of known concentration of *praziquantel RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_{19}H_{24}N_2O_2$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 150 mg of Praziquantel, add 70 ml of mobile phase, sonicate for 5 minutes, dilute with mobile phase to 100 ml, mix and filter. Dilute 3.0 ml of the solution to 25 ml with the mobile phase and mix.

Reference solution. A 0.018 per cent w/v solution of *praziquentel RS* in the mobile phase.

Chromatographic System
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase : a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

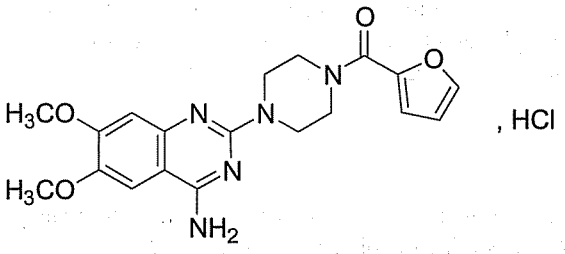
Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{24}N_2O_2$. in the tablets.

Storage. Store protected from light.

Prazosin Hydrochloride



$C_{19}H_{21}N_5O_4$, HCl Mol. Wt. 419.9

Prazosin Hydrochloride is 2-[4-(2-furoyl)piperazin-1-yl]-6,7-dimethoxyquinazolin-4-ylamine hydrochloride.

Prazosin Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{21}N_5O_4$, HCl, calculated on the anhydrous basis.

Category. Antihypertensive.

Dose. 500 µg to 1 mg two to three times daily.

Description. A white or almost white powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prazosin hydrochloride RS.*

B. Prepare a 0.05 per cent w/v solution of the substance under examination in a 0.1 per cent v/v solution of *hydrochloric acid* in *methanol.* Dilute separately 1 ml and 5 ml of this solution to 100 ml with the same acid solution (solutions A and B respectively). When examined in the range 220 nm to 280 nm (2.4.7), solution A shows an absorption maximum at about 247 nm; absorbance at the maximum, 0.66 to 0.70. When examined in the range 280 nm to 400 nm, solution B exhibits two maxima at about 330 nm and 343 nm; absorbances at the two maxima, 0.65 to 0.70 and 0.60 to 0.66 respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Solvent mixture. A mixture of 1 volume of *diethylamine,* 10 volumes of *methanol* and 10 volumes of *methylene chloride.*

Mobile phase. A mixture of 5 volumes of *diethylamine* and 95 volumes of *ethyl acetate.*

Test solution. Dissolve 10 mg of the substance under examination in 10 ml of solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *prazosin hydrochloride RS* in solvent mixture.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in warm air and examine under ultra-violet light at 254 nm. The principal spot in the chroma togram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. A 0.1 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Iron. To 1.0 g add dropwise about 1.5 ml of *nitric acid,* heat cautiously on a water-bath until fumes are no longer evolved. Ignite by slowly raising the temperature from 150° to 1000°, maintaining the final temperature for 1 hour. Cool, dissolve the residue in 20 ml of *2 M hydrochloric acid,* evaporate to about 5 ml, dilute to 25 ml with *2 M hydrochloric acid* and examine the resulting solution by atomic absorption spectrophotometry (2.4.2), measuring at 248 nm using an iron hollow-cathode light as a source of radiation, an air-acetylene flame and *iron standard solution (8 ppm Fe),* diluted as

necessary with *water* to prepare the standard solutions (100 ppm).

Reserve about 10 ml of the solution for the Nickel test.

Nickel. Examine the final solution prepared in the test for Iron by atomic absorption spectrophotometry (2.4.2), measuring at 232 nm using a nickel hollow-cathode light as a source of radiation, an air-acetylene flame and using *nickel standard solution (10 ppm Ni)*, diluted as necessary with *water* to prepare the standard solutions (50 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in the mobile phase and dilute to 50 ml with the mobile phase.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the mobile phase. Further dilute 1 ml of the solution to 10 ml with the mobile phase.

Reference solution (b). Dissolve 8 mg of *metoclopramide hydrochloride RS* in 1 ml of the test solution and dilute to 25 ml with the mobile phase. Further dilute 1 ml of the solution to 10 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of a solution containing 3.484 g per litre of *sodium pentanesulphonate* and 3.64 g per litre of *tetramethylammonium hydroxide* adjusted to pH 5.0 with *glacial acetic acid,*
- flow rate.1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The retention times are: prazocine about 9 minutes and metoclopramide about 5 minutes.The test is not valid unless the resolution between the peaks corresponding to prazocine and metoclopramide is at least 8.

Inject the test solution and reference solution (a). Continue the chromatography of the test solution for 6 times the retention time of the principal peak. Any secondary peak obtained with the test solution is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).The sum of all the impurities is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). Determine on the residue obtained in the test for Sulphated ash. Dissolve in sufficient *2 M nitric acid* to produce 50 ml. The resulting solution complies with the limit test for heavy metals, Method B (50 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent w/w, using 2.0 g dissolved in a mixture of equal volumes of *dichloromethane* and *methanol*.

Assay. Weigh accurately about 0.35 g, dissolve in 20 ml of *anhydrous formic acid* and 30 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04199 g of $C_{19}H_{21}N_5O_4$, HCl.

Storage. Store protected from light.

Prazosin Tablets

Prazosin Hydrochloride Tablets

Prazosin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of prazosin, $C_{19}H_{21}N_5O_4$.

Usual strengths. The equivalent of 500 µg; 1 mg; 2 mg; 5 mg of prazosin.

Identification

Shake a quantity of the powdered tablets containing about 10 mg of prazosin with a mixture of 10 ml of *dichloromethane* and 10 ml of *0.05 M potassium hydroxide*, filter the organic layer through cotton, evaporate to dryness and dry the residue at 60° at a pressure not exceeding 2 kPa at 60° for 2 hours.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prazosin hydrochloride RS* or with the reference spectrum of prazosin.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 95 volumes of *ethyl acetate* and 5 volumes of *diethylamine.*

Test solution. Shake a quantity of the powdered tablets containing 5 mg of prazosin with 2 ml of a mixture of 10 volumes of *dichloromethane*, 10 volumes of *methanol* and 1 volume of *diethylamine*, centrifuge and pass the supernatant liquid through a 0.5 µm PTFE (Polytetrafluoroethylene) filter.

Reference solution (a). Dilute 1 volume of test solution to 200 volumes with the same solvent mixture

Reference solution (b). Dilute 2 volumes of reference solution (a) to 5 volumes with the same solvent mixture.

Apply to the plate 20 µl of each solution. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than two such spots are more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Shake one tablet for 1 hour in a suitable volume of a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water* to produce a solution containing 0.002 per cent w/v solution of prazosin, centrifuge and use the supernatant liquid.

Reference solution. A 0.0022 per cent w/v solution of *prazosin hydrochloride RS* in the same solvent mixture.

Chromatographic system
- a stainless steel column 20 cm x 4 mm, packed with porous silica particles, (5 µm),
- mobile phase: 0.01 per cent w/v solution of *diethylamine* in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Calculate the content of $C_{19}H_{21}N_5O_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 2 mg of prazosin with 100 ml in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water* for 30 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.0022 per cent w/v solution of *prazosin hydrochloride RS* in a mixture of 96 volumes of *methanol*, 2 volumes of *glacial acetic acid* and 2 volumes of *water.*

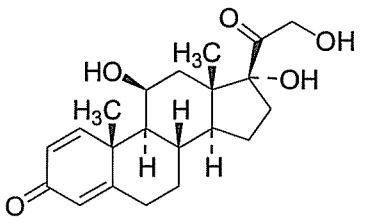
The chromatographic conditions as described under Uniformity of content may be used.

Calculate the content of $C_{19}H_{21}N_5O_4$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of prazosin.

Prednisolone



$C_{21}H_{28}O_5$ Mol. Wt. 360.4

Prednisolone is 11*β*,17*α*,21-trihydroxypregna-1,4-diene-3,20-dione.

Prednisolone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{21}H_{28}O_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Dose. Upto 30 mg daily, in divided doses.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisolone RS* or with the reference spectrum of prednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide.*

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *prednisolone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow

the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot. A.

C. Dissolve 2 mg in 2 ml of *sulphuric acid* by shaking and allow to stand for 5 minutes; an intense red colour is produced with a reddish brown fluorescence when examined in ultraviolet light at 365 nm. Pour the solution into 10 ml of *water* and mix; the colour fades and there is a yellow fluorescence under ultra-violet light (365 nm).

Tests

Specific optical rotation (2.4.22). +96.0° to +102°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 240 nm, 0.40 to 0.43.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 2 ml of *tetrahydrofuran* and dilute to 10ml with *water*.

Reference solution (a). Dissolve 2 mg of *prednisolone RS* and 2 mg of *hydrocortisone RS* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with base deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 220 ml of *tetrahydrofuran* and 700 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisolone, about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone and hydrocortisone is at least 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

Inject separately the solvent mixture of the test solution as a blank, the test solution and reference solution (b). Continue the chromatography of the test solution for 4.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak, is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and not more than one such peak has an area more than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not more than 2.0 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent). Ignore any peak obtained with the blank run and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 243.5 nm. Calculate the content of $C_{21}H_{28}O_5$ taking 415 as the specific absorbance at 243.5 nm.

Storage. Store protected from light and moisture.

Prednisolone Tablets

Prednisolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of prednisolone, $C_{21}H_{28}O_5$.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

Extract a quantity of the powdered tablets containing 30 mg of Prednisolone with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisolone RS* or with the reference spectrum of prednisolone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *prednisolone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 10 mg of Prednisolone with 25 ml of *methanol* for 10 minutes and mix with the aid of ultrasound for 2 minutes; filter the extract (Whatman GF/F is suitable), wash the filter with two 10-ml quantities of *methanol*, combine the filtrate and washings and evaporate to dryness using a rotary evaporator and a warm water-bath, dissolve the residue in 10 ml of *tetrahydrofuran* and dilute to 20 ml with *water*.

Reference solution (a). Dissolve 2 mg of *prednisolone RS* and 2 mg of *hydrocortisone RS* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with a 50 per cent v/v solution of *tetrahydrofuran*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 220 ml of *tetrahydrofuran* and 700 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 30 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisolone, about 14 minutes and hydrocortisone about 15.5 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisolone and hydrocortisone is not less than 2.2. If necessary, adjust the concentration of *tetrahydrofuran* in the mobile phase.

Inject separately the solvent mixture of the test solution as a blank, the test solution and reference solution (b). Continue the chromatography of the test solution for 4.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of the areas of all the peaks other than the principal peak is not greater than three times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak obtained with the blank run and any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent) and any peak with a retention time of 3 minutes or less.

Uniformity of content. (*For tablets containing 10 mg or less*) — Comply with the test stated under Tablets.

Powder one tablet, add 50 ml of *ethanol (95 per cent)*, shake for 30 minutes, add sufficient *ethanol (95 per cent)* to produce 100.0 ml. Centrifuge and pipette a suitable volume of the supernatant liquid containing 0.5 mg of Prednisolone and dilute to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{21}H_{28}O_5$ taking 415 specific absorbance at 240 nm.

Dissolution (2.5.2).

Apparatus. No. 1,

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium, filter and dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the filtrate at the maximum at about 240 nm

(2.4.7). Calculate the content of $C_{21}H_{28}O_5$ in the medium from the absorbance obtained from a solution of known concentration of *prednisolone RS*.

D. Not less than 70 per cent of the stated amount of $C_{21}H_{28}O_5$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Prednisolone, add 58 ml of *methanol*, shake for 10 minutes and add sufficient *water* to produce 100.0 ml. Mix well and filter.

Reference solution (a). A solution containing 0.005 per cent w/v of *prednisolone RS* and 0.0075 per cent w/v of *dexamethasone* (internal standard) in a mixture of 58 volumes of *methanol* and 42 volumes of *water*.

Reference solution (b). Prepare in the same manner as the test solution but adding 10 ml of a 0.075 per cent w/v solution of *dexamethasone* in *methanol* and 48 ml of *methanol* in place of 58 ml of methanol.

Chromatographic system

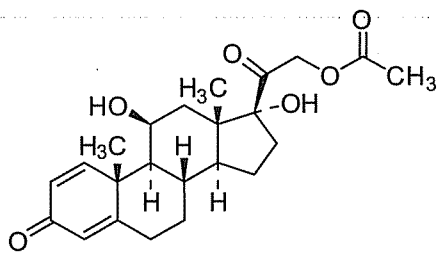
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a mixture of 42 volumes of *water* and 58 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

The assay is not valid unless the resolution factor between the peaks due to prednisolone and dexamethasone is greater than 2.5 and the column efficiency, determined using the peak due to prednisolone in the chromatogram obtained with the test solution is greater than 15,000 theoretical plates per metre.

Calculate the content of $C_{21}H_{28}O_5$ in the tablets.

Storage. Store protected from light.

Prednisolone Acetate



$C_{23}H_{30}O_6$ Mol.Wt. 402.5

Prednisolone Acetate is 11β,17α-dihydroxy-3,20-dioxopregna-1,4-dien-21-yl acetate.

Prednisolone Acetate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{23}H_{30}O_6$, calculated on the dried basis.

Category. Antiinflammtory; immunosuppressant.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisolone acetate RS* or with the reference spectrum of prednisolone acetate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Solvent mixture. 10 volumes of *methanol* and 90 volumes of *dichloromethane*.

Mobile phase. Add a mixture of 1.2 volumes of *water* and 8 volumes of *methanol* to a mixture of 15 volumes of *ether* and 77 volumes of *dichloromethane*.

Test solution. Dissolve 10 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *prednisolone acetate RS* in the solvent mixture.

Reference solution (b). Dissolve 10 mg of *prednisolone pivalate RS* in 10.0 ml of reference solution (a).

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray with *alcoholic solution of sulphuric acid*. Heat at 105° for 10 minutes or until the spots appear. Allow to cool and examine under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

C. Add about 2 mg of the substance under examination in 2 ml of *sulphuric acid* and shake to dissolve. Within 5 minutes, an intense red colour develops. When examined under ultraviolet light at 365 nm, a reddish-brown fluorescence is seen. Add the solution to 10 ml of *water* and mix. The colour fades and there is greenish-yellow fluorescence in ultraviolet light at 365 nm.

D. It gives the reaction of acetyl (2.3.1).

Tests

Specific optical rotation (2.4.22). +128° to +137°, determined in a 0.35 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. Equal volumes of *acetonitrile* and buffer solution pH 4 prepared by mixing 1 volume of *dilute hydrochloric acid*, 5 volumes of a 6.81 per cent w/v solution of *sodium acetate*, 15 volumes of a 3.73 per cent w/v solution of *potassium chloride* and 79 volumes of *water*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of *methanol*.

Reference solution (a). Dissolve 2 mg each of *prednisolone acetate RS* and *hydrocortisone acetate RS (prednisolone impurity A RS)* in 100 ml of the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100 ml with the solvent mixture. Dilute 2.0 ml of this solution to 10 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

The relative retention time with reference to prednisolone acetate for prednisolone acetate impurity B is about 0.4, for prednisolone acetate impurity A is about 1.1, for prednisolone acetate impurity C is about 2.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to prednisolone acetate and prednisolone acetate impurity A is not less than 2.0.

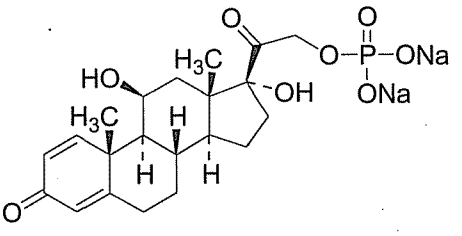
Inject the test solution and reference solution (b). Run the chromatogram 2.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to prednisolone acetate impurity A and B is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of secondary peak corresponding to prednisolone acetate impurity C is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore any peak with an area less than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Dissolve 0.1 g in 100.0 ml of *ethanol (95 per cent)*. Dilute 2 ml of this solution to 100 ml with the same solvent. Measure the absorbance at the maximum at about 243 nm (2.4.7). Calculate the content of $C_{23}H_{30}O_6$ taking 370 as the specific absorbance at 243 nm.

Storage. Store protected from light.

Prednisolone Sodium Phosphate



$C_{21}H_{27}Na_2O_8P$ Mol. Wt. 484.4

Predisolone Sodium Phosphate is 11β,17α, 21-trihydroxypregna-1,4-diene-3,20-dione-21-(dihydrogenphosphate) disodium salt.

Prednisolone Sodium Phosphate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{21}H_{27}Na_2O_8P$, calculated on the dried basis.

Category. Antiinflammtory; immunosuppressant.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisolone sodium phosphate RS*. If the spectra obtained in the solid state show differences, dissolve the substance under examination and the reference substance separately in the minimum volume of *ethanol (95 per cent)* evaporate to dryness on a water-bath and record the spectra again using the residues.

B. To about 40 mg add 2 ml of *sulphuric acid* and heat gently until white fumes are evolved. Add *nitric acid* dropwise, continue the heating until the solution is almost colourless and cool. Add 2 ml of *water*, heat until white fumes are again evolved, cool, add 10 ml of *water* and neutralise to *red litmus paper* with *dilute ammonia solution*. The solution complies with reaction A of sodium salts and reaction B of phosphates (2.3.1).

Tests

pH (2.4.24). 7.5 to 10.5 determined in 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +95.0° to +102.0°, determined in a 1.0 per cent w/v solution in a mixture of 9 volumes of *phosphate buffer pH 7.0* and 1 volume of *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 62.5 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dissolve 25 mg of *prednisolone sodium phosphate RS* and 25 mg of *prednisolone RS* in the mobile phase and dilute to 25.0 ml with the same solvent. Dilute 1.0 ml of the solution to 25.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: mix 1.36 g of *potassium dihydrogen phosphate* and 0.6 g of *hexylamine* and allow to stand for 10 minutes and dissolve in 185 ml of *water*, add 65 ml of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is 70 per cent to 90 per cent of the full scale of the recorder.

Equilibrate the column with the mobile phase for about 30 minutes.

Inject reference solution (a). The retention times are: prednisolone sodium phosphate, about 6.5 minutes and prednisolone, about 8.5 minutes. The test is not valid unless the resolution between the peaks due to prednisolone sodium phosphate and prednisolone is at least 4.5; if this resolution is not achieved, increase the concentration of *acetonitrile* or increase the concentration of *water* in the mobile phase.

Inject separately the test solution and reference solution (b). Continue the chromatography for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak other than that from the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent) and not more than one such peak has an area greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.025 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Inorganic phosphate. Not more than 1.0 per cent of phosphate, PO_4.

Dissolve 50 mg in sufficient *water* to produce 100 ml. To 10 ml of the resulting solution add 5 ml of *molybdovanadic reagent*, mix and allow to stand for 5 minutes. Any yellow colour in the solution is not more intense than that produced in a standard prepared at the same time in the same manner using 10 ml of *phosphate standard solution (5 ppm PO_4)*.

Water (2.3.43). Not more than 6.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient *water* to produce 100.0 ml and mix. Dilute 5.0 ml of the resulting solution to 250.0 ml with *water*. Measure the absorbance at the maximum at about 247 nm (2.4.7). Calculate the content of $C_{21}H_{27}Na_2O_8P$ taking 312 as the specific absorbance at 247 nm.

Storage. Store protected from light.

Prednisolone Sodium Phosphate Injection

Prednisolone Sodium Phosphate Injection is a sterile solution of Prednisolone Sodium Phosphate in Water for Injections.

Prednisolone Sodium Phosphate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of prednisolone phosphate, $C_{21}H_{29}O_8P$.

Usual strength. 20 mg per ml.

Description. A clear, colourless liquid.

Identification

A. In the Assay, the chromatogram obtained with the test solution corresponds to the peak due to prednisolone sodium phosphate in the chromatogram obtained with reference solution (a).

B. To a volume containing 0.2 mg of Prednisolone Sodium Phosphate slowly add 1 ml of *sulphuric acid* and allow to stand for 2 minutes. A deep red colour is produced.

Tests

pH (2.4.24). 7.0 to 8.0.

Bacterial endotoxins (2.2.3). Not more than 5.0 Endotoxin Units per mg of prednisolone phosphate.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute an accurately measured volume of the injection to obtain a solution containing 0.001 per cent w/v of prednisolone phosphate.

Reference solution (a). Weigh accurately about 10 mg of *prednisolone sodium phosphate RS*, dissolve in sufficient *water* to produce 100.0 ml (solution A) and dilute 10.0 ml of the solution to 100.0 ml with *water*.

Reference solution (b). Add 10 ml of a 0.01 per cent w/v solution of *betamethasone sodium phosphate RS* in *water* to 10 ml of solution A and dilute to 100 ml with *water*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (10 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 45 volumes of *methanol* and 55 volumes of *citro-phosphate buffer pH 5.0*,
- flow rate. 2.0 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume. 10 µl.

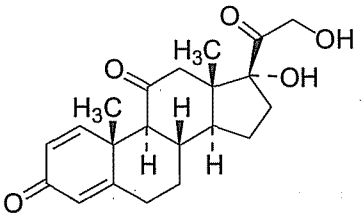
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to betamethasone sodium phosphate and prednisolone sodium phosphate is at least 2.5.

Inject alternatively the test solution and reference solution (a).

Calculate the content of $C_{21}H_{29}O_8P$ in the injection.

Storage. Store protected from light, in a single-dose or in multi-dose containers.

Prednisone



$C_{21}H_{26}O_5$ Mol. Wt. 358.4

Prednisone is 17α,21-dihydroxypregna-1,4-diene-3,11,20-trione.

Prednisone contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{21}H_{26}O_5$, calculated on the dried basis.

Category. Adrenocortical steroid.

Dose. 5 to 60 mg daily, in divided doses.

Description. A white or almost white, crystalline powder; odourless.

Identification

Tests A and B may be omitted if tests C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisone RS* or with the reference spectrum of prednisone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *prednisone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Dissolve 2 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced within 5 minutes, which exhibits a blue fluorescence in ultraviolet light at 365 nm. Pour the solution into 10 ml of *water*; the colour changes first to yellow and then fades gradually but the blue fluorescence in ultraviolet light remains.

D. Dissolve 1 mg in 1 ml of *ethanol (95 per cent)*, evaporate to dryness at a pressure not exceeding 0.7 kPa, add 5 ml of *1 M*

sodium hydroxide and heat at 70° for 30 minutes; not more than a slight yellow colour is produced (distinction from cortisone acetate).

Tests

Specific optical rotation (2.4.22). +167° to +175°, determined in a 1.0 per cent w/v solution in *dioxan*.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *methanol* at the maximum at about 240 nm, 0.40 to 0.43; the ratio of the absorbance at the maximum at about 240 nm to that at about 263 nm, 1.85 to 2.05.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in *methanol* and dilute to 10 ml with the same solvent.

Reference solution (a). Dissolve 2.0 mg of *prednisolone RS* and 2.0 mg of *prednisone RS* in methanol and dilute to 100 ml with the same solvent.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- column temperature. 45°,
- mobile phase: A. a mixture of 100 ml of *acetonitrile*, 200 ml of *methanol* and 650 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
  B. *acetonitrile*,
  –flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Time (min)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)	Comment
0	100	0	Isocratic
25	100	0	begin linear gradient
40	40	60	end chromatogram, change to 100B
41	0	100	being treatment with B
46	0	100	end treatment, return to 100A
47	100	0	begin equilibration with A
52	100	0	end equilibration, begin next chromatogram

Equilibrate the column with the mobile phase B for at least 30 minutes and then with mobile phase A for 5 minutes. For subsequent chromatograms, use the conditions described from 40.0 to 52.0 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisone, about 19 minutes and prednisolone about 23 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisone and prednisolone is at least 2.7. If necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately *methanol* as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak other than the principal peak is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak due to the blank run and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient *ethanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 238 nm. Calculate the content of $C_{21}H_{26}O_5$ taking 425 as the specific absorbance at 238 nm.

Storage. Store protected from light.

Prednisone Tablets

Prednisone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of prednisone, $C_{21}H_{26}O_5$.

Usual strengths. 5 mg; 10 mg.

Identification

Shake a quantity of the powdered tablets containing 30 mg of Prednisone with 10 ml of *chloroform*, filter and evaporate the

filtrate to dryness. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prednisone RS* or with the reference spectrum of prednisone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. Chloroform.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *prednisone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Prednisone with 10 ml of *methanol* for 10 minutes, mix with the aid of ultrasound for 2 minutes and filter the extract (Whatman GF/F is suitable).

Reference solution (a). Dissolve 2.0 mg of *prednisolone RS* and 2.0 mg of *prednisone RS* in *methanol* and dilute to 100 ml with the same solvent.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica(5 µm),
- column temperature. 45°,
- mobile phase: A. a mixture of 100 ml of *acetonitrile*, 200 ml of *methanol* and 650 ml of *water*, allowed to equilibrate, diluted to 1000 ml with *water* and mixed again,
  B. *acetonitrile*,
- flow rate. 2.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Time (min)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)	Comment
0	100	0	Isocratic,
25	100	0	begin linear gradient
40	40	60	end chromatogram, change to 100B
41	0	100	begin treatment with B
46	0	100	end treatment, return to 100A
47	100	0	begin equilibration with A
52	100	0	end equilibration, begin next chromatogram

Equilibrate the column with the mobile phase B for at least 30 minutes and then with mobile phase A for 5 minutes. For subsequent chromatograms use the conditions described from 40.0 to 52.0 minutes.

Adjust the sensitivity of the system so that the height of the principal peak in the chromatogram obtained with reference solution (b) is not less than 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are: prednisone, about 19 minutes and prednisolone about 23 minutes. The test is not valid unless the resolution between the peaks corresponding to prednisone and prednisolone is at least 2.7. If necessary, adjust the concentration of acetonitrile in mobile phase A.

Inject separately *methanol* as a blank, the test solution and reference solution (b). In the chromatogram obtained with the test solution: the area of any peak other than the principal

peak is not greater than 0.25 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.25 per cent); the sum of the areas of all the peaks, apart from the principal peak, is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.75 per cent). Ignore any peak due to the blank run and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, add 50 ml of *ethanol (95 per cent)*, shake for 30 minutes, add sufficient *ethanol (95 per cent)* to produce 100.0 ml. Centrifuge and pipette a suitable volume of the supernatant liquid equivalent to 0.5 mg of Prednisone and dilute to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{21}H_{26}O_5$ taking 415 as specific absorbance at 240 nm.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{21}H_{26}O_5$ in the medium from the absorbance obtained from a solution of known concentration of *prednisone RS*.

D. Not less than 70 per cent of the stated amount of $C_{21}H_{26}O_5$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. Dissolve an accurately weighed quantity of *acetanilide* in a 50 per cent v/v solution of *methanol* to obtain a solution having a known concentration of 0.11 mg per ml.

Test solution. Weigh and powder 20 tablets. To an accurately weighed quantity of the powder containing about 20 mg of Prednisone, add 5 ml of *water*, mix with the aid of ultrasound for one minute, add 50 ml of *methanol* and mix with the aid of ultrasound for I minute. Dilute with *water* to 100.0 ml and mix. To 5.0 ml of this solution add 5.0 ml of internal standard solution and dilute to 50.0 ml with a 50 per cent v/v solution of *methanol* and mix. Filter through a 5 µm filter and discard the first 20 ml of the filtrate.

Reference solution. Weigh accurately a suitable quantity of *prednisone RS* and dissolve in a 50 per cent v/v solution of *methanol* to obtain a solution having a concentration of about 0.2 mg per ml. To 5.0 ml of this solution add 5.0 ml of internal standard solution and dilute to 50.0 ml with a 50 per cent v/v solution of *methanol*.

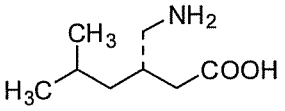
Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 to 10 µm),
- mobile phase: a suitable filtered mixture of 688 volumes of *water*, 250 volumes of *peroxide-free tetrahydrofuran* and 62 volumes of *methanol* such that at a flow rate of 1 ml per minute the retention times of prednisone and *acetanilide* are about 8 and 6 minutes respectively,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. Adjust the operating parameters such that the peak obtained is about 50 per cent of the full scale. The relative standard deviation for replicate injections is not more than 2.0 per cent and the resolution between prednisone and the internal standard is not less than 3.0.

Inject the test solution and the reference solution. Calculate the content of $C_{21}H_{26}O_5$ in the tablets.

Storage. Store protected from light.

Pregabalin



$C_8H_{17}NO_2$ Mol. Wt. 159.2

Pregabalin is (*S*)-4-amino-3-(2-methylpropyl)butyric acid.

Pregabalin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{17}NO_2$, calculated on the dried basis.

Category. Anticonvulsant.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pregabalin RS* or with the reference spectrum of pregabalin.

Tests

Specific optical rotation (2.4.22). +10° to +12.0°, determined on 1.0 per cent w/v solution.

Enantiomeric purity. Determine by liquid chromatography (2.4.14).

Merfey's reagent. Dissolve about 0.1502 g of merfey's reagent in 50 ml of *acetone*.

NOTE—Store the reagent at 2° to 8°.

Test solution (a). Dissolve about 100 mg of the substance under examination in 100.0 ml of *water*.

Test solution (b). To 2.0 ml of test solution (a), add 0.5 ml of merfey's reagent and 0.5 ml of *1 M sodium bicarbonate* in the test tube. Heat at 40° for 60 minutes. Allow to equilibrate to room temperature.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- mobile phase: a mixture of 62 volumes of a buffer solution prepared by diluting about 7.18 ml of *triethylamine* to 1000 ml *water*, adjusted to pH 3.0 with *orthophosphoric acid* and 38 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 340 nm,
- injection volume. 20 µl.

The retention time for S- Pregabalin is about 9 minutes and R-Pregabalin is about 12 minutes.

Inject test solution (b). The area of peak due to R-pregabalin is not more than 0.5 per cent the area of the S-pregabalin.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 95 volumes of *water* and 5 volumes of *acetonitrile*.

Test solution. Dissolve about 50 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution. Dilute 5.0 ml of the test solution to 100 ml with the solvent mixture. Further dilute 5.0 ml of this solution to 50 ml with the solvent mixture.

Use chromatographic system as described under Assay.

Inject the reference solution. The test is not valid unless the relative standard deviation of replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (1.0 per cent).

Heavy Metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 200 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.2 per cent w/v solution of *pregabalin RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- mobile phase: a mixture of 95 volumes of *water* and 5 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{17}NO_2$.

Storage. Store protected from light and moisture.

Pregabalin Capsules

Pregabalin Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pregabalin, $C_8H_{17}NO_2$.

Usual strengths. 25 mg; 50 mg; 75 mg; 100 mg; 150 mg; 225 mg; 300 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.05 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate and dilute, if necessary, with the dissolution medium.

Reference solution. A 0.0083 per cent w/v solution of *pregabalin RS* in the dissolution medium.

Use chromatographic system as described under Assay using injection volume 100 µl.

Inject the reference solution. The test is not valid unless the theoretical plates of the principal peak is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{17}NO_2$.

D. Not less than 70 per cent of the stated amount of $C_8H_{17}NO_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 1.2 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, adjust the pH to 6.9 with *dilute potassium hydroxide*.

Test solution. Mix the content of 10 capsules. Weigh and disperse a quantity containing about 15 mg of Pregabalin, in about 25 ml of the solvent mixture, sonicate for 30 minutes and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.015 per cent w/v solution of *pregabalin RS* in the solvent mixture.

Reference solution (b). Dissolve 4.5 mg of *lactam RS* in 10.0 ml of the solvent mixture.

Reference solution (c). Dissolve 750 mg of *pregabalin RS* in 30 ml of solvent mixture. Add about 5 ml of reference solution (b) and dilute to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3V),
- mobile phase: A. a mixture of 90 volumes of the solvent mixture and 10 volumes of *acetonitrile*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
25	35	65
30	100	0
40	100	0

Inject reference solution (a). The test is not valid unless the theoretical plates is not less than 3000, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject reference solution (a) and the test solution. The relative retention time with reference to lactam peak for lactose conjugate impurity is about 0.78. In the chromatogram obtained with the test solution, the area of the peak due to lactam multiplied with relative response factor, 0.07 is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent), the area of lactose conjugate impurity peak multiplied with relative response factor, 0.05 is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (2.5 per cent). Ignore any peak with an area less than 0.05 times of the area of principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix the contents of 20 capsules. Disperse a quantity of powder containing about 200 mg of Pregabalin with 35 ml of the mobile phase, sonicate for 10 minutes and dilute to 50 ml with the mobile phase.

Reference solution. A 0.4 per cent w/v solution of *pregabalin RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- mobile phase: a mixture of 92 volumes of solution containing 2.72 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, add 2 ml of *triethylamine* and adjusted to pH 6.0 with *orthophosphoric acid*, 5 volumes of *methanol* and 3 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates are not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_8H_{17}NO_2$ in the capsules.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of Pregabalin.

Pregelatinised Starch

Pregelatinised Starch is prepared from maize starch, potato starch or rice starch by mechanical processing in the presence of *water*, with or without heat, to rupture all or part of the starch granules and subsequent drying. It contains no added substances but it may be modified to render it compressible and to improve its flow characteristics.

Description. A white or yellowish-white powder.

Identification

A. *Microscopic*— In a mixture of equal volumes of *glycerol* and *water*, it presents irregular, translucent, white or yellowish-white flakes or pieces with an uneven surface. Under polarised light (between crossed nicol prisms), starch granules with a distinct black cross intersecting at the hilum may be seen.

B. Disperse 0.5 g in 2 ml of *water* without heating, add 0.05 ml of *iodine solution*; a reddish-violet to blue colour is produced.

Tests

pH (2.4.24). 4.5 to 7.0, determined in 3.0 per cent w/v solution in *carbon dioxide-free water*.

Oxidising substances. Transfer 4.0 g to a glass-stoppered, 125 ml conical flask and add 50.0 ml of a mixture of equal volumes of *water* and *methanol*. Insert the stopper and swirl for 5 minutes. Transfer to a glass-stoppered 50 ml centrifuge tube and centrifuge. Transfer 30.0 ml of the clear supernatant liquid to a glass-stoppered 125-ml conical flask. Add 1 ml of *glacial acetic acid* and 0.5 g to 1.0 g of *potassium iodide*. Insert the stopper, swirl, and allow to stand for 30 minutes in the dark. Add 1 ml of *starch solution* and titrate with *0.002 M sodium thiosulphate* until the starch-iodine colour disappears. Carry out a blank titration. Not more than 1.4 ml of *0.002 M sodium thiosulphate* is required (0.002 per cent, calculated as H_2O_2).

1 ml of 0.002 M *sodium thiosulphate* is equivalent to 0.034 mg of oxidising substances, calculated as H_2O_2.

Sulphur dioxide (2.3.40). Not more than 50 ppm.

Iron (2.3.14). Dissolve the residue obtained in the test for Sulphated ash in 20 ml of *dilute hydrochloric acid*, filter. 10 ml of the filtrate complies with the limit test for iron (20 ppm).

Foreign matter. Examined under a microscope using a mixture of equal volumes of *glycerol* and *water*, not more than traces of matter other than starch granules are present.

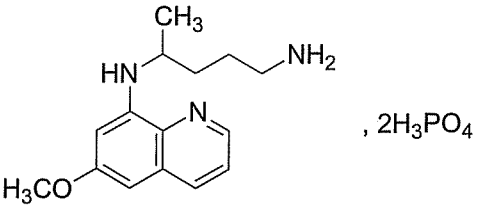
Sulphated ash (2.3.18). Not more than 0.6 per cent.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 130° for 90 minutes.

Microbial contamination (2.2.9). Total viable aerobic count is not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. It complies with the test for *Escherichia coli*.

Labelling. The label states the type of starch used as starting material.

Primaquine Phosphate



$C_{15}H_{21}N_3O,2H_3PO_4$ Mol. Wt. 455.3

Primaquine Phosphate is (*RS*)-8-(4-amino-1-methylbutylamino)-6-methoxyquinoline diphosphate.

Primaquine Phosphate contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{15}H_{21}N_3O,2H_3PO_4$, calculated on the dried basis.

Category. Antimalarial.

Dose. The equivalent of 15 mg of primaquine, once a day for 14 days.

Description. An orange, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *primaquine phosphate RS* or with the reference spectrum of primaquine phosphate.

B. When examined in the range 300 nm to 450 nm (2.4.7), a 0.015 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 332 nm and 415 nm; absorbance at about 332 nm, about 0.68 to 0.78 and at about 415 nm, 0.41 to 0.53. Dilute 5 ml of the solution to 50 ml with *0.01 M hydrochloric acid*. When examined in the range 215 nm to

310 nm, the resulting solution shows absorption maxima at about 225 nm, 265 nm and 282 nm; absorbances at the maxima are 0.74 to 0.77, 0.50 to 0.53 and 0.50 to 0.52, respectively.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel F254*.

Mobile phase. A mixture of 60 volumes of *chloroform*, 40 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Dissolve 0.2 g in 5 ml of *water*, dilute to 10 ml with *methanol* and dilute 1 volume of the resulting solution to 10 volumes with *methanol (50 per cent)*.

Reference solution. Dissolve 20 mg of *primaquine phosphate RS* in 5 ml of *water*, dilute to 10 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 50 mg in 5 ml of *water*, add 2 ml of *2 M sodium hydroxide* and shake with two quantities, each of 5 ml, of *chloroform*. The aqueous layer, acidified by the addition of *nitric acid*, gives reaction B of phosphates (2.3.1).

Tests

pH (2.4.24). 2.5 to 3.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Add 0.2 ml of *strong ammonia solution* to 1 ml of a 1.0 per cent w/v solution of the substance under examination, shake with 10 ml of the mobile phase and use the clear, lower layer.

Reference solution (a). Dilute 3 ml of the test solution to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 10 ml with the mobile phase and further dilute 1 ml of the resulting solution to 50 ml with the same solvent.

Reference solution (c). Add 0.2 ml of *strong ammonia solution* to 1 ml of a 1.0 per cent w/v solution of the *primaquine phosphate RS*, shake with 10 ml of the mobile phase and use the clear, lower layer.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with porous silica particles (10 µm),
- mobile phase: a mixture of 45 volumes of *chloroform*, 45 volumes of *hexane*, 10 volumes of *methanol* and 0.1 volume of *strong ammonia solution*,
- flow rate. 3 ml per minute,
- spectrophotometer set at 261 nm,
- injection volume. 20 µl.

The test is not valid unless in the chromatogram obtained with reference solution (c) there is a peak just before the principal peak with an area of about 6 per cent of that of the principal peak and the resolution between these peaks is not less than 2.0.

Inject each solution and record the chromatograms for at least twice the retention time of primaquine. The sum of the areas of any secondary peaks in the chromatogram obtained with the test solution is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a). Ignore any peak the area of which is less than that of the principal peak in the chromatogram obtained with reference solution (b).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in 40 ml of *anhydrous glacial acetic acid* with gentle heating. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02277 g of $C_{15}H_{21}N_3O,2H_3PO_4$.

Storage. Store protected from light and moisture.

Primaquine Tablets

Primaquine Phosphate Tablets

Primaquine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of primaquine, $C_{15}H_{21}N_3O$. The tablets are coated.

Usual strength. The equivalent of 2.5 mg of primaquine (13 mg of Primaquine Phosphate is approximately equivalent to 7.5 mg of primaquine).

Identification

A. Dissolve a quantity of the powdered tablets containing 60 mg of primaquine in a mixture of 10 ml of *water* and 2 ml of *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *chloroform*. Wash the chloroform extracts with *water*, dry over *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 2 ml of *chloroform*. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *primaquine phosphate RS* or with the reference spectrum of primaquine.

B. Extract a quantity of the powdered tablets containing 25 mg of primaquine with 10 ml of *water* and filter. To 2 ml of the filtrate add 3 ml of *water* and 1 ml of a 5 per cent w/v solution

of *ceric ammonium sulphate* in *2 M nitric acid*; a deep violet colour is produced immediately.

Tests

Uniformity of content. Comply with the tests stated under Tablets.

Transfer one tablet into a suitable container, add 5 ml of *hydrochloric acid*, disperse the tablet in about 25 g of crushed ice and add sufficient *water* to produce 50.0 ml. Carry out the nitrite titration (2.3.31), using *0.01 M sodium nitrite* as titrant. Carry out a blank titration.

1 ml of *0.01 M sodium nitrite* is equivalent to 0.002594 g of $C_{15}H_{21}N_3O$.

Other tests. Comply with the tests stated under Tablets.

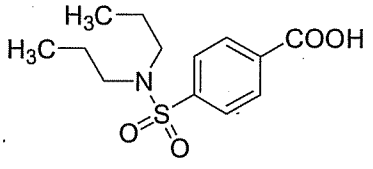
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of primaquine, dissolve in 20 ml of *water*, add 5 ml of *2 M sodium hydroxide* and extract with four quantities, each of 25 ml of *chloroform*. Combine the chloroform extracts and evaporate to a volume of about 10 ml. Add 40 ml of *anhydrous glacial acetic acid*, Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01297 g of $C_{15}H_{21}N_3O$.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of primaquine.

Probenecid



$C_{13}H_{19}NO_4S$ Mol. Wt. 285.4

Probenecid is 4-(dipropylsulphamoyl)benzoic acid.

Probenecid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{13}H_{19}NO_4S$, calculated on the dried basis.

Category. Uricosuric agent and for delaying renal excretion of penicillins and cephalosporins.

Dose. 250 mg to 500 mg twice daily.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *probenecid RS* or with the reference spectrum of probenecid.

B. When examined in the range 210 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *0.1 M hydrochloric acid* shows absorption maxima at about 223 nm and 248 nm; absorbance at about 248 nm is about 0.33.

C. Dissolve 0.2 g in about 0.6 ml of *2 M ammonia* and add 3 ml of 1.7 per cent w/v solution of *silver nitrate*; a white precipitate is produced which is soluble in an excess of *dilute ammonia solution*.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *2 M sodium hydroxide* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Acidity. Add 2.0 g to 100 ml of *water*, heat on a water-bath for 30 minutes, cool, filter and dilute with *water* to 100.0 ml. Titrate 50.0 ml of the solution with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Not more than 0.5 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 55 volumes of *toluene*, 20 volumes of *di-isopropyl ether*, 15 volumes of *chloroform* and 10 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *acetone*.

Reference solution. A 0.005 per cent w/v solution of the substance under examination in *acetone*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13).1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g and dissolve in 100 ml of *ethanol (95 per cent)*, shaking well and heating gently if

necessary. Cool and titrate with *0.1 M sodium hydroxide*, using *bromothymol blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02854 g of $C_{13}H_{19}NO_4S$.

Storage. Store protected from moisture.

Probenecid Tablets

Probenecid Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of probenecid, $C_{13}H_{19}NO_4S$.

Usual strength. 500 mg.

Identification

A. Triturate a quantity of the powdered tablets containing 0.5 g of Probenecid with *ethanol (95 per cent)*, filter and concentrate the filtrate by evaporation on a water-bath. Cool, filter and recrystallise the residue from *ethanol (50 per cent)*. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *probenecid RS* or with the reference spectrum of probenecid.

B. When examined in the range 210 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 225 nm and 248 nm.

C. The residue obtained in test A melts at about 199° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 55 volumes of *toluene*, 20 volumes of *di-isopropyl ether*, 15 volumes of *chloroform* and 10 volumes of *glacial acetic acid*.

Test solution. Extract a quantity of the powdered tablets containing 0.2 g of Probenecid with 20 ml of *acetone*, filter and use the filtrate.

Reference solution. Dilute 1 ml of the test solution to 200 ml with *acetone*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus. No. 1,

Medium. 900 ml of *phosphate buffer pH 7.6*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter, Dilute 4.0 ml of the filtrate to 100.0 ml with *0.1 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 244 nm (2.4.7). Similarly measure the absorbance of a solution of a known concentration of *probenecid RS*. Calculate the content of $C_{13}H_{19}O_4S$.

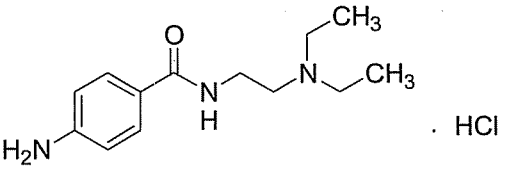
D. Not less than 80 per cent of the stated amount of $C_{13}H_{19}NO_3S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Probencid, add 200 ml of *ethanol (95 per cent)* and 5 ml of *1 M hydrochloric acid*, heat on a water-bath at 70° for 30 minutes, shaking occasionally. Cool, add sufficient *ethanol (95 per cent)* to produce 250.0 ml and filter. To 5.0 ml of the filtrate add 5 ml of *0.1 M hydrochloric acid*, dilute to 250.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 248 nm (2.4.7). Calculate the content of $C_{13}H_{19}NO_4S$ taking 332 as specific absorbance at 248 nm.

Storage. Store protected from moisture.

Procainamide Hydrochloride



$C_{13}H_{21}N_3O,HCl$ Mol. Wt. 271.8

Procainamide Hydrochloride is 4-amino-*N*-[2-(diethylamino) ethyl)benzamide hydrochloride.

Procainamide Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_{21}N_3O,HCl$, calculated on the dried basis.

Category. Antiarrhythmic.

Dose. Orally, 500 mg to 1.5 g daily, in divided doses; by slow intravenous injection

Description. A white or very slightly yellow, crystalline powder; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procainamide hydrochloride RS* or with the reference spectrum of procainamide hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum at about 273 nm; absorbance at about 273 nm, 0.58 to 0.61.

C. Dilute 1 ml of a solution, prepared by dissolving 2.5 g in sufficient *carbon dioxide-free water* to produce 25 ml, to 2 ml with *water*. 1 ml of this solution gives the reaction of primary aromatic amines (2.3.1).

D. A 2 per cent w/v solution gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

pH (2.4.24). 5.6 to 6.3, determined in a 10.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *1-butanol*, 30 volumes of *water* and 15 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *ethanol (95 per cent)*.

Reference solution. A 0.005 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 75 ml of *water* and 10 ml of *hydrochloric acid* and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02718 g of $C_{13}H_{21}N_3O$, HCl.

Storage. Store protected from light.

Procainamide Injection

Procainamide Hydrochloride Injection

Procainamide Injection is a sterile solution of Procainamide Hydrochloride in Water for Injections. It may contain Sodium Metabisulphite as a stabilising agent.

Procainamide Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of procainamide hydrochloride, $C_{13}H_{21}N_3O$, HCl.

Usual strength. 100 mg per ml.

Identification

A. Dilute a volume containing 0.25 g of Procainamide Hydrochloride to 25 ml with *water*, make alkaline with *5 M sodium hydroxide* and extract with two quantities, each of 5 ml, of *chloroform*. Filter the combined extracts through *anhydrous sodium sulphate*, evaporate the filtrate to dryness using a rotatory evaporator and dissolve the residue in 5 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procainamide hydrochloride RS* or with the reference spectrum of procainamide.

B. Dilute a suitable volume of the injection with *water* to produce a solution containing 0.0005 per cent w/v of Procainamide Hydrochloride. Absorbance of the resulting solution at about 280 nm, about 0.30 (2.4.7).

C. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.5.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *1-butanol*, 30 volumes of *water* and 15 volumes of *glacial acetic acid*.

Test solution. Dilute a volume of the injection containing 0.1 g of Procainamide Hydrochloride to 5 ml with *methanol*.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To a volume containing about 0.25 g of Procainamide Hydrochloride add 45 ml of *6 M hydrochloric acid* and boil for 1 minute. Carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02718 g of $C_{13}H_{21}N_3O,HCl$.

Storage. Store protected from light.

Procainamide Tablets

Procainamide Hydrochloride Tablets

Procainamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of procainamide hydrochloride, $C_{13}H_{21}N_3O,HCl$.

Usual strength. 250 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.25 g of Procainamide Hydrochloride with 25 ml of *water*, make alkaline with *5 M sodium hydroxide* and extract with two quantities, each of 5 ml, of *chloroform*. Filter the combined extracts through *anhydrous sodium sulphate*, evaporate the filtrate to dryness using a rotatory evaporator and dissolve the residue in 5 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procainamide hydrochloride RS* or with the reference spectrum of procainamide.

B. Triturate a quantity of the powdered tablets containing 1 g of Procainamide Hydrochloride with 10 ml of *water* and filter. The filtrate gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *1-butanol*, 30 volumes of *water* and 15 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.4 g of Procainamide Hydrochloride with 20 ml of *methanol (90 per cent)* for 15 minutes and filter.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

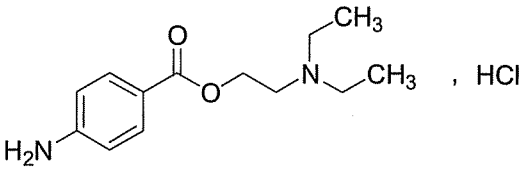
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.25 g of Procainamide Hydrochloride, add 100 ml of *6 M hydrochloric acid*, shake for 15 minutes and boil for 1 minute. Carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02718 g of $C_{13}H_{21}N_3O,HCl$.

Storage. Store protected from light and moisture.

Procaine Hydrochloride



$C_{13}H_{20}N_2O_2,HCl$ Mol. Wt. 272.8

Procaine Hydrochloride is 2-(diethylamino)ethyl 4-aminobenzoate hydrochloride.

Procaine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{13}H_{20}N_2O_2,HCl$, calculated on the dried basis.

Category. Local anaesthetic.

Dose. To be adjusted according to the site of operation and response of the patient.

Description. Colourless crystals or a white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procaine hydrochloride RS* or with the reference spectrum of procaine hydrochloride.

B. To 0.2 ml of a 5 per cent w/v solution add 2 ml of *water* and 0.5 ml of *1 M sulphuric acid*, shake and add 1 ml of a 0.1 per cent w/v solution of *potassium permanganate*; the colour is immediately discharged.

C. To about 5 mg add 0.5 ml of *fuming nitric acid*, evaporate to dryness on a water-bath, cool, dissolve the residue in 5 ml of *acetone* and add 1 ml of *0.1 M ethanolic potassium hydroxide*; only a brownish red colour develops.

D. Dilute 1 ml of a 5 per cent w/v solution to 100 ml with *water*; 2 ml of the solution gives the reaction of primary aromatic amines (2.3.1).

E. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 5.0 to 6.5, determined in a 2.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *dibutyl ether*, 16 volumes of *n-hexane* and 4 volumes of *glacial acetic acid*.

Test solution. A 10 per cent w/v solution of the substance under examination in *water*.

Reference solution. A 0.005 per cent w/v solution of *4-aminobenzoic acid* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° for 10 minutes and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. The principal spot remains on the line of application.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and titrate slowly with *0.1 M sodium nitrite*, stirring constantly. Determine the end point potentiometrically (2.4.25).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02728 g of $C_{13}H_{20}N_2O_2$, HCl.

Storage. Store protected from light.

Procaine and Adrenaline Injection

Procaine Hydrochloride and Adrenaline Bitartrate Injection; Procaine Hydrochloride and Epinephrine Bitartrate Injection

Procaine and Adrenaline Injection is a sterile solution of Procaine Hydrochloride and Adrenaline Bitartrate in Water for Injections.

Procaine and Adrenaline Injection contains not less than 1.9 per cent and not more than 2.1 per cent w/v of procaine hydrochloride, $C_{13}H_{20}N_2O_2$, HCl and the equivalent of not less than 0.00175 per cent and not more than 0.00225 per cent w/v of adrenaline, $C_9H_{13}NO_3$.

Description. A clear, colourless solution.

Identification

A. To 5 ml add 5 ml of *water* and 10 ml of *picric acid solution*, shake gently and set aside for 1 hour; the crystalline precipitate, after washing with *water* and drying at 100°, melts at about 134° (2.4.21).

B. To 5 ml add 1 ml of *hydrochloric acid*, cool to 0°, add 5 ml of *sodium nitrite solution* and pour the mixture into 2 ml of *2-naphthol solution* containing 1 g of *sodium acetate*; an orange-red colour is produced.

C. To 10 ml add 4 ml of *disodium hydrogen phosphate solution* and sufficient *0.1 M iodine* to produce a distinct brown colour. Add *0.1 M sodium thiosulphate* to remove the excess of iodine; a pink colour is produced.

Tests

pH (2.4.24). 3.0 to 5.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For procaine hydrochloride* — To 10.0 ml of the injection add 0.5 g of *sodium carbonate* and extract with three quantities, each of 20 ml, of a mixture of 1 volume of *2-propanol* and 3 volumes of *chloroform* until complete extraction of procaine is effected. Shake the combined extracts with 5 ml of *water*, wash the water with the solvent mixture and add the washing to the combined extracts. Shake the combined extracts and washings with 10.0 ml of *0.1 M hydrochloric acid*, separate the acid layer, wash the combined extracts and washings with 5 ml of *water*, add the aqueous extract to the separated acid layer and titrate with *0.1 M sodium hydroxide*, using *methyl red-methylene blue solution* as indicator.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.02728 g of $C_{13}H_{20}N_2O_2$,HCl.

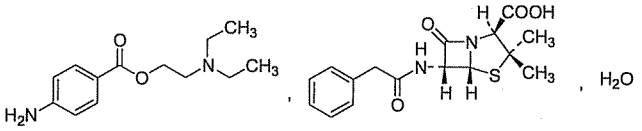
For adrenaline — To 10.0 ml of the injection add 20 mg of *sodium metabisulphite*, 0.1 ml of *ferrous sulphate-citrate solution*, 1 ml of *glycine buffer solution* and mix. Allow to stand for 10 minutes, extract with 10 ml of *ether*, allow to separate, reject the *ether* and measure the absorbance of a 4-cm layer of the solution at about 540 nm (2.4.7). Calculate the content of adrenaline, $C_9H_{13}NO_3$, from a reference curve prepared by treating suitable aliquots of a solution of *adrenaline bitartrate RS* in the same manner.

1 mg of adrenaline bitartrate is equivalent to 0.0005497 g of $C_9H_{13}NO_3$.

Storage. Store protected from light.

Labelling. The label states the strength as "Procaine Hydrochloride, 2 per cent w/v; Adrenaline, 0.002 per cent w/v".

Procaine Penicillin



$C_{13}H_{20}N_2O_2,C_{16}H_{18}N_2O_4S,H_2O$ Mol. Wt. 588.7

Procaine Penicillin is 2-diethylaminoethyl 4-aminobenzoate (6*R*)-6-(2-phenylacetamido)penicillanate monohydrate.

Procaine Penicillin contains not less than 51 per cent and not more than 59.6 per cent of penicillin G, calculated as $C_{16}H_{18}N_2O_4S$, and not less than 37.5 per cent and not more than 43.0 per cent of procaine, $C_{13}H_{20}N_2O_2$, both calculated on the anhydrous basis.

Category. Antibacterial.

Dose. By intramuscular injection, 300 to 900 mg daily (300 mg of Procaine Penicillin is approximately equivalent to 200 mg of benzylpenicillin).

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procaine penicillin RS* or with the reference spectrum of procaine penicillin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution (a).

C. A turbid solution of 0.1 g in 2 ml of *2 M hydrochloric acid* gives the reaction of primary aromatic amines (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5, determined in a solution prepared by shaking 50 mg in sufficient *carbon dioxide-free water* to produce 15 ml until dissolution is complete.

Specific optical rotation (2.4.22). +165° to +180°, determined in a solution prepared by dissolving 0.25 g in sufficient of a mixture of 3 volumes of *acetone* and 2 volumes of *water* to produce 25 ml.

Water (2.3.43). 2.8 to 4.2 per cent, determined on 0.5 g.

Assay. Determine the contents of benzyl penicillin and procaine by liquid chromatography (2.4.14).

Test solution. Weigh accurately 70 mg of the substance under examination and dissolve in 30 ml of the mobile phase with the aid of ultrasound for about 2 minutes. Dilute to 50.0 ml with the mobile phase.

Reference solution (a). Weigh accurately about 56 mg of *benzyl penicillin potassium RS* and 40 mg of *procaine hydrochloride RS* and dissolve in 25 ml of the mobile phase with the aid of ultrasound for about 2 minutes. Dilute to 50.0 ml with the mobile phase.

Reference solution (b). Mix 1 volume of a 0.24 per cent w/v solution of *phenoxymethylpenicillin potassium RS* in the mobile phase with 3 volumes of reference solution (a).

Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 50 volumes of a solution prepared by mixing 14 g of *potassium dihydrogen phosphate* in 6.5 g of *tetrabutylammonium hydroxide (40 per cent)* and adjusting the pH to 7.0 with *1 M potassium hydroxide* or *dilute phosphoric acid*, 25 volumes of *acetonitrile* and 25 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between benzyl penicillin potassium and phenoxymethylpenicillin potassium is not less than 2. The relative retention time of procaine with reference to benzyl penicillin is about 2.2.

Inject the test solution and reference solution (a). Record the peak responses of the main peaks of benzyl penicillin and procaine. Calculate the content of benzyl penicillin, $C_{16}H_{18}N_2O_4S$ and the content of procaine, $C_{13}H_{20}N_2O_2$ using the factor of 0.866 for converting the content of procaine hydrochloride to that of procaine.

Procaine Penicillin intended for use in the manufacture of Parenteral Preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.10 Endotoxin Unit per mg.

Procaine Penicillin intended for use in the manufacture of Parenteral Preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from moisture. If the material is intended for use in the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) where applicable, that the contents are free from bacterial endotoxins; (2) where applicable, that the contents are sterile.

Fortified Procaine Penicillin Injection

Procaine Penicillin with Benzylpenicillin Injection

Fortified Procaine Penicillin Injection is a sterile mixture of five parts of Procaine Penicillin and one part of Benzylpenicillin Potassium or Benzylpenicillin Sodium, together with suitable dispersing and buffering agents. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

Storage. The constituted injection should be used within 24 hours (4 days if a buffering agent is present) of preparation when stored at a temperature not exceeding 20° or within 7 days (14 days if a buffering agent is present) when stored at a temperature between 2° and 8°.

Fortified Procaine Penicillin Injection contains a quantity of total penicillins calculated as $C_{16}H_{17}N_2NaO_4S$ and equivalent to not less than 60.0 per cent and not more than 74.0 per cent of the stated amounts of procaine penicillin and benzylpenicillin potassium or benzylpenicillin sodium and a quantity of procaine, $C_{13}H_{20}N_2O_2$, equivalent to not less than 36.0 per cent and not more than 44.0 per cent of the stated amount of procaine penicillin.

Usual strengths. Procaine Penicillin, 300 mg (300,000 Units) and Benzylpenicillin Potassium or Benzylpenicillin Sodium 60 mg (100,000 Units), Procaine Penicillin 3 g (3,000,000 Units) and Benzylpenicillin Potassium or Benzylpenicillin Sodium, 600 mg (1,000,000 Units).

Description. A white or almost white powder.

The contents of the sealed container comply with the tests stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

A. Dissolve 10 mg in 10 ml of *water* and add 0.5 ml of *neutral red solution*. Add sufficient *0.01 M sodium hydroxide* to give a permanent orange colour and then add 1 ml of *penicillinase solution*; a red colour is produced rapidly.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Give the reaction of primary aromatic amines (2.3.1), producing a bright, orange-red precipitate.

Tests

Water (2.3.43). Not more than 3.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 10 containers equivalent to about 70 mg of procaine penicillin and dissolve in 30 ml of the mobile phase with the aid of ultrasound for about 2 minutes. Dilute to 50.0 ml with the mobile phase.

Reference solution (a). Weigh accurately about 56 mg of *benzylpenicillin potassium RS* and 40 mg of *procaine hydrochloride RS* and dissolve in 25 ml of the mobile phase with the aid of ultrasound for about 2 minutes. Dilute to 50.0 ml with the mobile phase.

Reference solution (b). Mix 1 volume of a 0.24 per cent w/v solution of *phenoxymethylpenicillin potassium RS* in the mobile phase with 3 volumes of reference solution (a).

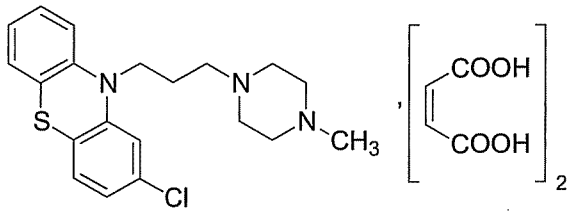
Chromatographic system
- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 50 volumes of a solution containing 1.4 per cent w/v of *potassium dihydrogen orthophosphate* and 0.65 per cent w/v *tetrabutyl ammonium hydroxide* adjusted to pH 7.0 with *1 M potassium hydroxide* or *orthophosphoric acid*, 25 volumes of *acetonitrile* and 25 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (b). The resolution between benzylpenicillin potassium and phenoxymethylpenicillin potassium is not less than 2. The relative retention time of procaine with reference to benzylpenicillin is about 2.2.

Inject the test solution and reference solution (a). Record the peak responses of the main peaks of benzyl penicillin and procaine. Calculate the total penicillin content as benzylpenicillin, $C_{16}H_{18}N_2O_4S$ and the content of procaine, $C_{13}H_{20}N_2O_2$ using the factor of 0.866 for converting the content of procaine hydrochloride to that of procaine.

Labelling. The label states (1) the quantities of Procaine Penicillin and Benzylpenicillin Potassium or Benzylpenicillin Sodium contained in it; (2) the names of any added dispersing and buffering agents; (3) "for intramuscular injection only".

Prochlorperazine Maleate



$C_{20}H_{24}ClN_3S,2C_4H_4O_4$ Mol. Wt. 606.1

Prochlorperazine Maleate is 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine di(hydrogen maleate).

Prochlorperazine Maleate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{24}ClN_3S$, $2C_4H_4O_4$, calculated on the dried basis.

Category. Antipsychotic; antiemetic.

Dose. As antipsychotic, 15 to 100 mg daily, in divided doses; as antiemetic, 10 to 30 mg daily, in divided doses.

Description. A white or pale yellow, crystalline powder; practically odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. To 20 mg add 5 ml of *water* and 1 ml of *1 M sodium hydroxide*, shake and extract with 10 ml of *ether*. Wash the ether extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.2 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prochlorperazine maleate RS* or with the reference spectrum of prochlorperazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol (95 per cent)* containing 0.01 per cent v/v of *strong ammonia solution* shows an absorption maximum at about 258 nm and a less well-defined maximum at about 313 nm; absorbance at about 258 nm, about 0.6.

C. Complies with the test for identification of phenothiazines (2.3.3).

Test solution. A solution containing 0.1 per cent w/v of the substance under examination in a mixture of equal volumes of *methanol* and *chloroform*.

Reference solution. A 0.1 per cent w/v solution of *prochlorperazine maleate RS* in the same solvent mixture.

Apply 4 µl of each solution.

D. Triturate 0.2 g with a mixture of 3 ml of *water* and 1 ml of *10 M sodium hydroxide* and shake with three quantities, each of 5 ml, of *ether*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat on a water-bath for 15 minutes; no colour develops. To the remainder of the aqueous layer add 2 ml of *bromine solution*, heat on a water-bath for 15 minutes, then heat to boiling and cool. To 0.1 ml of the solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat on a water-bath for 15 minutes; a blue colour develops.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a freshly prepared saturated solution.

Related substances. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase A.

NOTE— Prepare the test solution immediately before use.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, in powder, dissolve in 50 ml of *anhydrous glacial acetic acid*, heating gently on a water-bath, allow to cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03031 g of $C_{20}H_{24}ClN_3S,2C_4H_4O_4$.

Storage. Store protected from light and moisture.

Prochlorperazine Tablets

Prochlorperazine Maleate Tablets

Prochlorperazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of prochlorperazine maleate, $C_{20}H_{24}ClN_3S,2C_4H_4O_4$.

Usual strengths. 5 mg; 25 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Prochlorperazine Maleate add 10 ml of *water* and 2 ml of *1 M sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with

prochlorperazine maleate RS or with the reference spectrum of prochlorperazine.

B. To a quantity of the powdered tablets containing 5 mg of Prochlorperazine Maleate add 5 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

C. Shake a quantity of the powdered tablets containing 0.2 g of Prochlorperazine Maleate with 2 ml of *water* and 1 ml of *5 M sodium hydroxide*, mix and extract with three quantities, each of 10 ml, of *ether*. Dry the combined extracts with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *methanol* and add a solution of 0.15 g of *picric acid* in 10 ml of *methanol*. The precipitate, after washing with a small quantity of *methanol*, melts at about 255°, with decomposition (2.4.21).

Tests

Related substances. Comply with the test for Related substances in Phenothiazines (2.3.5), using mobile phase A.

NOTE— Prepare the following solutions freshly.

Test solution. Extract a quantity of the powdered tablets containing 0.1 g of Prochlorperazine Maleate with 10 ml of *methanol* containing 0.5 per cent v/v of *strong ammonia solution* and filter.

Reference solution. Dilute 1.0 ml of the test solution to 200.0 ml with the same solvent.

Apply to the plate 20 µl of each solution.

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

NOTE— Protect the solutions from light throughout the Assay.

Crush one tablet and extract with three quantities, each of 10 ml, of *ethanol* containing 1 per cent v/v of *strong ammonia solution*. Filter the extracts and to the combined filtrates add sufficient *ethanol* to produce 50.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *ethanol*. Dilute further with *ethanol*, if necessary, to give a final solution containing 10 µg of Prochlorperazine Maleate per ml and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{20}H_{24}ClN_3S$, $2C_4H_4O_4$ taking 620 as the specific absorbance at 258 nm.

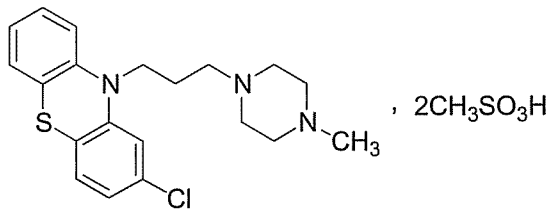
Other tests. Comply with the tests stated under Tablets.

Assay. *Protect the solutions from light throughout the Assay.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Prochlorperazine Maleate and extract with three quantities, each of 10 ml, of *ethanol* containing 1 per cent v/v of *strong ammonia solution*. Filter the extracts and to the combined filtrates add sufficient *ethanol* to produce 100.0 ml. Dilute 10.0 ml to 50.0 ml with *ethanol*, dilute 10.0 ml of this solution to 50.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{20}H_{24}ClN_3S$, $2C_4H_4O_4$ taking 620 as the specific absorbance at 258 nm.

Storage. Store protected from light and moisture.

Prochlorperazine Mesylate



$C_{20}H_{24}ClN_3S,2CH_4SO_3$ Mol. Wt. 566.2

Prochlorperazine Mesylate is 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine di(methanesulphonate).

Prochlorperazine Mesylate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{20}H_{24}ClN_3S,2CH_4SO_3$, calculated on the dried basis.

Category. Antipsychotic; antiemetic.

Dose. As antipsychotic, by intramuscular injection, 12.5 to 25 mg, twice or thrice daily; as antiemetic, by intramuscular injection, 12.5 mg.

Description. A white or almost white powder; odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prochlorperazine mesylate RS* or with the reference spectrum of prochlorperazine mesylate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *ethanol* containing 0.01 per cent v/v of *strong ammonia solution* shows an absorption maximum at about 258 nm and a less well-defined maximum at about 313 nm; absorbance at about 258 nm, about 0.6.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

D. Mix 50 mg with 0.2 g of powdered *sodium hydroxide*, heat to fusion and continue the heating for a few seconds longer. Cool, add 0.5 ml of *water* and a slight excess of *2 M hydrochloric acid* and warm; sulphur dioxide is evolved which turns moistened *starch iodate paper* blue.

Tests

pH (2.4.24). 2.0 to 3.0, determined in a 2.0 per cent w/v solution.

Related substances. Complies with the test for Related substances in Phenothiazines (2.3.5), using mobile phase A.

Test solution. Dissolve the substance under examination in *methanol* containing 0.5 per cent v/v of *strong ammonia solution*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.5 g, dissolve in 10 ml of *water*, add 5 ml of *1 M sodium hydroxide* and extract with successive quantities of 50, 25, 25 and 25 ml of *ether*. Wash the combined *ether* extracts with 5 ml of *water*, shake the washings with 5 ml of *ether*, add the *ether* to the combined ether extracts and evaporate to dryness. Add 2 ml of *ethanol*, evaporate to dryness. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02831 g of $C_{20}H_{24}ClN_3S, 2CH_4SO_3$.

Storage. Store protected from light and moisture.

Prochlorperazine Injection

Prochlorperazine Mesylate Injection

Prochlorperazine Injection is a sterile solution of Prochlorperazine Mesylate in Water for Injections free from dissolved air.

Prochlorperazine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of prochlorperazine mesylate, $C_{20}H_{24}ClN_3S,2CH_4SO_3$.

Usual strength. 12.5 mg per ml.

Identification

A. To a volume containing 0.1 g of Prochlorperazine Mesylate add 20 ml of *water* and 2 ml of *10 M sodium hydroxide*. Shake and extract with 25 ml of *ether*. Wash the ether layer with two quantities, each of 5 ml, of *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness. Dissolve the residue in 1 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prochlorperazine mesylate RS* treated in the same manner or with the reference spectrum of prochlorperazine.

B. To a volume containing 5 mg of Prochlorperazine Mesylate add carefully 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

Tests

pH (2.4.24). 5.5 to 6.5.

Related substances. Carry out the test for Related substances in Phenothiazines (2.3.5), using mobile phase A.

Test solution. Use the injection under examination.

Reference solution (a). Dilute 1.0 ml of the test solution to 40 ml with *methanol* containing 0.5 per cent v/v of *strong ammonia solution* immediately before use.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the *methanol-ammonia mixture* immediately before use.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

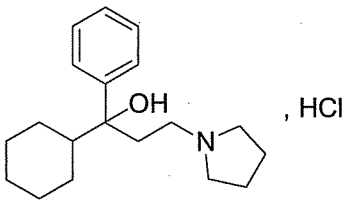
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *Protect the solutions from light throughout the Assay.*

To an accurately measured volume containing about 25 mg of Prochlorperazine Mesylate add sufficient *ethanol* containing 0.01 per cent v/v of *strong ammonia solution* to produce 200.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with the *ammoniacal ethanol* and measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7). Calculate the content of $C_{20}H_{24}ClN_3S, 2CH_4SO_3$ taking 635 as the specific absorbance at 258 nm.

Storage. Store protected from light.

Procyclidine Hydrochloride



$C_{19}H_{29}NO,HCl$ Mol. wt. 323.9

Procyclidine Hydrochloride is (*RS*)-1-cyclohexyl-1-phenyl-3-pyrrolidin-1-ylpropan-1-ol hydrochloride.

Procyclidine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{29}NO,HCl$, calculated on the dried basis.

Category. Antiparkinsonian.

Dose. Initially, 2.5 mg thrice daily, after meals increasing at intervals of two to three days by 2.5 to 5 mg daily; maximum, 30 mg daily.

Description. A white, crystalline powder, odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procyclidine hydrochloride RS*.

B. Dissolve 0.25 g in 10 ml of *water*, make alkaline with *5 M ammonia* and extract with three quantities, each of 10 ml, of *ether*. Dry the combined ethereal extracts over *anhydrous sodium sulphate*, filter, remove the ether and scratch the residue with a glass rod to induce solidification. The residue melts at about 85° (2.4.21).

C. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 1.0 per cent w/v solution.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *ether* and 1 volume of *strong ammonia solution*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform*.

Reference solution (a). A 0.004 per cent w/v solution of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride RS* in *chloroform*.

Reference solution (b). A 0.01 per cent w/v solution of the substance under examination in *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm. Any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-hydrochloride-1-one in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray the plate with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

B. Determine by gas chromatography (2.4.13).

Test solution. Add 5 ml of *1.25 M sodium hydroxide* to 20 ml of a 0.015 per cent w/v solution of the substance under examination and mix. Extract with two quantities, each of 20 ml, of *ether*, add to the combined extracts 5 ml of a 0.06 per cent w/v solution of *triphenylethylene* (internal standard) in *ether*, shake with *anhydrous sodium sulphate* and filter. Evaporate the filtrate and dissolve the residue in 1 ml of *ether*.

Reference solution (a). Prepare in the same manner as the test solution but using 20 ml of a 0.5 per cent w/v solution of the substance under examination and omitting the addition of the internal standard solution.

Reference solution (b). Prepare in the same manner as the test solution but using 20 ml of a 0.5 per cent w/v solution of the substance under examination.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (such as HP chromosorb W) coated with 10 per cent w/w of modified polyethylene glycol 20M (such as SP-1000) and 2 per cent w/w of *potassium hydroxide*,
- temperature :
  column. 240°,
  inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

The ratio of the sum of the areas of any secondary peaks to the area of the peak due to the internal standard in the chromatogram obtained with reference solution (b) is not more than the ratio of the area of the principal peak to the area of the internal standard peak in the chromatogram obtained with the test solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.7 g, dissolve in 75 ml of *anhydrous glacial acetic acid*, warm if necessary to effect solution and cool. Add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03239 g of $C_{19}H_{29}NO,HCl$.

Storage. Store protected from moisture.

Procyclidine Tablets

Procyclidine Hydrochloride Tablets

Procyclidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of procyclidine hydrochloride, $C_{19}H_{29}NO,HCl$.

Usual strengths. 2.5 mg; 5 mg.

Identification

A. Dissolve a quantity of the powdered tablets containing about 25 mg of Procyclidine Hydrochloride in 10 ml of *water*, shake with 20 ml of *ether* and discard the ether layer. Make the aqueous layer alkaline with *2 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *ether*. Wash the combined ether extracts with two quantities, each of 10 ml, of *water*, dry by shaking with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. If necessary, induce crystallization by scratching with a glass rod.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *procyclidine hydrochloride RS*.

B. The powdered tablets give the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *ether* and 1 volume of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Procyclidine Hydrochloride with 5 ml of *chloroform* and filter.

Reference solution (a). A 0.001 per cent w/v solution of *1-phenyl-3-pyrrolidinopropan-1-one hydrochloride RS* in *chloroform*.

Reference solution (b). Dilute 1 volume of test solution to 200 volumes with *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate at 105° for 15 minutes and examine in ultraviolet light at 254 nm. Any spot corresponding to 1-phenyl-3-pyrrolidinopropan-1-one in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) (0.2 per cent). Spray the plate with *dilute potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any spot due to excipients on the line of application.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 2.5 mg of Procyclidine Hydrochloride, transfer to a 100-ml volumetric flask, add 10.0 ml of *water* and mix, and dilute to volume with a 0.025 per cent w/v solution of *bromocresol purple* in 2 per cent v/v solution of *glacial acetic acid*. Allow the undissolved particles to settle. Transfer 5.0 ml of the supernatant solution to a separating funnel, extract with 20.0 ml of *chloroform* and filter the extract, discarding the first 5 ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 405 nm (2.4.7), using as the blank a solution prepared by treating 0.5 ml of *water* and 4.5 ml of a 0.025 per cent w/v solution of *bromocresol purple* in the same manner beginning at the words "extract with 20 ml of *chloroform* .....".

Calculate the content of $C_{19}H_{29}NO,HCl$ from the absorbance obtained by repeating the operation using *procyclidine hydrochloride RS* in place of the powdered tablets.

Storage. Store protected from moisture.

Progesterone Injectable Suspension

Progesterone Injectable Suspension is a sterile suspension of Progesterone in Water for Injections.

Progesterone Injectable Suspension contains not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of progesterone, $C_{21}H_{30}O_2$.

Usual Strengths. 25 mg per ml; 50 mg per ml; 100 mg per ml; 200 mg per ml.

Identification

A. Filter a volume of suspension containing about 100 mg of progesterone, through a medium-porosity, sintered-glass crucible, filter again if the fluid is not clear. Wash with several 5.0 ml portions of *water*, evaporate on a steam bath, dry the residue at 105°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *progesterone RS* or with the reference spectrum of progesterone.

B. Melting point (2.4.21). 126° to 131°.

Tests

pH (2.4.24). 4.0 to 7.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 85 volumes of *ethanol (95 per cent)* and 15 volumes of *water*.

Internal standard solution. A 0.66 per cent w/v solution of *methyltestosterone RS* in the solvent mixture.

Test solution. Shake a volume of suspension containing about 25 mg of progesterone with 16 ml of the solvent mixture into a polytef-lined, screw-capped, 25 ml test tube until the solution

is clear. Add 2.0 ml of the internal standard solution and dilute to 25 ml with the solvent mixture.

Reference solution. A 0.25 per cent w/v solution of *progesterone RS* in the solvent mixture. To 4.0 ml of this solution, add 2.0 ml of the internal standard solution and dilute to 10 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 100 cm x 2.1 mm, packed with octadecylsilane chemically bonded to porous silica (30-50 µm),
- mobile phase: a mixture of 72 volumes of *water* and 28 volumes of *isopropyl alcohol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 5 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to progesterone and methyltestosterone is not less than 3.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent.

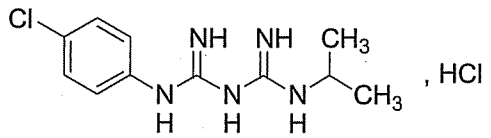
Inject the test solution and the reference solution.

Calculate the content of $C_{21}H_{30}O_2$ in the suspension.

Storage. Store in single dose or multiple dose containers, preferably of Type 1 glass.

Proguanil Hydrochloride

Chloroguanide Hydrochloride



$C_{11}H_{16}ClN_5, HCl$ Mol. Wt. 290.2

Proguanil Hydrochloride is 1-(4-chlorophenyl)-5-isopropylbiguanide hydrochloride.

Proguanil Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{11}H_{16}ClN_5, HCl$, calculated on the dried basis.

Category. Antimalarial.

Dose. Suppressive, 100 to 300 mg daily.

Description. A white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *proguanil hydrochloride RS* or with the reference spectrum of proguanil hydrochloride.

B. To 10 ml of a saturated solution add 0.25 ml of *potassium ferrocyanide solution*; a white precipitate is produced which dissolves on addition of a few drops of *dilute nitric acid.*

C. Dissolve 5 mg in 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *5 M sodium hydroxide* and 1 ml of *bromine solution*; a deep red colour is produced.

D. Gives the reactions of chlorides (2.3.1).

Tests

Acidity or alkalinity. To 35 ml of *water* maintained at 60° to 65° add 0.2 ml of *methyl red solution*, neutralise with *0.01 M sodium hydroxide* or *0.01 M hydrochloric acid*, add 0.4 g of the substance under examination and stir until dissolved. The resulting solution is not acidic and requires for neutralisation not more than 0.2 ml of *0.01 M hydrochloric acid.*

4-Chloroaniline. Dissolve 0.1 g in 1 ml of *2 M hydrochloric acid*, add sufficient *water* to produce 20 ml, cool to 5°, add 1 ml of *0.05 M sodium nitrite*, allow to stand at 5° for 5 minutes, add 2 ml of a 5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 10 minutes. Add 2 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, dilute to 50 ml with *water* and allow to stand for 30 minutes. Any magenta colour produced is not more intense than that obtained by treating in the same manner and at the same time 20 ml of a solution containing 1.25 µg of *4-chloroaniline.*

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 25 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01451 g of $C_{11}H_{16}ClN_5, HCl$.

Storage. Store protected from light and moisture.

Proguanil Tablets

Proguanil Hydrochloride Tablets; Chloroguanide Hydrochloride Tablets

Proguanil Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of proguanil hydrochloride, $C_{11}H_{16}ClN_5, HCl$.

Usual strength. 100 mg.

Identification

A. Boil a quantity of the powdered tablets containing 0.5 g of Proguanil Hydrochloride with 5 ml of *dilute hydrochloric acid*, cool and filter. To the filtrate add a slight excess of *sodium hydroxide solution*, extract with 30 ml of *ether* and evaporate the ethereal extract; the residue, after drying at 105°, melts at about 131° (2.4.21).

Dissolve the residue obtained in test A in the minimum quantity of *dilute hydrochloric acid*; the solution diluted with *water* to about 40 ml and neutralised if necessary, with cautious addition of *dilute ammonia solution*, complies with the following tests.

B. To 10 ml add 0.25 ml of *potassium ferrocyanide solution*; a white precipitate is produced which dissolves on addition of a few drops of *dilute nitric acid*.

C. To 0.5 ml add 5 ml of a warm 1.0 per cent w/v solution of *cetrimide* and add 1 ml of *5 M sodium hydroxide* and 1 ml of *bromine solution*; a deep red colour is produced.

Tests

4-Chloroaniline. To a quantity of the powdered tablets containing about 0.1 g of Proguanil Hydrochloride add 5 ml of *ethanol (95 per cent)* and shake for 10 minutes. Add 2.5 ml of *2 M hydrochloric acid* and 15 ml of *water*, mix and filter through a wetted filter paper, washing the filter with 5 ml of *water*. Cool to 5°, add 1 ml of *0.05 M sodium nitrite*, allow to stand at 5° for 5 minutes, add 2 ml of a 5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 10 minutes. Add 2 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, dilute to 50 ml with *water* and allow to stand for 30 minutes. Any magenta colour produced is not more intense than that obtained by treating in the same manner and at the same time 20 ml of a solution containing 1.25 µg of *4-chloroaniline*.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Proguanil Hydrochloride, add 5 ml of *water* and warm on a water-bath with stirring until a smooth paste is obtained. Add 50 ml of *water*, continue warming for 10 minutes, cool, add sufficient *water* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water* and to 10.0 ml of the resulting solution add 70 ml of *water*, 5 ml of a 20 per cent w/v solution of *cetrimide* and 1 ml of *2-propanol*. Adjust the temperature of the solution to 20° and add 2 ml of *alkaline sodium hypobromite solution* and sufficient *water* to produce 100.0 ml. Allow to stand at 20° for 25 minutes and measure the absorbance of the resulting solution at the maximum at about 480 nm (2.4.7).

Calculate the content of $C_{11}H_{16}ClN_5$,HCl from the absorbance obtained by repeating the operation using 10 ml of a 0.01 per cent w/v solution of *proguanil hydrochloride RS* beginning at the words "add 70 ml of *water*,....".

Storage. Store protected from light and moisture.

Promazine Tablets

Promazine Hydrochloride Tablets

Promazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of promazine hydrochloride, $C_{17}H_{20}N_2S$,HCl.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Promazine Hydrochloride, add 10 ml of *water* and 2 ml of *1 M sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promazine hydrochloride RS* or with the reference spectrum of promazine hydrochloride.

B. To a quantity of the powdered tablets containing 5 mg of Promazine Hydrochloride, add 5 ml of *sulphuric acid* and allow to stand for 5 minutes. An orange colour is produced.

C. Triturate a quantity of the powdered tablets containing 0.2 g of Promazine Hydrochloride with 4 ml of *methanol*, centrifuge, filter the supernatant liquid, heat almost to boiling, immediately add 2 ml of a boiling 3.5 per cent w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals with three quantities of *methanol*, dissolve in 10 ml of hot *methanol* and repeat the crystallisation and washing. The rust red crystals so obtained, after drying at 105° for 1 hour, melts at about 144° (2.4.21).

Tests

Related substances. Comply with the test for Related substances in Phenothiazines (2.3.5), using mobile phase (a) and the following freshly prepared solutions.

Test solution. Extract a quantity of the powdered tablets containing 0.1 g of Promazine Hydrochloride with 10 ml of *methanol* and filter.

Reference solution. Dilute 1 ml of test solution to 200 ml with *methanol*.

Other tests. Comply with the tests stated under Tablets.

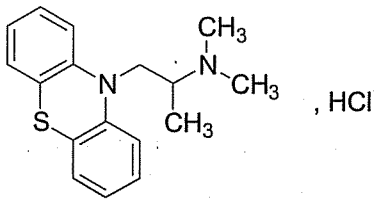
Assay.

NOTE—Carry out the test protected from light.

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Promazine Hydrochloride and triturate with 10 ml of *2 M hydrochloric acid* and add 200 ml of *water*. Shake for 15 minutes, add sufficient *water* to produce 500 ml and centrifuge about 50 ml of the mixture. To 5 ml of the clear, supernatant liquid add 10 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 100 ml. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 251 nm.

Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the specific absorbance at 251 nm.

Promethazine Hydrochloride



$C_{17}H_{20}N_2S,HCl$ Mol. Wt. 320.9

Promethazine Hydrochloride is (*RS*)-dimethyl(2-phenothiazin-10-ylpropyl)amine hydrochloride.

Promethazine Hydrochloride contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2S$, HCl, calculated on the dried basis.

Category. Antiemetic.

Dose. 20 to 50 mg daily, in single or divided doses.

Description. A white or faintly yellowish, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promethazine hydrochloride RS* or with the reference spectrum of promethazine hydrochloride.

B. Complies with the test for Identification of Phenothiazines (2.3.3).

C. Dissolve 0.1 g in 3 ml of *water* and add 1 ml of *nitric acid* dropwise; a precipitate is produced which dissolves rapidly to give a red solution which becomes orange and then yellow. Heat the solution to boiling; it becomes orange and an orange-red precipitate is produced.

D. Gives reaction B of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 5.0, determined in a 10.0 per cent w/v solution prepared immediately before use.

Related substances. Carry out the test for Related substances in Phenothiazines (2.3.5), protected from bright light using mobile phase B.

Prepare the following solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine.*

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (b). A 0.02 per cent w/v solution of *isopromethazine hydrochloride RS* in the same solvent mixture.

Apply to the plate 10 µl of each solution.

Any spot corresponding to isopromethazine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Record the volume added between the two inflections.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03209 g of $C_{17}H_{20}N_2S,HCl$.

Storage. Store protected from light and moisture.

Promethazine Injection

Promethazine Hydrochloride Injection

Promethazine Injection is a sterile solution of Promethazine Hydrochloride in Water for Injections free from dissolved air. It may contain suitable stabilising agents.

Promethazine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of promethazine hydrochloride, $C_{17}H_{20}N_2S$, HCl.

Usual strengths. 25 mg in 1 ml; 50 mg in 2 ml.

Identification

A. To a volume containing 0.1 g of Promethazine Hydrochloride add 20 ml of *water* and 2 ml of *10 M sodium hydroxide*. Shake and extract the mixture with 25 ml of *ether*. Wash the ether layer with two quantities, each of 5 ml, of *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness. Dissolve the residue in 1 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promethazine hydrochloride RS* treated in the same manner or with the reference spectrum of promethazine.

B. To a volume containing 0.2 g of Promethazine Hydrochloride add sufficient *potassium carbonate* to saturate the solution, extract with two quantities, each of 10 ml, of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 ml of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol*, previously warmed to about 50°. Cool, scratch the sides of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The residue, after washing with *methanol* and drying, melts at about 160° (2.4.21).

C. To a volume containing 5 mg of Promethazine Hydrochloride add carefully 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

Tests

pH (2.4.24). 5.0 to 6.0.

Related substances. Carry out the test for Related substances in Phenothiazines (2.3.5), using mobile phase B.

Test solution. Dilute a volume of the injection with a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* to contain 1 per cent w/v of Promethazine Hydrochloride.

Reference solution (a). Dilute 1 volume of the test solution to 40 volumes with the same solvent mixture.

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *isopromethazine hydrochloride RS* in the same solvent mixture.

Apply separately to the plate 10 µl of each solution.

Any spot corresponding to isopromethazine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *Carry out the following procedure protected from light.*

To an accurately measured volume containing about 25 mg of Promethazine Hydrochloride add sufficient *0.01 M hydrochloric acid* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with *0.01 M hydrochloric acid*, dilute 10.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 249 nm (2.4.7). Calculate the content of $C_{17}H_{20}N_2S$, HCl taking 910 as the specific absorbance at 249 nm.

Storage. Store protected from light.

Promethazine Syrup

Promethazine Hydrochloride Syrup; Promethazine Hydrochloride Oral Solution; Promethazine Oral Solution

Promethazine Syrup is a solution containing Promethazine Hydrochloride in a suitable flavoured vehicle.

Promethazine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of promethazine hydrochloride, $C_{17}H_{20}N_2S$, HCl.

Usual strength. 5 mg per 5 ml.

Identification

Carry out the method for identification of phenothiazines (2.3.3).

Test solution. A solution prepared by diluting a suitable volume of the syrup with *water* to contain 0.08 per cent w/v of Promethazine Hydrochloride. Apply 5 µl to the plate.

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. *Carry out the following procedure protected from light.*

Weigh accurately a quantity containing about 10 mg of Promethazine Hydrochloride, add 25 ml of *water* and 5 ml of a 5 per cent w/v solution of *sodium hydroxide*. Extract the mixture with two quantities, each of 50 ml, of *chloroform*, shaking vigorously for 1 minute each time, evaporate the combined extracts to dryness at about 30° at a pressure of

2 kPa and dissolve the residue in sufficient *0.1 M hydrochloric acid* to produce 50.0 ml (solution A). Dilute 10 ml of solution A to 50.0 ml with *water* (solution B). To a further 10 ml of solution A add 5 ml of *peroxyacetic acid solution*, allow to stand for 10 minutes and add sufficient *water* to produce 50.0 ml (solution C). Measure the absorbance of solution C at the maximum at about 336 nm (2.4.7), using solution B as the blank and measure the absorbance of solution B at the same wavelength using *water* as the blank. Repeat the procedure using a 0.02 per cent w/v solution of *promethazine hydrochloride RS* in *0.1 M hydrochloric acid* in place of solution A and beginning at the words "Dilute 10 ml of.....".

Determine the weight per ml of the syrup (2.4.29), and calculate the content of $C_{17}H_{20}N_2S$, HCl, weight in volume. The result is not valid unless the absorbance of solution B is less than 0.10.

Storage. Store protected from light and moisture.

Promethazine Tablets

Promethazine Hydrochloride Tablets

Promethazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of promethazine hydrochloride, $C_{17}H_{20}N_2S$, HCl. The tablets are coated.

Usual strengths. 10 mg; 25 mg; 50 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Promethazine Hydrochloride add 10 ml of *water* and 2 ml of *1 M sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promethazine hydrochloride RS* treated in the same manner or with the reference spectrum of promethazine.

B. Dissolve a quantity of the powdered tablets containing 0.2 g of Promethazine Hydrochloride in 2 ml of *water*, filter, add sufficient *potassium carbonate* to saturate the solution, extract with two quantities, each of 10 ml, of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 ml of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol*, previously warmed to about 50°. Cool, scratch the sides of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The residue, after washing with *methanol* and drying, melts at about 160° (2.4.21).

C. To a quantity of the powdered tablets containing 5 mg of Promethazine Hydrochloride add 5 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

Tests

Related substances. Carry out the test for Related substances in Phenothiazines (2.3.5), using mobile phase B.

Test solution. A solution freshly prepared by extracting a quantity of the powdered tablets containing 0.1 g of Promethazine Hydrochloride with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filtering.

Reference solution (a). Dilute 1.0 ml of the test solution to 40 ml with the same solvent.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the same solvent.

Reference solution (c). A 0.01 per cent w/v solution of *isopromethazine hydrochloride RS* in the same solvent.

Apply to the plate 10 µl of each solution. Any spot corresponding to isopromethazine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

Crush one tablet, add 1 ml of *dilute hydrochloric acid* and 30 ml of *water* and shake for 15 minutes. Add sufficient *water* to produce 50.0 ml and centrifuge. Complete the procedure described in the Assay beginning at the words "To 5.0 ml of the clear supernatant liquid....".

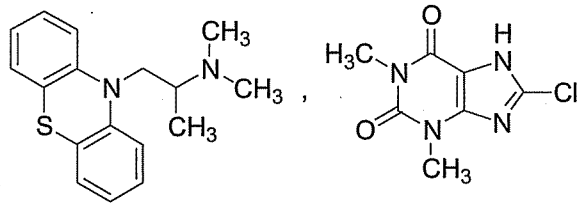
Other tests. Comply with the tests stated under Tablets.

Assay. *Carry out the following procedure protected from light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Promethazine Hydrochloride with 10 ml of *2 M hydrochloric acid* and add 200 ml of *water*. Shake for 15 minutes, add sufficient *water* to produce 500.0 ml and centrifuge about 50 ml of the mixture. To 5.0 ml of the clear supernatant liquid add 10 ml of *0.1 M hydrochloric acid* and sufficient *water* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 249 nm (2.4.7). Calculate the content of $C_{17}H_{20}N_2S$, HCl taking 910 as the specific absorbance at 249 nm.

Storage. Store protected from light and moisture.

Promethazine Theoclate



$C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$ Mol. Wt. 499.0

Promethazine Theoclate is the (*RS*)-dimethyl(2-phenothiazin-10-ylpropyl)amine salt of 8-chlorotheophylline.

Promethazine Theoclate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2S$, $C_7H_7ClN_4O_2$, calculated on the dried basis.

Category. Antiemetic.

Dose. 25 to 50 mg daily, in single or divided doses.

Description. A white or almost white powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Shake 0.15 g with 2.5 ml of *water*, add 1 ml of *5 M ammonia* and extract with 30 ml of *ether*. Wash the ether extract with 10 ml of *water*, dry with *anhydrous sodium sulphate* and evaporate to dryness. Dissolve the residue in 1 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promethazine RS* or with the reference spectrum of promethazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0007 per cent w/v solution in *ethanol* containing 0.01 per cent v/v of *strong ammonia solution* shows an absorption maximum at about 255 nm; absorbance is about 0.53.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

D. Shake 0.4 g with 10 ml of *water*, add 4 ml of *5 M ammonia*, shake with two quantities, each of 30 ml, of *ether* and add 4 ml of *hydrochloric acid* to the aqueous solution; a white precipitate is produced. Filter, wash with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness; a reddish residue remains which becomes purple on exposure to the vapour of ammonia.

E. Fuse 50 mg of the residue obtained in test D with 0.5 g of *anhydrous sodium carbonate*, boil the residue with 5 ml of *water*, acidify to *litmus paper* with *nitric acid* and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Chlorides (2.3.12). Shake 1.5 g with 50 ml of *water* for 2 minutes and filter. 25 ml of the filtrate complies with the limit test for chlorides (330 ppm).

Related substances. Carry out the test for Related substances in Phenothiazines (2.3.5), protected from bright light, using mobile phase B.

NOTE-Prepare the following solutions immediately before use.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine*.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (b). A 0.02 per cent w/v solution of *isopromethazine hydrochloride RS* in the same solvent mixture.

Apply to the plate 10 µl of each solution. Any spot corresponding to isopromethazine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 1.0 g and dissolve in 200 ml of *acetone*. Titrate with *0.1 M perchloric acid*, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04990 g of $C_{17}H_{20}N_2S,C_7H_7ClN_4O_2$.

Storage. Store protected from light and moisture.

Promethazine Theoclate Tablets

Promethazine Theoclate Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of promethazine theoclate, $C_{17}H_{20}N_2S$, $C_7H_7ClN_4O_2$.

Usual strengths. 25 mg; 50 mg.

Identification

A. To a quantity of the powdered tablets containing 40 mg of Promethazine Theoclate add 10 ml of *water* and 2 ml of *1 M sodium hydroxide*, shake and extract with 15 ml of *ether*. Wash the ether extract with 5 ml of *water*, dry with *anhydrous sodium sulphate*, evaporate to dryness and dissolve the residue in 0.4 ml of *chloroform*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promethazine theoclate RS* treated in the same manner or with the reference spectrum of promethazine.

B. Dissolve a quantity of the powdered tablets containing 0.2 g of Promethazine Theoclate in 2 ml of *water*, filter, add sufficient *potassium carbonate* to saturate the solution, extract with two quantities, each of 10 ml, of *ether* and evaporate the combined extracts to dryness. Dissolve the residue in 2 ml of *methanol* and pour into a solution of 0.4 g of *picric acid* in 10 ml of *methanol*, previously warmed to about 50°. Cool, scratch the sides of the tube to induce crystallisation, allow to stand for 3 to 4 hours and filter. The residue, after washing with *methanol* and drying, melts at about 160° (2.4.21).

C. To a quantity of the powdered tablets containing 5 mg of Promethazine Theoclate add 5 ml of *sulphuric acid* and allow to stand for 5 minutes; a red colour is produced.

D. Extract a quantity of the powdered tablets containing 0.2 g of Promethazine Theoclate with *chloroform*, filter and evaporate the filtrate to dryness. Shake the residue with 10 ml of *water*, add 4 ml of *5 M ammonia* and extract with two quantities, each of 30 ml, of *ether*. Wash the combined extracts with 10 ml of *water*. Combine the aqueous layer and washings, add 4 ml of *hydrochloric acid*; a white precipitate is produced. Filter, wash the residue with *water* and dry at 105°. Dissolve 10 mg of the residue in 1 ml of *hydrochloric acid*, add 0.1 g of *potassium chlorate* and evaporate to dryness; a reddish residue remains which becomes purple on exposure to the vapour of ammonia.

Tests

Related substances. Carry out the test for related substances in phenothiazines (2.3.5), using mobile phase B.

Test solution. A solution freshly prepared by extracting a quantity of the powdered tablets containing 0.1 g of Promethazine Theoclate with 10 ml of a mixture of 95 volumes of *methanol* and 5 volumes of *diethylamine* and filtering.

Reference solution (a). Dilute 1 volume of the test solution to 40 volumes with the same solvent mixture.

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with the same solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *isopromethazine hydrochloride RS* in the same solvent mixture.

Apply to the plate 10 µl of each solution. Any spot corresponding to isopromethazine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Tablets.

Assay. *Carry out the following procedure protected from light.*

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Promethazine Theoclate, add 5 ml of *water* and 1 ml of *strong ammonia solution* and allow to stand for 5 minutes. Add 50 ml of *ethanol*, shake for 5 minutes and filter, washing the residue with five quantities, each of 5 ml, of *ethanol*. Add sufficient *ethanol* to the filtrate to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with *ethanol* and dilute 10.0 ml of this solution to 100.0 ml with *ethanol*. Measure the absorbance of the resulting solution at the maximum at about 255 nm (2.4.7). Calculate the content of $C_{17}H_{20}N_2S$, $C_7H_7ClN_4O_2$ taking 755 as the specific absorbance at 255 nm.

Storage. Store protected from light and moisture.

Propane

C_3H_8 Mol. Wt. 44.1

Propane is dimethylmethane.

Propane contains not less than 98.0 per cent of C_3H_8.

CAUTION—Propane is highly flammable and explosive.

Identification

Determine by infrared absorption spectrophotometry (2.4.6), the solution give maxima at the wavelength of 3.4 µm, 6.8 µm and 7.2 µm.

Tests

Water (2.3.43). Not more than 0.001 per cent with the following modifications (a) provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas

dispersion tube connected to a sample cylinder; (b) dilute the reagent with *anhydrous methanol* to give a water equivalence factor of between 0.2 and 1.0 mg per ml, age this diluted solution for not less than 16 hours before sanitation; (c) obtain a 100 g sample as directed under inhalation preparation, and introduce the sample into the titration vessel through the gas dispersion tube at a rate of about 100 ml of gas per minute.

High-boiling residues. Not more than 5 µg per ml, determined by the following method. Prepare a cooling coil from copper tubing (about 6 mm outside diameter × about 6.1 m long) to fit into a vacuum-jacketed flask. Immerse the cooling coil in a mixture of dry ice and *acetone* in a vacuum-jacketed flask, and connect one end of the tubing to the propellant sample cylinder. Carefully open the sample cylinder valve, flush the cooling coil with about 50 ml of the propellant, and discard this portion of liquefied propellant. Continue delivering liquefied propellant from the cooling coil, and collect it in a previously chilled 1000-ml sedimentation cone until the cone is filled to the 1000-ml mark. Allow the propellant to evaporate, using a warm water bath maintained at about 40° to reduce evaporating time. When all of the liquid has evaporated, rinse the sedimentation cone with two 50-ml portions of *pentane*, and combine the rinsings in a tared 150-ml evaporating dish. Transfer 100 ml of the *pentane* solvent to a second tared 150-ml evaporating dish, place both evaporating dishes on a water bath, evaporate to dryness, and heat the dishes in an oven at 100° for 60 minutes. Cool the dishes in a desiccator, and weigh. Repeat the heating for 15-minute periods until successive weighings are within 0.1 mg, and calculate the weight of the residue obtained from the propellant as the difference between the weights of the residues in the two evaporating dishes.

Acidity of residue. Add 10 ml of *water* to the residue obtained in the test for High boiling residues, mix by swirling for about 30 seconds, add 2 drops of *methyl orange solution*, insert the stopper in the tube, and shake vigorously; no pink or red color appears in the aqueous layer.

Sulphur. Carefully open the container valve to produce a moderate flow of gas. Do not direct the gas stream toward the face, but deflect a portion of the stream toward the nose, the odour free from the characteristic odour of sulphur compounds.

Assay. Determine by Gas chromatography (2.4.13).

Test solution. Connect 1 Propane cylinder to the chromatograph through a suitable sampling valve and a flow control valve downstream from the sampling valve. Flush the liquid sample through the sampling valve, taking care to avoid entrapment of gas or air in the sampling valve.

Chromatographic system

- an aluminum column 6 m x 3 mm, packed with 10 per cent of liquid phase G30 (Tetraethylene glycol dimethyle ether) on non-acid-washed support S1C (A support prepared from crushed firebrick and calcined or burned with a clay binder above 900° with subsequent acid-wash. It may be silanized),
- a thermal-conductivity detector,
- flow rate. 50 ml per minute using nitrogen as carrier gas.

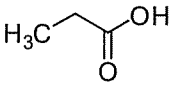
Inject the test solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 1.0.

Inject 2 µl of test solution.

Calculate the percentage purity by dividing 100 times the propane response by the sum of all of the responses in the chromatogram.

Storage. Store protected from moisture and prevent exposure to excessive heat.

Propionic Acid



$C_3H_6O_2$ Mol. Wt. 74.1

Propionic Acid contains not less than 99.5 per cent and not more than 100.5 per cent of $C_3H_6O_2$.

Tests

Weight per ml (2.4.29). 0.988 to 0.993.

Distilling range (2.4.8). 138.5° to 142.5°.

Heavy metals (2.3.13). To the residue obtained in the test for Nonvolatile residue, add 8 ml of *0.1 M hydrochloric acid*, warm gently until solution is complete, dilute with *water* to 100 ml. 10 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm).

Limit of nonvolatile residue. Evaporate 20 g in a tared dish, and dry at 105° for 1 hour, the weight of the residue does not exceed 2 mg.

Readily oxidizable substances. Dissolve 15 g of *sodium hydroxide* in 50 ml of *water*, cool, add 6 ml of *bromine*, stirring to effect complete solution, and dilute with *water* to 2000 ml. Transfer 25.0 ml of this solution to a glass-stoppered, 250-ml conical flask containing 100 ml of *water*, and add 10 ml of 20.0 per cent w/v solution *sodium acetate* and 10.0 ml of *Propionic Acid*. Allow to stand for 15 minutes, add 5 ml of 25.0 per cent w/v solution of *potassium iodide* and 10 ml of *hydrochloric acid*, and titrate with *0.1 M sodium thiosulphate* just to the disappearance of the brown color. Carry out a blank titration. The difference between the volume of *0.1 M sodium thiosulphate* required for the blank and that required for the test solution is not more than 2.2 ml.

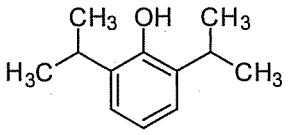
Aldehydes. Transfer 10.0 ml to a glass-stoppered, 250-ml conical flask containing 50 ml of *water* and 10.0 ml of 1.25 per cent w/v solution of *sodium bisulphite*, insert the stopper, and shake vigorously. Allow the mixture to stand for 30 minutes, then titrate with *0.1 M iodine* to the same brownish yellow endpoint obtained with a blank treated with the same quantities of the same reagents. The difference between the volume of *0.1 M iodine* required for the blank and that required for the test solution is not more than 1.75 ml.

Assay. Weigh accurately about 1.5 g and dissolve with 100 ml of recently boiled and cooled *water* in a 250-ml conical flask, and titrate with *0.5 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator .

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.03704 g of $C_3H_6O_2$.

Storage. Store protected from moisture.

Propofol



$C_{12}H_{18}O$ Mol. Wt. 178.3

Propofol is 2,6-diisopropylphenol.

Propofol contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{18}O$.

Category. General anaesthetic.

Description. A colourless or very light yellow, clear liquid.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propofol RS* or with the reference spectrum of propofol.

Tests

Refractive index (2.4.27). 1.5125 to 1.5145.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 1.0 g of the substance under examination in 10 ml of *hexane*.

Test solution (b). Dissolve 0.24 g of the substance under examination in 100 ml of *hexane*.

Reference solution (a). A solution containing 5 mg of the substance under examination and 15 µl containing 15 mg of *2,6-bis(1-methylethyl)benzene-1,4-dione RS (propofol impurity J RS)* in 50 ml of *hexane*.

Reference solution (b). Dilute 0.1 ml of *propofol for peak identification RS* (containing 3,3',5,5'-tetrakis(1-methylethyl)biphenyl-4,4'-diol (propofol impurities E) and 2-(1-methylethoxy)-1,3-bis(1-methylethyl)benzene (propofol impurities G) to 1.0 ml with *hexane*.

Reference solution (c). Dilute 1.0 ml of test solution (a) to 100 ml with *hexane*. Dilute 1.0 ml of this solution to 10 ml with *hexane*.

Reference solution (d). A 0.24 per cent w/v solution of *propofol RS* in *hexane*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with silica gel (5 µm),
- mobile phase: a mixture of 1 volume of *anhydrous ethanol*, 7.5 volumes of *acetonitrile* and 990 volumes of *hexane*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 275 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to propofol impurity J and propofol is not less than 4.0. The relative retention times with reference to propofol for propofol impurity G is about 0.5, for oxydibenzene (propofol impurity G) is about 0.6, for 2-(1-methylethenyl)-6-(1-methylethyl)phenol (propofol impurity B) is about 0.7, for 4-hydroxy-3,5-bis(1-methylethyl)benzoic acid (propofol impurity F) is about 2.3, for 2,5-bis(1-methylethyl)phenol (propofol impurity D) is about 2.5 , for 1-methylethyl 4-hydroxy-3,5-bis(1-methylethyl)benzoate (propofol impurity P) is about 2.9, for 2,4-bis(1-methylethyl)phenol (propofol impurity A) is about 3.0, for 2-(1-methylethyl)phenol (propofol impurity C) is about 3.4, for propofol impurity E is about 4.0, for 3-(1-methylethyl)phenol (propofol impurity F) is about 5.8 and for 4-(1-methylethyl)phenol (propofol impurity H) is about 6.4.

Inject test solution (a), reference solution (a), (b) and (c). Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with test solution (a), the area of peak due to propofol impurity G is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent); the area of the peak due to propofol impurity E is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.01 per cent). The area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent). The sum of the areas of all the secondary peaks is not more than 3 times the area of the

principal peak in the chromatogram obtained with reference solution (c) (0.3 per cent). Ignore any peak other than propofol impurity E with an area less than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.03 per cent).

Impurities J, K, L and O. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 40 mg of the substance under examination in 10 ml of *dichloromethane.*

Reference solution (a). Dilute 1.0 ml of the test solution to 100 ml with *dichloromethane.* Further dilute 1.0 ml of this solution to 10 ml with *dichloromethane.*

Reference solution (b). Dilute 5 µl containing 5 mg of *propofol impurity J RS* to 100 ml with *dichloromethane.* Dilute 1.0 ml of this solution to 25 ml with *dichloromethane.*

Reference solution (c). Dissolve 4 mg of *propofol RS* in 1.0 ml of reference solution (b).

Chromatographic system
- a fused-silica capillary column 30 m x 0.32 mm, coated with polymethylphenylsiloxane (0.5 µm),
- temperature:
  Column 80° from 0-3 minutes, 80°-120° from 3-25 minutes and 210° from 25-40 minutes,
  Injection port at 100° and detector at 270°,
- a flame ionisation detector,
- flow rate. 1.7 ml per minute using nitrogen as carrier gas.

Inject 1 µl of reference solution (c). The test is not valid unless the peak- to- valley ratio is not less than 3.0 where H_p is the height above the baseline of the peak due to propofol impurity J and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to propofol. The relative retention time with reference to propofol for 1-(1-methylethoxy)-2-(1-methylethyl)benzene (propofol impurity K) is about 0.76, for 2,2-dimethyl-4-(1-methylethyl)-1,3-benzodioxole (propofol impurity L) is about 0.81 , for propofol impurity J is about 1.01 and for 2-(1-methylethyl)-6-propylphenol (propofol impurity O) is about 1.03.

Inject 1 µl of the test solution, reference solution (a) and (c). In the chromatogram obtained with the test solution the area of the peak due to propofol impurities J, K, L and O, for each impurity, is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances.

Inject test solution (b) and reference solution (d).

Calculate the content of $C_{12}H_{18}O$.

Storage. Store protected from light under an inert gas.

Propofol Injection

Propofol Injection is a sterile emulsion of Propofol in a suitable base.

Propofol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of propofol, $C_{12}H_{18}O$.

Usual strength. 10 mg per ml.

Identification

A. Extract a volume of the injection containing 0.1 g of Propofol with three 25 ml quantities of *hexane* and filter the combined extracts using a glass fibre filter (such as Whatman GF/C). Extract the filtrate with two 20 ml quantities of *methanol* (90 per cent) and evaporate the combined extracts under reduced pressure. Dissolve the residue obtained in 2 ml of *ethanol* and evaporate to dryness under reduced pressure. Dry the residue at 50° over *phosphorus pentoxide* for 1 hour. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propofol RS* or with the reference spectrum of propofol.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 8.5.

Propofol quinone and propofol dimer. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 80 mg of Propofol in 100 ml of *propan-2-ol.*

Reference solution. A solution containing 0.0008 per cent w/v of *propofol RS*, 0.00008 per cent w/v of *2,6-bis(1-methylethyl)-1,4-benzoquinone RS* (*propofol impurity J RS*) and 0.0002 per cent w/v of *3,3',5,5'-tetrakis (1-methylethyl)biphenyl-4,4'-diol RS* (*propofol dimer RS*) in *propan-2-ol* containing 6.8 per cent v/v of *water.*

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a mixture of 40 volumes of *tetrahydrofuran* and 60 volumes of *water,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative retention time with reference to propofol for propofol impurity J is about 0.8 and for propofol dimer is about 2.5.

Inject the test solution and the reference solution. Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to propofol quinone (propofol impurity J) is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.1 per cent) and the area of the peak due to propofol dimer is not more than the area of the corresponding peak in the chromatogram obtained with the reference solution (0.25 per cent).

Globule size. Not more than 5000 globules more than or equal to 2 µm per ml of a 0.01 per cent v/v dilution in a filtered 0.9 per cent w/v solution of *sodium chloride*. By means of suitable equipment, pass the solution through a 50 µm aperture and count the number of globules by monitoring the change in electrical current between two electrodes immersed on either side of the aperture (such as Coulter Counter). Repeat the procedure using the 0.9 per cent w/v solution of *sodium chloride* only to determine the background count.

Free fatty acid. Not more than 7 millimoles per litre, when determined by the following method.

To 5 ml of a well shaken injection add 25 ml of a mixture of 40 volumes of *propan-2-ol*, 10 volumes of *n-heptane* and 1 volume of *0.5 sulphuric acid* and shake for 1 minute. Allow to stand for 10 minutes, add 15 ml of *n-heptane* and 15 ml of *water*. Mix by inverting the container 10 times and allow to stand for 15 minutes. To 15 ml of the upper phase add 5 ml of *nile blue A solution* and pass a stream of *nitrogen*, previously passed through *0.1 M sodium hydroxide*, through the solution. Titrate with 0.02 M sodium hydroxide, using a microburette. Calculate the content of free fatty acid from a calibration curve prepared from quantities of 1, 2, 4, 6 and 8 ml of a 1.282 per cent w/v solution of *palmitic acid* in *n-heptane*, each diluted to 50 ml with *n- heptane*. Carry out the method described above, using 5 ml of each solution and beginning at the words 'add 25 ml of a mixture'.

Lysolecithin. Not more than 0.2 per cent w/v.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 0.1 g of Propofol in 100 ml of the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *lysolecithin* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm X 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 1.4 volumes of *orthophosphoric acid*, 40 volumes of *water* and 60 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 250 µl.

Inject the test solution and the reference solution.

Calculate the content of lysolecithin.

Bacterial endotoxins (2.2.3). Not more than 1.65 Endotoxin Units per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing 80 mg of Propofol to 100 ml with the *propan-2-ol*.

Reference solution (a). A 0.08 per cent w/v solution of *propofol RS* in in *propan-2-ol* containing 6.8 per cent v/v of *water*.

Reference solution (b). A solution containing 0.0008 per cent w/v of *propofol RS* and 0.00008 per cent w/v of *propofol impurity J RS* in *propan-2-ol* containing 6.8 per cent v/v of *water*.

The chromatographic system described under Propofol quinone and propofol dimer may be used but using a detection wavelength of 275 nm and injecting 20 µl of each solution.

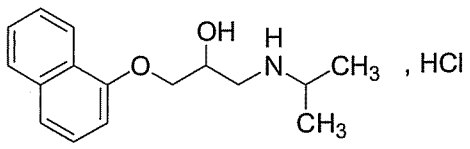
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to propofol and propofol impurity J is not less than 2.5.

Inject the test solution and reference solution (b).

Calculate the content of $C_{12}H_{18}O$ in the injection.

Storage. Store at a temperature not exceeding 30°. It should not be allowed to freeze.

Propranolol Hydrochloride



$C_{16}H_{21}NO_2,HCl$ Mol. Wt. 295.8

Propranolol Hydrochloride is (*RS*)-1-[(1-methylethyl)amino]-3-(naphthalen-1-yloxy)propan-2-ol hydrochloride.

Propranolol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{21}NO_2$, HCl, calculated on the dried basis.

Category. Antihypertensive, antianginal and antiarrhythmic agent.

Dose. Orally, 20 mg to 2 g daily, in divided doses; the initial dose should not exceed 40 mg; by slow intravenous injection, 3 to 10 mg.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propranolol hydrochloride RS* or with the reference spectrum of propranolol hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows absorption maxima at about 290 nm, 306 nm and 319 nm.

C. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than degree 6 of the appropriate range of reference solutions (2.4.1).

pH (2.4.24). 5.0 to 6.0, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). –1.0° to +1.0°, determined in a 4.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 10.0 ml of the mobile phase.

Reference solution. Dilute 2.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 1.6 g of *sodium laurylsulphate* and 0.31 g of *tetrabutylammonium dihydrogen phosphate* in a mixture of 1 ml of *sulphuric acid*, 450 ml of *water* and 550 ml of *acetonitrile*, adjust to pH 3.3 using *dilute sodium hydroxide solution*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 292 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (0.1 per cent) and the sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (0.4 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 25 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02958 g of $C_{16}H_{21}NO_2$, HCl.

Storage. Store protected from moisture.

Propranolol Injection

Propranolol Hydrochloride Injection

Propranolol Injection is a sterile solution of Propranolol Hydrochloride in Water for Injections containing Citric Acid.

Propranolol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of propranolol hydrochloride, $C_{16}H_{21}NO_2$, HCl.

Usual strength. 1 mg per ml.

Identification

A. Make alkaline with *1 M sodium hydroxide* a volume containing 10 mg of Propranolol Hydrochloride and extract with three quantities, each of 5 ml, of *ether*. Wash the combined *extracts* with *water* until the washings are free from alkali, dry with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propranolol hydrochloride RS* treated in the same manner or with the reference spectrum of propranolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows absorption maxima at about 290 nm, 306 nm and 319 nm.

Tests

pH (2.4.24). 3.0 to 3.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 100 mg of Propranolol Hydrochloride in 100 ml of *acetonitrile*.

Reference solution. Dilute 1 ml of the test solution to 500 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 1.15 g of *sodium dodecyl sulphate*, 10 ml of a mixture of 1 volume of *sulphuric acid* and 9 volumes of *water*, 20 ml of a 1.7 per cent w/v solution of *tetrabutylammonium dihydrogen orthophosphate*, 370 ml of *water* and 600 ml of *acetonitrile*, adjusted to pH 3.3 with *2 M sodium hydroxide*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 292 nm,
- injection volume. 10 µl.

Inject the test solution and the reference solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than four times the area of the principal peak in the chromatogram obtained with the reference solution (0.8 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 2 mg of Propranolol Hydrochloride add sufficient *methanol* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{16}H_{21}NO_2$, HCl taking 206 as the specific absorbance at 290 nm.

Storage. Store protected from light, in a single dose containers.

Propranolol Tablets

Propranolol Hydrochloride Tablets

Propranolol Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of propranolol hydrochloride, $C_{16}H_{21}NO_2$, HCl.

Usual strengths. 10 mg; 40 mg.

Identification

A.Suspend a quantity of the powdered tablets containing 0.1 g of Propranolol Hydrochloride in 20 ml of *water*, filter, make the filtrate alkaline with *1 M sodium hydroxide* and extract with three quantities, each of 10 ml, of *ether*. Wash the combined extracts with *water* until the washings are free from alkali, dry with *anhydrous sodium sulphate*, filter, evaporate the filtrate to dryness and dry the residue at 50° at a pressure of 2 kPa for 1 hour.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propranolol hydrochloride RS* treated in t he same manner or with the reference spectrum of propranolol.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows absorption maxima at about 290 nm, 306 nm and 319 nm.

Tests

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

Transfer one tablet to a 100-ml volumetric flask, add 5 ml of *dilute hydrochloric acid* and allow to stand, swirling occasionally, until it is disintegrated. Add about 70 ml of *methanol* and shake well for about 1 minute. Dilute to volume with *methanol,* mix and centrifuge an aliquot of the solution. Dilute a suitable volume of the clear solution with *methanol* to produce a solution containing 20 µg of Propranolol Hydrochloride per ml. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7), using *methanol* as the blank. Calculate the content of $C_{16}H_{21}NO_2$, HCl taking 206 as the specific absorbance at 290 nm.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Dilute a suitable volume of the filtrate with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{16}H_{21}NO_2$,HCl in the medium taking 206 as the specific absorbance at 290 nm.

D. Not less than 75 per cent of the stated amount of $C_{16}H_{21}NO_2$, HCl.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 100 mg of Propranolol Hydrochloride in 100.0 ml of *methanol* and filter.

Reference solution. Dilute 1 ml of the test solution to 500 ml with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 1.15 g of *sodium dodecyl sulphate*, 10 ml of a mixture of 1 volume of *sulphuric*

acid and 9 volumes of *water*, 20 ml of a 1.7 per cent w/v solution of *tetrabutylammonium dihydrogen orthophosphate*, 370 ml of *water* and 600 ml of *acetonitrile*, adjusted to pH 3.3 with *2 M sodium hydroxide*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 292 nm,
- injection volume. 10 µl.

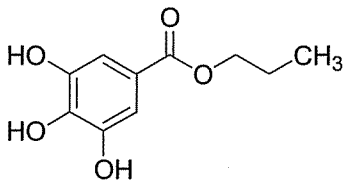
Inject the test solution and the reference solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than four times the area of the principal peak in the chromatogram obtained with the reference solution (0.8 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Propranolol Hydrochloride and shake with 20 ml of *water* for 10 minutes. Add 50 ml of *methanol*, shake for a further 10 minutes, add sufficient *methanol* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_{16}H_{21}NO_2$, HCl taking 206 as the specific absorbance at 290 nm.

Storage. Store protected from light and moisture.

Propyl Gallate



$C_{10}H_{12}O_5$ Mol. Wt. 212.2

Propyl Gallate is propyl 3,4,5-trihydroxybenzoate.

Category. Pharmaceutical aid (antioxidant).

Description. A white or creamy white, crystalline powder; odourless or almost odourless.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 275 nm; absorbance at about 275 nm, about 0.49.

B. Dissolve 10 mg in 50 ml of *water*, cool and add 5 ml of *dilute ammonia solution*; a red colour is produced which becomes brown on standing and is restored on shaking.

C. Dissolve 5 mg in 50 ml of *water* and add 0.05 ml of *ferric chloride test solution*; a bluish black colour is produced.

Tests

Chlorides (2.3.12). Shake 1.0 g with 50 ml of *water* for 5 minutes and filter. 25 ml of the filtrate complies with the limit test for chlorides (500 ppm).

Sulphates (2.3.17). Shake 0.8 g with 100 ml of *water* for 5 minutes and filter. 15 ml of the filtrate complies with the limit test for sulphates (0.12 per cent).

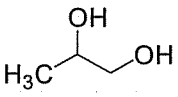
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Storage. Store protected from light and moisture, in non-metallic containers.

Propylene Glycol

1, 2-Propanediol



$C_3H_8O_2$ Mol. Wt. 76.1

Propylene Glycol is (*RS*)-propane-1,2-diol.

Category. Pharmaceutical aid (humectant; solvent).

Description. A clear, colourless, viscous liquid; practically odourless; hygroscopic.

Identification

A. To 0.5 ml of a 0.01 per cent w/v solution, cooled in ice, add 5 ml of a cooled mixture of 10 ml of *water* and 90 ml of *sulphuric acid*. Heat for 10 minutes on a water-bath at 70°, cool and add 0.2 ml of a 3 per cent w/v solution of *ninhydrin* in a 2.5 per cent w/v solution of *sodium metabisulphite*; a violet colour slowly appears.

B. Heat 0.15 ml with 0.1 g of *boric acid*; a pleasant odour develops.

C. Add 1 ml to 0.5 g of *potassium bisulphate* and heat gently; a fruity odour develops and when the solution is heated to dryness, no sharp, acrid smell of acrolein is perceptible.

Tests

Appearance of solution. The substance under examination is clear (2.4.1), and colourless (2.4.1).

Acidity. Mix 10 ml with 40 ml of *water* and add 0.1 ml of *bromothymol blue solution*. The solution is greenish yellow and not more than 0.05 ml of *0.1 M sodium hydroxide* is required to change the colour to blue.

Boiling range (2.4.8). 184° to 189°.

Relative density (2.4.29). 1.035 to 1.040.

Refractive index (2.4.27). 1.431 to 1.433.

Heavy metals (2.3.13). Dilute 3 ml to 12 ml with *water*. The resulting solution complies with the limit test for heavy metals, Method D (5 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Oxidising substances. To 10 ml add 5 ml of *water*, 2 ml of *potassium iodide solution* and 2 ml of *1 M sulphuric acid* and allow to stand in a ground-glass-stoppered flask protected from light for 15 minutes. Titrate the liberated iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not more than 0.2 ml of *0.05 M sodium thiosulphate* is required.

Reducing substances. Mix 1 ml with 1 ml of *6 M ammonia* and heat on a water-bath at 60° for 5 minutes; the solution is not yellow. Immediately add 0.15 ml of *0.1 M silver nitrate*; the solution does not change its appearance within 5 minutes.

Ethylene glycol and diethylene glycol. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 2 g of the substance under examination in sufficient *ethanol (95 per cent)* to produce 100 ml.

Reference solution. Dissolve 2 g of the substance under examination, 0.02 g of *ethylene glycol* and 0.02 g of *diethylene glycol* in *ethanol (95 per cent)* and dilute to 100 ml with the same solvent.

Chromatographic system
- a glass column 1.5 m x 3 mm, packed with 12 per cent Sorbitol on untreated siliceous earth (Such as Chromosorb W-NAW (SINS),
- temperature:
  column: 165°,
  inlet port and detector at 260°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 3 µl or other suitable volume of the test solution. Record the chromatograms adjusting the sensitivity so that the height of the peak due to propylene glycol is more than 50 per cent of the full-scale deflection in the chromatograms. Inject the same volume of reference solution and record the chromatograms. The order of elution is propylene glycol, ethylene glycol and diethylene glycol. The test is not valid unless in the chromatogram obtained with the reference solution, the resolution between the peaks due to propylene glycol (first peak) and ethylene glycol (second peak) is not less than 1.0.

No peaks corresponding to ethylene glycol and diethylene glycol are obtained in the chromatogram obtained with the test solution.

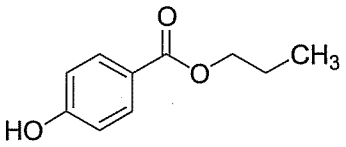
Sulphated ash (2.3.18). Not more than 0.01 per cent w/w, determined by the following method. Heat 50 g until it burns, and ignite. Allow to cool, moisten the residue with *sulphuric acid* and ignite; repeat the operations.

Water (2.3.43). Not more than 0.2 per cent, determined on 5.0 g.

Storage. Store protected from moisture.

Propylparaben

Propyl Hydroxybenzoate



$C_{10}H_{12}O_3$ Mol. Wt. 180.2

Propylparaben is propyl 4-hydroxybenzoate.

Propylparaben contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{12}O_3$, calculated on the dried basis.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white, crystalline powder; odourless.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *ethanol (95 per cent)* shows an absorbance maximum at about 258 nm; absorption at 258 nm, 0.44 to 0.47.

B. To about 10 mg in a test-tube add 1 ml of *sodium carbonate solution*, heat to boiling for 30 seconds and cool (solution A). To a further 10 mg in a test-tube add 1 ml of *sodium carbonate solution*; the substance partly dissolves (solution B). Add at the same time to each of the solutions A and B 5 ml of *aminophenazone solution* and 1 ml of *potassium ferricyanide solution* and mix. Solution B is yellow to orange-brown. Solution A is orange to red and the colour is clearly more intense than any similar colour that may be obtained with solution B.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity. Dissolve 1.0 g in sufficient *ethanol (95 per cent)* to produce 10 ml. To 2 ml of the solution add 3 ml of *ethanol (95 per cent)*, 5 ml of *carbon dioxide-free water* and 0.1 ml of *bromocresol green solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *acetone*.

Reference solution (a). Dilute 0.5 ml of the test solution to 100.0 ml with *acetone*.

Reference solution (b). Dissolve 10 mg of *ethyl parahydroxybenzoate RS* in 1.0 ml of the test solution and dilute to 10.0 ml with *acetone*.

Apply 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any secondary spot is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (0.5 per cent) The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated principal spots.

Chlorides (2.3.12). Heat 2.0 g with 100 ml of *water*, cool, add sufficient *water* to restore the original volume, and filter. 25 ml of the filtrate complies with the limit test for chlorides (500 ppm).

Sulphates. To 10 ml of the filtrate obtained in the test for Chlorides add 0.15 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride solution*; no turbidity is produced within 10 minutes.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

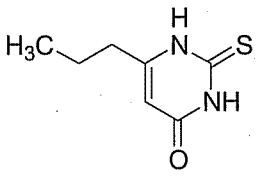
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *silica gel* for 5 hours.

Assay. To 1.0 g add 20.0 ml of *1 M sodium hydroxide*. Heat at about 70° for 1 hour. Cool rapidly in an ice bath. Titrate the excess *sodium hydroxide* with *0.5 M sulphuric acid*, continuing the titration until the second point of inflexion and determining the end-point potentiometrically (2.4.25).

1 ml of *1 M sodium hydroxide* is equivalent to 0.1802 g of $C_{10}H_{12}O_3$.

Storage. Store protected from moisture.

Propylthiouracil



$C_7H_{10}N_2OS$ Mol. Wt. 170.2

Propylthiouracil is 2,3-dihydro-6-propyl-2-thioxopyrimidin-4(1*H*)-one.

Propylthiouracil contains not less than 98.0 per cent and not more than 100.5 per cent of $C_7H_{10}N_2OS$, calculated on the dried basis.

Category. Antithyroid.

Dose. 300 to 450 mg daily, in divided doses.

Description. A white or pale cream-coloured crystals or crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propylthiouracil RS* or with the reference spectrum of propylthiouracil.

B. Examine the chromatograms obtained in the test for Related substances in ultraviolet light at 254 nm before exposure of the plate to iodine vapour. The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. To about 20 mg add 8 ml of *bromine water* and shake for a few minutes. Boil until the mixture is decolorised, allow to cool and filter. Add 2 ml of *barium chloride solution*; a white precipitate is produced. Add 5 ml of *2 M sodium hydroxide*; the precipitate does not become violet.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *chloroform*, 12 volumes of *2-propanol* and 0.2 volume of *glacial acetic acid*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.01 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 0.1 per cent w/v solution of *propylthiouracil RS* in *methanol*.

Reference solution (c). A 0.0005 per cent w/v solution of *thiourea* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 10 minutes. By both methods of visualisation, any spot corresponding to thiourea in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (c) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Arsenic (2.3.10). Dissolve 2.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in oven at 105°.

Assay. Weigh accurately about 0.3 g, add 30 ml of *water* and 30.0 ml (n_1 ml) of *0.1 M sodium hydroxide*, boil and shake until solution is complete. Add 50 ml of *0.1 M silver nitrate* with stirring, boil gently for 5 minutes, cool and titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25) (n_2 ml). Record the total volume ($n_1 + n_2$ ml) of *0.1 M sodium hydroxide* added.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.008511 g of $C_7H_{10}N_2OS$.

Storage. Store protected from light and moisture.

Propylthiouracil Tablets

Propylthiouracil Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of propylthiouracil, $C_7H_{10}N_2OS$.

Usual strength. 50 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 20 ml of *methanol* for 10 minutes, filter and evaporate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propylthiouracil RS* or with the reference spectrum of propylthiouracil.

B. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 60 ml of *methanol* for 20 minutes, dilute to 100 ml with *methanol* and filter. Dilute 5 ml of the filtrate to 250 ml with *methanol*. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 274 nm.

C. Extract a quantity of the powdered tablets in a continuous extraction apparatus (2.1.8) with *ether* and evaporate the solution to dryness. The residue, after drying at 105°, melts at about 219° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *chloroform*, 12 volumes of *2-propanol* and 0.2 volume of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of Propylthiouracil with 5 ml of *methanol* for 15 minutes, filter and use the filtrate.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Reference solution (b). A 0.001 per cent w/v solution of thiourea in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Expose the plate to iodine vapour for 10 minutes. By both methods of visualisation, any spot corresponding to thiourea in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Comply with the tests stated under Tablets

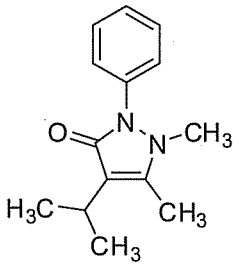
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Propylthiouracil, dissolve in a mixture of 20 ml of *0.1 M sodium hydroxide* and 75 ml of *water* with the aid of gentle heat. Cool, add 4 g of *sodium acetate*, just acidify the solution to *litmus paper* with *6 M acetic acid*, add 0.5 ml of a freshly prepared

0.5 per cent w/v solution of *1,5-diphenylcarbazone* in *ethanol (95 per cent)* and titrate with *0.02 M mercuric nitrate* until a pinkish violet colour persists for 2 to 3 minutes.

1 ml of *0.02 M mercuric nitrate* is equivalent to 0.006808 g of $C_7H_{10}N_2OS$.

Storage. Store protected from light and moisture.

Propyphenazone



$C_{14}H_{18}N_2O$ Mol. Wt. 230.3

Propyphenazone is 4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one.

Propyphenazone contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{14}H_{18}N_2O$, calculated on the dried basis.

Category. Analgesic; antipyretic.

Dose. 1.5 to 3 g daily, in divided doses.

Description. A white or slightly yellowish, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propyphenazone RS* or with the reference spectrum of propyphenazone.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 2 g in sufficient of a mixture of equal volumes of *ethanol (95 per cent)* and *carbon dioxide-free water* to produce 50 ml (solution A). To 1 ml of solution A add 0.1 ml of *ferric chloride solution*; a brownish red colour is produced which becomes yellow on addition of 1 ml of *2 M hydrochloric acid*.

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.1 ml of *phenolphthalein solution*; the solution is colourless. Add 0.2 ml of *0.01 M sodium hydroxide*; the solution is pink. Add 0.4 ml of *0.01 M hydrochloric acid*; the solution becomes colourless. Add 0.2 ml of *methyl red solution*; the solution is orange or red.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 45 volumes of *cyclohexane*, 45 volumes of *ethyl acetate* and 10 volumes of *1-butanol*.

Test solution (a). An 8 per cent w/v solution of the substance under examination in *methanol*.

Test solution (b). A 1.6 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (a). A 0.016 per cent w/v solution of the substance under examination in *methanol*.

Reference solution (b). A 1.6 per cent w/v solution of *propyphenazone RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of hot air for 15 minutes and examine in ultraviolet light at 254 nm. Spray the plate with a mixture of equal volumes of *potassium ferricyanide solution* and *ferric chloride solution*. By both methods of visualisation, any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Arsenic (2.3.10). Mix 1.0 g with 10 ml of a 2 per cent w/v solution of *magnesium nitrate* in *ethanol* in a silica or platinum dish, evaporate on a water-bath and heat gradually in order to incinerate. If the material remains incompletely carbonised, moisten with a small quantity of *nitric acid* and ignite again. Cool, add 3 ml of *hydrochloric acid* and heat on a water-bath to dissolve the residue. The resulting solution complies with the limit test for arsenic (10 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. Weigh accurately about 0.2 g of the dried material, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02303 g of $C_{14}H_{18}N_2O$.

Storage. Store protected from moisture.

Protamine Sulphate

Protamine Sulphate is a purified mixture of the sulphates of basic peptides prepared from the sperm or mature testes of suitable species of fish. It binds with heparin in solution, inhibiting its anticoagulant activity. It is prepared in conditions designed to minimise the degree of Microbial contamination.

Each mg of Protamine Sulphate precipitates not less than 100 Units of *heparin sodium RS*, calculated on the dried basis.

Category. Heparin antidote.

Dose. By slow intravenous injection over a 10-minute period, 1 mg for every 100 Units of heparin remaining in the patient if given within 15 minutes; maximum 50 mg.

Description. A white or almost white powder; hygroscopic.

Identification

A. Produces a precipitate under the conditions of the Assay.

B. Dissolve 0.2 g in 5 ml of *water* and dilute to 10 ml with the same solvent (solution A). To 0.5 ml of solution A add 4.5 ml of *water*, 1 ml of a 10 per cent w/v solution of *sodium hydroxide* and 1 ml of a 0.02 per cent w/v solution of *1-naphthol* and mix. Cool to 5° and add 0.5 ml of *alkaline sodium hypobromite solution*; an intense red colour is produced.

C. Heat 2 ml of solution A on a water-bath at 60°, add 0.1 ml of *mercuric sulphate solution* and mix; no precipitate is produced. Cool the mixture in ice; a white precipitate is produced.

D. Gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. To 2.5 ml of solution A add 7.5 ml of *water*. The resulting solution is not more opalescent than opalescence standard OS2 (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Specific optical rotation (2.4.22). –65.0° to –85.0°, determined at 20° in a 1.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Light absorption (2.4.7). Dilute 2.5 ml of solution A to 5.0 ml with *water*. Absorbance of the resulting solution at 260 to 280 nm is not more than 0.1.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Iron (2.3.14). Dissolve 2.0 g in *water* with the aid of heat and dilute to 20 ml with *water*. The resulting solution complies with the limit test for iron (20 ppm).

Mercury. Add 20 ml of a mixture of equal volumes of *nitric acid* and *sulphuric acid* to 2.0 g in a 250-ml flask fitted with a ground-glass stopper, boil under a reflux condenser for 1 hour, cool and carefully dilute with *water*. Boil until nitrous fumes are no longer evolved. Cool, carefully dilute the solution to 200 ml with *water*, mix and filter. Transfer 50 ml of the filtrate to a separating funnel. Shake with successive small quantities of *chloroform* until the *chloroform* layer remains colourless. To the aqueous layer add 25 ml of *1 M sulphuric acid*, 115 ml of *water* and 10 ml of a 20 per cent w/v solution of *hydroxylamine hydrochloride*. Titrate with *dithizone solution*; after each addition, shake the mixture 20 times and towards the end-point of the titration allow to separate and discard the chloroform layer. Titrate until a greenish blue colour is produced. Calculate the content of mercury using the equivalent of mercury in µg per ml of titrant determined in the standardisation of the dithizone solution (10 ppm).

Nitrogen (2.3.30). 21.0 to 26.0 per cent, calculated on the dried basis, determined by Method C.

Sulphates. 16 to 24 per cent, determined by the following method. Dissolve 0.15 g in 15 ml of *water* in a beaker, add 5 ml of *2 M hydrochloric acid* and heat to boiling. Slowly add to the boiling solution 10 ml of *barium chloride solution*. Cover and heat on a *water*-bath for 1 hour. Filter and wash the precipitate several times with small quantities of hot *water*. Dry and ignite the residue to constant weight at 600°.

1 g of the residue is equivalent to 0.4117 g of SO_4.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity, using 0.5 mg dissolved in 0.5 ml of water for injections.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Prepare solutions of the substance under examination in *water* containing (1) 0.015 per cent w/v (2) 0.01 per cent w/v and (3) 0.005 per cent w/v. Titrate each of the solutions in duplicate with a 174 IU per ml or a suitable dilution of *heparin sodium RS* using the following procedure. Introduce an accurately measured volume of the solution to be titrated, for example 1.5 ml, into the cell of a suitable spectrophotometer, set the instrument at a suitable wavelength (none is critical) in the visible range and add the titrant in small volumes until there is a sharp increase in the absorbance. Note the volume of titrant added.

Carry out three independent assays. For each individual titration, calculate the number of Units of heparin titrated per mg of the substance under examination. Calculate the result of the assay as the average of the 18 values. Test the linearity of the response by standard statistical methods. The assay is

not valid unless the standard deviations calculated for the results obtained with each test solution are less than 5 per cent of the average result.

Protamine Sulphate intended for use in the manufacture of Parenteral Preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 7.0 Endotoxin Units per mg of protamine sulphate.

Protamine Sulphate intended for use in the manufacture of Parenteral Preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility. Complies with the test for sterility (2.2.11).

Storage. Store protected from moisture. If it is intended for use in the manufacture of Parenteral Preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are intended for use in the manufacture of Parenteral Preparations.

Protamine Sulphate Injection

Protamine Sulphate Injection is a sterile solution of Protamine Sulphate in Water for Injections.

Protamine Sulphate Injection contains not less than 80.0 per cent of the stated amount of protamine sulphate.

Usual strengths. 50 mg in 5 ml; 100 mg in 10 ml.

Identification

A. Produces a precipitate under the conditions of the Assay.

B. Dilute a suitable volume with *water* to give a solution containing 0.2 per cent w/v solution of Protamine Sulphate. To 5 ml of this solution add 1 ml of a 10 per cent w/v solution of *sodium hydroxide* and 1 ml of a 0.02 per cent w/v solution of *1-naphthol* and mix. Cool to 5° and add 0.5 ml of *alkaline sodium hypobromite solution*; an intense red colour is produced.

C. Heat 2 ml on a water-bath at 60°, add 0.1 ml of *mercuric sulphate solution* and mix; no precipitate is produced. Cool the mixture in ice; a white precipitate is produced.

D. Gives reaction A of sulphates (2.3.1).

Tests

pH (2.4.24). 2.5 to 3.5.

Optical rotation (2.4.22). –0.52° to –0.68°, determined in a solution prepared by diluting the injection with *0.5 M hydrochloric acid* so as to contain 0.8 per cent w/v of Protamine Sulphate.

Light absorption. Dilute the injection, if necessary, with *water* to produce a solution containing 1 per cent w/v solution of Protamine Sulphate. Absorbance of the resulting solution at 260 to 280 nm, not more than 0.1 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 7.0 Endotoxin Units per mg of protamine sulphate.

Abnormal toxicity. Complies with the test for abnormal toxicity (2.2.1), using a volume containing 0.5 mg of Protamine Sulphate.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

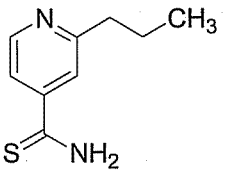
Assay. Prepare solutions of the substance under examination in *water* containing (1) 0.015 per cent w/v (2) 0.01 per cent w/v and (3) 0.005 per cent w/v. Titrate each of the solutions in duplicate with a 174 IU per ml or a suitable dilution of *heparin sodium RS* using the following procedure. Introduce an accurately measured volume of the solution to be titrated, for example 1.5 ml, into the cell of a suitable spectrophotometer, set the instrument at a suitable wavelength (none is critical) in the visible range and add the titrant in small volumes until there is a sharp increase in the absorbance. Note the volume of titrant added.

Carry out three independent assays. For each individual titration, calculate the number of Units of heparin titrated per mg of the substance under examination. Calculate the result of the assay as the average of the 18 values. Test the linearity of the response by standard statistical methods. The assay is not valid unless the standard deviations calculated for the results obtained with each test solution are less than 5 per cent of the average result.

Storage. Store protected from light, in single dose containers.

Labelling. The label states (1) that the dose is calculated from the results of determinations of the amount required to produce an acceptable blood-clotting time in the patient; (2) the approximate number of Units of heparin activity 1 ml is capable of neutralising.

Prothionamide



$C_9H_{12}N_2S$ Mol. wt. 180.3

Prothionamide is 2-propyl-4-pyridinecarbothioamide.

Prothionamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_{10}N_2S$,calculated on the dried basis.

Category. Antitubercular.

Description. A yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prothionamide RS*.

B. When examined in the range 230 nm to 350 nm (2.4.7), a 0.002 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum only at about 291 nm. The absorbance at 291 nm is about 0.78.

Tests

Acidity. Dissolve 2.0 g in 20 ml of *methanol*, heating to about 50°, and add 20 ml of *water*. Cool slightly, shake until crystallisation occurs, if any and allow to cool to room temperature. Add 60 ml of *water* and titrate with *0.1 M sodium hydroxide* using 0.2 ml of *cresol red solution* as indicator. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the indicator to red.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *prothionamide RS* in the mobile phase. Dilute 1 ml of the solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of a buffer solution prepared by mixing 2.0 ml of *triethylamine* with 1000 ml of *water*, adjusting the pH to 6.0 with *dilute orthophosphoric acid* and 40 volumes of *acetonitrile,*
- flow rate.1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject the reference solution.The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any individual impurity peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy Metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances using the following solutions.

Test solution. Dissolve 50.0 mg of the substance under examination in the mobile phase and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of this solution to a 50.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *prothionamide RS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 5000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_9H_{12}N_2S$.

Storage. Store protected from light and moisture.

Prothionamide Tablets

Prothionamide Tablets contain not less than 90.0 per cent not more than 110.0 per cent of the stated amount of prothionamide, $C_9H_{12}N_2S$.

Usual strength. 250 mg.

Identification

A. Extract a quantity of the powdered tablets containing 25 mg of Prothionamide with 5 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *prothionamide RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 2,

Medium. 900 ml *0.1 M hydrochloric acid,*

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the dissolution medium if necessary, at the maximum at about 290 nm (2.4.7). Calculate the content of $C_9H_{12}N_2S$ in the medium from the absorbance obtained from a solution of known concentration of *prothionamide RS* in the same medium.

D. Not less than 75 per cent of the stated amount of $C_9H_{12}N_2S$.

Related substances. Determine by liquid chromatography (2.4.14) as described under Assay using the following solutions.

Test solution. Weigh accurately a quantity of the powdered tablets containing 50 mg of Prothionamide disperse in the mobile phase, shake, dilute to 100 ml with the mobile phase and filter.

Reference solution. A solution containing 0.025 per cent w/v of *prothionamide RS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the mobile phase.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternatively the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any individual impurity peak is not more than the area of the peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all such impurities is not more than twice the area of the peak in the chromatogram obtained with the reference solution (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Prothionamide, disperse in the mobile phase, shake and dilute to 100.0 ml with the mobile phase. Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *prothionamide RS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by mixing 2.0 ml of *triethylamine* with 1000 ml of *water* and adjusting the pH to 6.0 with *dilute orthophosphoric acid*, and 40 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

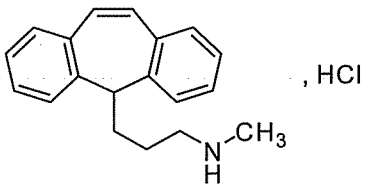
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 the column efficiency is not less than 5000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_9H_{12}N_2S$ in the tablets.

Storage. Store protected from light and moisture.

Protriptyline Hydrochloride



$C_{19}H_{21}N,HCl$ Mol. Wt. 299.8

Protriptyline Hydrochloride is 3-(5*H*-dibenzo[*a*,*d*]cyclohept-5-yl)propyl(methyl)amine hydrochloride.

Protriptyline Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{21}N,HCl$, calculated on the dried basis.

Category. Tricyclic antidepressant.

Description. A white to yellowish white powder.

Identification

A. Dissolve 0.1 g in 10 ml of *water*, make alkaline with *1M sodium hydroxide*, extract with 5 ml of *chloroform*, dry with *anhydrous sodium sulphate* and evaporate the solvent using a current of nitrogen. On the oily residue, determine by the infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *protriptyline hydrochloride RS* or with the reference spectrum of protriptyline hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.5, determined in a 1.0 per cent w/v solution.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.7 g, dissolve in 0.5 ml of *acetic anhydride* and 20 ml of *anhydrous glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1M perchloric acid* is equivalent to 0.02998 g of $C_{19}H_{21}N$,HCl.

Protriptyline Tablets

Protriptyline Hydrochloride Tablets

Protriptyline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of protriptyline hydrochloride, $C_{19}H_{21}N$, HCl.

Usual strength. 5 mg.

Identification

A. When examined in the range 230 to 350 nm (2.4.7), a solution obtained in the Assay exhibits a maximum only at 292 nm.

B. Shake a quantity of the powdered tablets containing about 5 mg of Protriptyline Hydrochloride with 20 ml of *methanol* and filter. To 1 ml of the filtrate add 1 ml of a 2.5 per cent w/v solution of *sodium hydrogen carbonate*, 1 ml of a 2 per cent w/v solution of *sodium periodate* and 1 ml of a 0.3 per cent w/v solution of *potassium permanganate*. Allow to stand for 15 minutes, acidify with *1 M sulphuric acid* and extract with 10 ml of *2,2,4-trimethylpentane*, washing the extract with 10 ml of *0.25 M sulphuric acid*. The absorbance of trimethylpentane solution does not exhibit a maxima at 265 nm (2.4.7) (distinction from amitriptyline and nortriptyline).

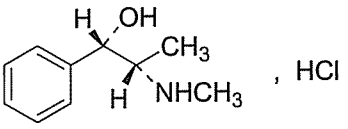
C. Triturate a quantity of the powdered tablets containing about 0.1 g of Protriptyline Hydrochloride with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dissolve part of the residue in 3 ml of *water* and add 0.05 ml of a 2.5 per cent w/v solution of *quinhydrone* in *methanol*. A red colour develops.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Triturate 10 tablets for 15 minutes with 100 ml of a solution prepared by mixing 1 volume of *1 M hydrochloric acid* and 9 volumes of *methanol* , transfer to a graduated flask using sufficient of the solvent mixture to produce 250 ml, mix and filter. Dilute a volume of the filtrate containing about 1 mg of Protriptyline Hydrochloride to 100 ml with the solvent mixture and measure the absorbance at the maximum at 292 nm (2.4.7). Calculate the content of $C_{19}H_{21}N$, HCl taking 465 as the absorbance.

Pseudoephedrine Hydrochloride



$C_{10}H_{15}NO$,HCl Mol. Wt. 201.7

Pseudoephedrine Hydrochloride is (1*S*,2*S*)-2-methylamino-1-phenylpropan-1-ol hydrochloride.

Pseudoephedrine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{15}NO$, HCl, calculated on the dried basis.

Category. Sympathomimetic.

Dose. 60 mg three to four times daily.

Description. A white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pseudoephedrine hydrochloride RS* or with the reference spectrum of pseudoephedrine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.1 per cent w/v solution shows absorption maxima at about 251 nm, 257 nm and 263 nm; absorbances at the maxima, about 0.75, about 0.98 and about 0.78, respectively.

C. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is not more than very faintly opalescent and colourless (2.4.1).

pH (2.4.24). 4.6 to 6.0, determined in a 5.0 per cent w/v solution.

Specific optical rotation (2.4.22). +61.0° to +62.5°, determined in a 5.0 per cent w/v solution using a 2-dm tube.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the substance under examination in the mobile phase and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dissolve 20 mg of *ephedrine hydrochloride RS* (*pseudoephedrine impurity A RS*) in 20.0 ml of the mobile phase. Dilute 1.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (c). Dissolve 10 mg of *ephedrine hydrochloride RS* (*pseudoephedrine impurity A RS*) in 5 ml of the test solution and dilute to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with phenylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 6 volumes of *methanol* and 94 volumes of 1.2 per cent w/v solution of *ammonium acetate*, adjusted to pH 4.0 with *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 257 nm,
- injection volume. 20 µl.

Inject the test solution, reference solution (a), (b) and (c). Run the chromatogram 1.5 times the retention time of pseudoephedrine. The relative retention time with reference to pseudoephedrine for pseudoephedrine impurity A is about 0.89. The test is not valid unless the resolution between the peaks due to pseudoephedrine impurity A and pseudoephedrine is not less than 2.0. In the chromatogram obtained with the test solution the area of the peak due to pseudoephedrine impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of all the secondary peaks other than pseudoephedrine impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.5 g, dissolve in a mixture of 50 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02017 g of $C_{10}H_{15}NO$, HCl.

Storage. Store protected from light and moisture.

Pseudoephedrine Syrup

Pseudoephedrine Hydrochloride Syrup

Pseudoephedrine Syrup is a solution of Pseudoephedrine Hydrochloride in a suitable flavoured vehicle.

Pseudoephedrine Syrup contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pseudoephedrine hydrochloride, $C_{10}H_{15}NO$, HCl.

Usual strength. 30 mg per 5 ml.

Identification

A. Shake a quantity of the syrup containing 120 mg of Pseudoephedrine Hydrochloride with two quantities, each of 30 ml, of *ether*, and discard the ether layer. Add 4 ml of *1 M sodium hydroxide* to the aqueous layer and extract with two quantities, each of 10 ml, of *ether*. Dry the combined ether extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pseudoephedrine hydrochloride RS* treated in the saame manner or with the reference spectrum of pseudoephedrine.

B. The residue obtained in test A melts at about 118° (2.4.21).

C. Dissolve 50 mg of the residue obtained in test A in 10 ml of *0.1 M hydrochloric acid*; it is dextro-rotatory.

Tests

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. To an accurately measured volume of the syrup containing about 0.12 g of Pseudoephedrine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* mix, add sufficient *0.1 M hydrochloric acid* to produce 100.0 ml, filter and use the filtrate.

Reference solution. A 0.12 per cent w/v solution of *pseudoephedrine hydrochloride RS* in *0.1 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.2 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *ethanol* and 15 volumes of a 0.4 per cent w/v solution of *ammonium acetate*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution and record the chromatogram. The test is not valid unless the relative standard deviation is not more than 2.0 per cent and the tailing factor is not more than 1.5.

Inject alternately the test solution and the reference solution.

Determine the weight per ml of the syrup (2.4.29), and calculate the content of $C_{10}H_{15}NO$, HCl weight in volume.

Storage. Store protected from light and moisture.

Pseudoephedrine Tablets

Pseudoephedrine Hydrochloride Tablets

Pseudoephedrine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pseudoephedrine hydrochloride, $C_{10}H_{15}NO$, HCl.

Usual strength. 60 mg.

Identification

A. Shake a quantity of the powdered tablets containing 60 mg of Pseudoephedrine Hydrochloride with 10 ml of *water* and filter. Shake the filtrate with 10 ml of *ether*, discard the ether layer. Add 1 ml of *1 M sodium hydroxide* to the aqueous layer and extract with two quantities, each of 10 ml, of *ether*. Dry the combined ether extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pseudoephedrine hydrochloride RS* treated in the same manner or with the reference spectrum of pseudoephedrine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 40 volumes of *butyl acetate*, 20 volumes of *acetone*, 20 volumes of *1-butanol*, 10 volumes of *5 M ammonia* and 10 volumes of *methanol*.

Test solution (a). Add 25 ml of *methanol* to a quantity of the powdered tablets containing 0.5 g of Pseudoephedrine Hydrochloride, shake for 5 minutes, filter, wash the filter with *methanol* and evaporate the combined filtrate and washings to dryness. Dissolve the residue as completely as possible in 5 ml of *methanol*, centrifuge and use the supernatant liquid.

Test solution (b). Dilute 1 volume of the test solution to 10 volumes with *methanol*.

Reference solution (a). Dilute 1 volume of the test solution to 100 volumes with *methanol*.

Reference solution (b). A 1.0 per cent w/v solution of *pseudoephedrine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of warm air, spray with a solution containing 0.3 g of *ninhydrin* in a mixture of 100 ml *1-butanol* and 3 ml of *glacial acetic acid* and heat at 120° for 20 minutes. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any yellow spot near the line of application.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. To a quantity of the powdered tablets containing about 0.12 g of Pseudoephedrine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* mix with the aid of ultrasound for 15 minutes, add sufficient *methanol* to produce 100.0 ml, filter and use the filtrate.

Reference solution. A 0.12 per cent w/v solution of *pseudoephedrine hydrochloride RS* in *methanol (50 per cent)*.

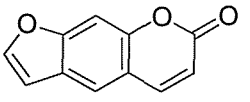
Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: *0.005 M dioctyl sodium sulphosuccinate* in a mixture of 65 volumes of *methanol*, 35 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate: 1.5 ml per minute,
- spectrophotometer set at 258 nm,
- injection volume. 20 µl.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{10}H_{15}NO$,HCl in the tablets.

Storage. Store protected from light and moisture.

Psoralen



$C_{11}H_6O_3$ Mol. Wt. 186.2

Psoralen is 7*H*-furo[3,2-*g*][1]benzopyran-7-one, obtained from the fruits of *Psoralea carylifolia* Linn. (Fam. Leguminosae) and from the leaves of *Ficus carica* (Fam. Urticaceae) or prepared by synthesis.

Psoralen contains not less than 95.0 per cent and not more than 101.0 per cent of $C_{11}H_6O_3$, calculated on the dried basis.

Category. Topical pigmenting agent.

Description. Colourless needles; odourless.

Identification

A. Dissolve 1 mg in 5 ml of *ethanol (95 per cent)* and add 15 ml of a mixture containing 43 volumes of *water*, 5 volumes of *acetic acid* and 3 volumes of *propylene glycol*; a blue fluorescence is visible in ultraviolet light at 365 nm.

B. Dissolve 1 mg in 2 ml of *ethanol (95 per cent)* and add 0.1 ml of *0.1 M sodium hydroxide*; a yellow fluorescence is visible in ultraviolet light at 365 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *benzene* and 10 volumes of *ethyl acetate*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *chloroform*.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *chloroform*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

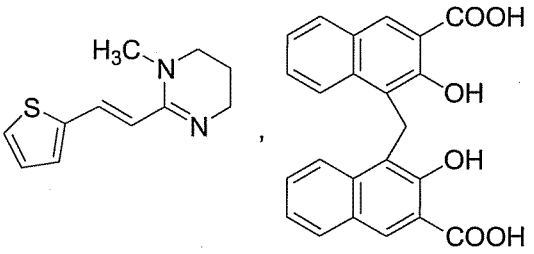
Assay. Weigh accurately about 0.1 g and dissolve in sufficient *methanol* to produce 100.0 ml. Dilute 2.0 ml of this solution to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 247 nm (2.4.7).

Calculate the content of $C_{11}H_6O_3$ from the absorbance obtained by repeating the operation using a final solution of 20 µg per ml of *psoralen RS* in *methanol* in place of the substance under examination.

Storage. Store protected from light and moisture.

Pyrantel Pamoate

Pyrantel Embonate



$C_{11}H_{14}N_2S, C_{23}H_{16}O_6$ Mol. Wt. 594.7

Pyrantel Pamoate is 1,4,5,6-tetrahydro-1-methyl-2-[(*E*)-2-(2-thienyl)vinyl]pyrimidine hydrogen; 4,4′-methylene bis(3-hydroxynaphthalene-2-carboxylate).

Pyrantel Pamoate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{34}H_{30}N_2O_6S$, calculated on the dried basis.

Category. Anthelmintic.

Dose. 10 mg per kg

Description. A pale yellow or yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyrantel pamoate RS* or with the reference spectrum of pyrantel pamoate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Prepare the solutions immediately before use and protected from light.

Solvent mixture. Mix 25 volumes of *glacial acetic acid*, 25 volumes of *water* and 10 volumes of *diethylamine* with cooling.

Test solution. Dissolve 80 mg of the substance under examination in 7 ml of the solvent mixture and dilute to 100.0 ml with mobile phase.

Reference solution (a). Dissolve 10.0 mg of *1-methyl-2-[(Z)-2-(thiophen-2-yl)-1,4,5,6-tetrahydropyrimidine RS* (*pyrantel impurity A RS*) in the solvent mixture, add 2.5 ml of the test solution and dilute to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica gel (5 µm),

- mobile phase: 7.2 volumes of the solvent mixture and 92.8 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 288 nm,
- injection volume. 20 µl.

Inject reference solution (a). Run the chromatogram for 4 times the retention time of pyrantel. The test is not valid unless the resolution between the peaks due to pyrantel and pyrantel impurity A is at least 4.0. The relative retention times with reference to pyrantel pamoate are about 0.5 for pamoic acid, about 1.3 for pyrantel impurity A and about 1.8 for ((*E*)-*N*-{methylamino)propyl]-3-(thiophen-2-yl)prop-2-enamide) pyrantel impurity B.

Inject the test solution, reference solutions (a) and (b). For the calculation of content, multiply the peak area of pyrantel impurity B by 0.4. In the chromatogram obtained with the test solution, the area of the peak due to pyrantel impurity A is not more than the corresponding peak in the chromatogram obtained with reference solution (a) (0.5 per cent); the area of the peak due to pyrantel impurity B is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent); the area any other secondary peak is not more than 0.2 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks other than those of pyrantel impurities A and B is not more than 0.6 times the area of the principal peak in the chromatogram obtained with the reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Chlorides (2.3.12). To 0.7 g, add 10 ml of *dilute nitric acid* and 30 ml of *water*. Heat on water-bath for 5 minutes and cool. The resulting solution complies with the limit test for chlorides (360 ppm).

Sulphates (2.3.17). To 0.5 g, add 2.5 ml of *dilute nitric acid* and dilute to 50 ml with *water*. Heat on water bath for 5 minutes. 15 ml complies with the limit test for sulphates (0.1 per cent).

Iron (2.3.14). Ignite 0.54 g at 800° for 2 hours. Dissolve the residue in 2.5 ml of *dilute hydrochloric acid* with gentle heating for 10 minutes and cool. The resulting solution complies with the limit test for iron (75 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in vacuum at 60° for 3 hours.

Assay. Weigh accurately about 0.5 g, add 10 ml of *acetic anhydride* and 50 ml *glacial acetic acid*. Heat at 50° and stir for 10 minutes. Allow to cool (a clear solution is not obtained). Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05947 g of $C_{34}H_{30}N_2O_6S$.

Storage. Store protected from light.

Pyrantel Pamoate Oral Suspension

Pyrantel Pamoate Oral Suspension is a suspension of Pyrantel Pamoate in a suitable flavoured aqueous vehicle.

Pyrantel Pamoate Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pyrantel, $C_{11}H_{14}N_2S$.

Usual strength. 50 mg per ml

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. The upper layer of a mixture obtained by shaking 20 volumes of *methyl isobutyl ketone*, 10 volumes of *formic acid* and 10 volumes of *water*.

Test solution. Dilute a known volume of the oral suspension with sufficient *0.05 M methanolic ammonia* to produce a solution containing about 8 mg of pyrantel per ml. Shake the mixture by mechanical means and centrifuge. Use the clear supernatant liquid.

Reference solution. A solution of *pyrantel pamoate RS* in *0.05 M methanolic ammonia* containing 0.008 per cent w/v of *pyrantel*.

Apply to the plate 100 µl of each solution. Allow the mobile phase to rise to about 17 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the major peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 6.0

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14).

NOTE— Carry out the test protected from light.

Test solution. Weigh accurately a quantity of the oral suspension containing about 60 mg of pyrantel, disperse in

60 ml of *water* and add sufficient *water* to produce 100.0 ml. Dilute 1.0 ml of the well-stirred suspension to 25.0 ml with the mobile phase to obtain a clear solution. Mix and filter.

Reference solution. Weigh accurately about 20 mg of *pyrantel pamoate RS* and dissolve in sufficient mobile phase to produce 25.0 ml. Dilute 10.0 ml of the resulting solution to 100.0 ml with the mobile phase and mix.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with porous silica particles (5 to 10 µm),
- mobile phase: a mixture of 92.8 volumes of *acetonitrile*, 3 volumes of *acetic acid*, 3 volumes of *water* and 1.2 volumes of *diethylamine*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 288 nm,
- injection volume. 20 µl.

Inject the reference solution. The resolution between pyrantel and pamoic acid is not less than 10.0, the column efficiency for the pyrantel peak is not less than 8000 theoretical plates, the tailing factor for the pyrantel peak is not greater than 1.3 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

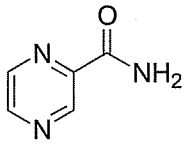
Inject the test solution and the reference solution. Continue the chromatography for a period not less than 2.5 times the retention times of pyrantel base. The relative retention times for pamoic acid and pyrantel base are about 0.6 and 1.0 respectively.

Determine the weight per ml (2.4.29) of the suspension and calculate the content of $C_{11}H_{14}N_2S$, weight in volume.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of pyrantel.

Pyrazinamide



$C_5H_5N_3O$ Mol. Wt. 123.1

Pyrazinamide is pyrazine-2-carboxamide.

Pyrazinamide contains not less than 99.0 per cent and not more than 100.5 per cent of $C_5H_5N_3O$, calculated on the anhydrous basis.

Category. Antitubercular.

Dose. Up to 35 mg per kg of body weight daily, in divided doses.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyrazinamide RS* or with the reference spectrum of pyrazinamide.

B. Dissolve 50 mg in *water* and dilute to 100 ml with the same solvent (solution A). Dilute 1 ml of solution A to 10 ml. When examined in the range 290 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 310 nm. Dilute 2 ml of solution A to 100 ml with *water*. When examined in the range 230 nm to 290 nm, the solution shows an absorption maximum at about 268 nm; absorbance at about 268 nm, between 0.64 and 0.68.

C. Boil 20 mg with 5 ml of *sodium hydroxide solution*; ammonia, recognisable by its odour, is evolved.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* (solution B) is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 25 ml of solution B add 0.05 ml of *phenolphthalein solution* and 0.2 ml of *0.01 M sodium hydroxide*; the solution is red. Add 1 ml of *0.01 M hydrochloric acid*; the solution is colourless. Add 0.15 ml of *methyl red solution*; the solution is red.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 60 volumes of *1-butanol*, 20 volumes of *glacial acetic acid* and 20 volumes of *water.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Reference solution. A 0.002 per cent w/v solution of the substance under examination in a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.4.19). Not more than 0.5 per cent, determined on 5.0 g.

Assay. Weigh accurately about 0.3 g and transfer to the flask of an ammonia distillation apparatus. Add 200 ml of *water* and 75 ml of *sodium hydroxide solution*. Boil gently for 20 minutes, collecting the distillate in 50.0 ml of *0.05 M sulphuric acid*. Boil vigorously to complete the distillation of the ammonia and titrate the excess of acid with *0.1 M sodium hydroxide*, using *methyl red solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of acid required to neutralise the ammonia formed.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.01231 g of $C_5H_5N_3O$.

Storage. Store protected from moisture.

Pyrazinamide Tablets

Pyrazinamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pyrazinamide, $C_5H_5N_3O$.

Usual strengths. 250 mg; 500 mg; 750 mg; 1000 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.25 g of Pyrazinamide with 20 ml of *ethanol*, filter, evaporate the filtrate to dryness and dry the residue at 105° for 30 minutes.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyrazinamide RS* or with the reference spectrum of pyrazinamide.

B. Shake a quantity of the powdered tablets containing 50 mg of Pyrazinamide with 50 ml of *water*, dilute to 100 ml with *water* and filter (solution A). Dilute 1 ml of solution A to 10 ml with *water*. When examined in the range 290 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 310 nm. Dilute 2 ml of solution A to 100 ml with *water*. When examined in the range 230 nm to 290 nm, the resulting solution shows an absorption maximum at about 268 nm; absorbance at 268 nm, between 0.64 and 0.68.

C. Boil a quantity of the powdered tablets containing 20 mg of Pyrazinamide with 5 ml of *sodium hydroxide solution*; ammonia, recognisable by its odour, is evolved.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *1-butanol*, 20 volumes of *glacial acetic acid* and 20 volumes of *water*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Pyrazinamide with 50 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*, filter, evaporate to dryness and dissolve the residue in sufficient of the same solvent mixture to produce 10 ml.

Reference solution. Dilute 1 volume of the test solution to 500 volumes with a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

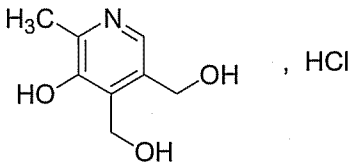
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Pyrazinamide, add 200 ml of *water*, allow to stand for 10 minutes, swirling occasionally, mix with the aid of ultrasound for 10 minutes and dilute to 500.0 ml with *water*. Filter and discard the first 20 ml of the filtrate. Dilute 5.0 ml of the filtrate to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 268 nm (2.4.7). Calculate the content of $C_5H_5N_3O$ taking 650 as the specific absorbance at 268 nm.

Storage. Store protected from moisture.

Pyridoxine Hydrochloride

Vitamin B_6



$C_8H_{11}NO_3,HCl$ Mol. Wt. 205.6

Pyridoxine Hydrochloride is 5-hydroxy-6-methylpyridine-3,4-dimethanol hydrochloride.

Pyridoxine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_{11}NO_3,HCl$, calculated on the dried basis.

Category. B group vitamin; sideroblastic anaemia therapy; anti-isoniazid neuropathy.

Dose. In deficiency states, prophylactic, upto 2 mg daily; therapeutic, 20 to 50 mg upto three times daily. In multi- vitamin

preparations for oral use, prophylactic, 500 µg to 1.5 mg daily; therapeutic, 1.5 to 3 mg daily. In sideroblastic anaemia, 100 to 400 mg daily, in divided doses. In isoniazid neuropathy, prophylactic, 10 mg daily; therapeutic, 50 mg three times daily.

Description. A white or almost white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B may be omitted if tests A, C and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyridoxine hydrochloride RS* or with the reference spectrum of pyridoxine hydrochloride.

B. When examined in the range 250 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at 288 nm to 296 nm; absorbance at the maximum, 0.420 to 0.445. A solution prepared by diluting 1 ml of a 0.1 per cent w/v solution in *0.1 M hydrochloric acid* to 100 ml with *0.025 M standard phosphate buffer* shows absorption maxima at 248 nm to 256 nm and at 320 nm to 327 nm; absorbances at the maxima, 0.175 to 0.195 and 0.345 to 0.365, respectively.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. A 5 per cent w/v solution gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 2.4 to 3.0, determined in a 5.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *acetone*, 13 volumes of *dichloromethane*, 13 volumes of *tetrahydrofuran* and 9 volumes of *strong ammonia solution*.

Test solution (a). A 10 per cent w/v solution of the substance under examination in *water*.

Test solution (b). A 1 per cent w/v solution of the substance under examination *water*.

Reference solution (a). A 0.025 per cent w/v solution of the substance under examination in *water*.

Reference solution (b). A 1 per cent w/v solution of *pyridoxine hydrochloride RS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *sodium carbonate* in a mixture of 70 volumes of *water* and 30 volumes of *ethanol (95 per cent)*. Dry in a current of air, spray with a 0.1 per cent w/v solution of *2,6-dichloroquinone-4-chloroimide* in *ethanol (95 per cent)* and examine immediately. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spots remaining on the line of application.

Heavy metals (2.3.13). 12 ml of a 5.0 per cent w/v solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in a mixture of 5 ml of *anhydrous glacial acetic acid* and 6 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator, until a green colour is produced. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02056 g of $C_8H_{11}NO_3$, HCl.

Storage. Store protected from moisture.

Pyridoxine Tablets

Pyridoxine Hydrochloride Tablets

Pyridoxine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of pyridoxine hydrochloride, $C_8H_{11}NO_3$, HCl.

Usual strength. 5 mg.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Pyridoxine Hydrochloride with 50 ml of *0.025 M standard phosphate buffer* for 15 minutes and dilute to 100 ml with the same solvent. Mix, filter and dilute 5 ml of the filtrate to 100 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution exhibits two maxima, at about 254 nm and 324 nm.

B. Triturate a quantity of the powdered tablets containing 20 mg of Pyridoxine Hydrochloride with 50 ml of *water* and allow to stand for 20 minutes. To 1 ml of the supernatant liquid add 10 ml of a 5 per cent w/v solution of *sodium acetate*, 1 ml of *water* and 1 ml of a 0.5 per cent w/v solution of *2,6-dichloroquinone-4-chloroimide* in *ethanol (95 per cent)* and

shake; a blue colour is produced which fades rapidly and becomes brown. Repeat the operation but adding 1 ml of a 0.3 per cent w/v solution of *boric acid* in place of 1 ml of *water*; no blue colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *acetone*, 13 volumes of *dichloromethane*, 13 volumes of *tetrahydrofuran* and 9 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Pyridoxine Hydrochloride with 10 ml of *water* for 15 minutes, filter and use the filtrate.

Reference solution. Dilute 1 ml of test solution to 200 ml with *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *sodium carbonate* in a mixture of 70 volumes of *water* and 30 volumes of *ethanol (95 per cent)*. Dry it in a current of air, spray with a 0.1 per cent w/v solution of *2,6-dichloroquinone-4-chloroimide* in *ethanol (95 per cent)* and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Uniformity of content. Comply with the test stated under Tablets.

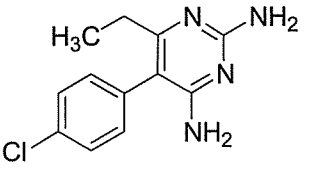
Powder one tablet, add 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 15 minutes, swirling occasionally. Cool, dilute to 100.0 ml with *0.1 M hydrochloric acid*, filter, discarding the first 20 ml of the filtrate. If necessary, dilute quantitatively and stepwise with *0.1 M hydrochloric acid* to produce a solution containing 10 µg of the pyridoxine hydrochloride per ml and measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_8H_{11}NO_3$, HCl taking 430 as the specific absorbance at 290 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Pyridoxine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* and heat on a water-bath for 15 minutes, swirling occasionally. Cool, dilute to 100.0 ml with *0.1 M hydrochloric acid* and filter, discarding the first 20 ml of the filtrate. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7). Calculate the content of $C_8H_{11}NO_3$, HCl taking 430 as the specific absorbance at 290 nm.

Storage. Store protected from light and moisture.

Pyrimethamine



$C_{12}H_{13}ClN_4$ Mol. Wt. 248.7

Pyrimethamine is 5-(4-chlorophenyl)-6-ethylpyrimidine-2,4-diamine.

Pyrimethamine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{13}ClN_4$, calculated on the dried basis.

Category. Antimalarial.

Dose. Used only in combination with sulphadoxine (25 mg pyrimethamine and 500 mg sulphadoxine) or dapsone (12.5 mg pyrimethamine and 100 mg dapsone).

Description. Colourless crystals or an almost white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyrimethamine RS* or with the reference spectrum of pyrimethamine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 0.14 g in sufficient *ethanol* to produce 100 ml, dilute 10 ml of this solution to 100 ml with *0.1 M hydrochloric acid* and dilute 10 ml of the solution to 100 ml with the same solvent. When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 272 nm; absorbance at about 272 nm, 0.43 to 0.46.

Tests

Appearance of solution. Dissolve 0.25 g in sufficient of a mixture of 3 volumes of *dichloromethane* and 1 volume of *methanol* to produce 10 ml. The resulting solution is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or alkalinity. Shake 1.0 g with 50 ml of *water* for 2 minutes and filter (solution A). To 10 ml of solution A add

0.05 ml of *phenolphthalein solution*; the solution is colourless and not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour to pink. Add 0.4 ml of *0.01 M hydrochloric acid* and 0.05 ml of *methyl red solution*; the solution is red or orange.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 76 volumes of *toluene*, 12 volumes of *glacial acetic acid*, 8 volumes of *1-propanol* and 4 volumes of *chloroform*.

Prepare the following solutions immediately before use.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of a mixture of 90 volumes of *chloroform* and 10 volumes of *methanol*.

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of the same solvent mixture.

Reference solution (a). A 0.0025 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *pyrimethamine RS* in the same solvent mixture.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphates (2.3.17). 25 ml of Solution A complies with the limit test for sulphates. Use a mixture of 5.0 ml of *sulphate standard solution (10 ppm SO_4)* and 10 ml of *distilled water* to prepare the standard (100 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.2 g, dissolve in 25 ml of *anhydrous glacial acetic acid*, heating gently, cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02487 g of $C_{12}H_{13}ClN_4$.

Storage. Store protected from light and moisture.

Pyrimethamine Tablets

Pyrimethamine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of pyrimethamine, $C_{12}H_{13}ClN_4$.

Usual strength. 25 mg.

Identification

A. Shake a quantity of the powdered tablets containing 50 mg of Pyrimethamine with 50 ml of *ethanol (95 per cent)* for 20 minutes, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pyrimethamine RS* or with the reference spectrum of pyrimethamine.

B. Extract the powdered tablets with *1 M sulphuric acid*, filter and add *potassium tetraiodomercurate solution* to the filtrate. A creamy white precipitate is produced.

C. Extract a quantity of the powdered tablets containing 50 mg of Pyrimethamine with two 10 ml quantities of *chloroform* and evaporate the combined extracts to dryness. The residue melts at about 240° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 90 volumes of *chloroform* and 10 volumes of *methanol*.

Mobile phase. A mixture of 4 volumes of *chloroform*, 8 volumes of *1-propanol*, 12 volumes of *glacial acetic acid* and 76 volumes of *toluene*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg Pyrimethamine with 5 ml of solvent mixture and filter.

Reference solution. Dilute 1 ml of the test solution to 400 ml with the solvent mixture.

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 10 cm. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.25 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.025g of Pyrimethamine, add 50 ml of hot *0.1 M hydrochloric acid* and heat on a water-bath for 10 minutes, swirling occasionally. Mix with the aid of ultrasound for 30 minutes, remove and cool to room temperature. Add sufficient *0.1 M hydrochloric acid* to produce 100 ml, filter and dilute 5 ml of the filtrate to 100 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 272 nm (2.4.7). Calculate the content of $C_{12}H_{13}ClN_4$ taking 316 as the specific absorbance at 272 nm.

Pyrimethamine and Sulphadoxine Tablets

Pyrimethamine and Sulphadoxine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of pyrimethamine, $C_{12}H_{13}ClN_4$, and of sulphadoxine, $C_{12}H_{14}N_4O_4S$.

Usual strength. Pyrimethamine 25 mg and sulphadoxine 500 mg.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 4 volumes of *chloroform*, 4 volumes of *n-heptane*, 1 volume of *glacial acetic acid* and 4 volumes of a mixture of 1 volume of *methanol* and 19 volumes of *ethanol*.

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Pyrimethamine with 50 ml of a 2 per cent w/v solution of *strong ammonia solution* in *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *pyrimethamine RS* in *methanol*.

Reference solution (b). A 1.0 per cent w/v solution of *sulphadoxine RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. One of the principal spots in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a) and the other corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Other tests. Complies with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 25 mg of Pyrimethamine and 500 mg of Sulphadoxine and shake with 35 ml of *acetonitrile* for 30 minutes in a 100-ml volumetric flask. Dilute to volume with the mobile phase, mix and filter. To 25.0 ml of the filtrate add 2.0 ml of solution A prepared by dissolving 0.1 g of *phenacetin* (internal standard) in 100 ml of *acetonitrile* and sufficient of the mobile phase to produce 50.0 ml.

Reference solution. Weigh accurately about 25 mg of *pyrimethamine RS* and 500 mg of *sulphadoxine RS*, add 35 ml of *acetonitrile* and sufficient of the mobile phase to produce 100.0 ml and mix. To 25.0 ml add 2.0 ml of solution A and add sufficient of the mobile phase to produce 50.0 ml and mix well.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 4 volumes of a 1 per cent v/v solution of *glacial acetic acid* and 1 volume of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention times with reference to phenacetin for sulphadoxine is about 0.7 and for pyrimethamine is about 1.3.

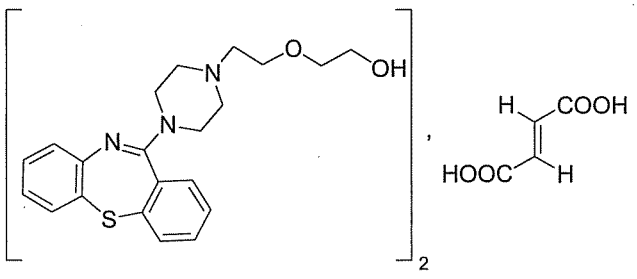
Inject the test solution and the reference solution.

Calculate the content of $C_{12}H_{13}ClN_4$ and the content of $C_{12}H_{14}N_4O_4S$ in the tablets.

Storage. Store protected from light and moisture.

Q

Quetiapine Fumarate



$(C_{21}H_{25}N_3O_2S)_2,C_4H_4O_4$ Mol. Wt. 883.1

Quetiapine Fumarate is 2-[2-(4-dibenzo[*b,f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol fumarate

Quetiapine Fumarate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{42}H_{50}N_6O_4S_2,C_4H_4O_4$ calculated on the dried basis.

Dose. 300 to 450 mg per day.

Category. Antipsychotic.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *quetiapine fumarate RS*. If the spectra obtained show differences, dissolve the substance under examination and the reference substance in *methanol* with the aid of ultrasound. if necessary. Evaporate the solvent under nitrogen at 50° for 2 hours and determine on the residues.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Fumaric acid. 12.6 to 13.6 per cent.

Weigh accurately about 0.35 g, dissolve in 80 ml of *dimethylformamide*. Titrate with *0.1 M tetra butyl ammonium hydroxide,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetra butyl ammonium hydroxide* is equivalent to 0.0058 g of fumaric acid.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.001 per cent w/v solution of *quetiapine fumarate RS* in the mobile phase.

Reference solution (b). A 0.05 per cent w/v solution of *fumaric acid* in the mobile phase.

Reference solution (c). Dissolve 1 mg of *quetiapine fumarate RS* and 1.5 mg of *2-(4-Dibenzo[b,f][1,4] thiazepine-11-yl-Piperazin-1-yl)-ethanol RS* (*quetiapine impurity A RS*) in 100 ml of the mobile phase. Dilute 5 ml of the solution to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as C8-Novapak),
- mobile phase: a mixture of 500 volumes of *methanol*, 400 volumes of *water*, 100 volumes of *acetonitrile* and 0.4 volume of *triethylamine,* adjusted to pH 7.0 with *orthophosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks corresponding to quetiapine impurity A and quetiapine in the chromatogram obtained with the reference solution (c) is not less than 1.5.

Inject the test solution, reference solution (a) and (b). Run the chromatograms for three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (a) (1.0 per cent). Ignore the peak due to fumaric acid.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *quetiapine fumarate RS* in the mobile phase.

Use the chromatographic system described in the test for Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $(C_{21}H_{25}N_3O_2S)_2,C_4H_4O_4$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Quetiapine Tablets

Quetiapine Fumarate Tablets

Quetiapine Tablets contain Quetiapine Fumarate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quetiapine, $C_{21}H_{25}N_3O_2S$.

Dose. 25 mg; 50 mg; 100 mg.

Identification

In the Assay, the principal peak in the chromatogram of the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 45 minutes.

Test solution. Withdraw about 10 ml of the medium and centrifuge. Dilute the supernatant liquid suitably with *0.1 M hydrochloric acid* to obtain a solution containing about 10 µg per ml, of quetiapine.

Reference solution. Dissolve about 58 mg of *quetiapine fumarate RS* in 15 ml of the dissolution medium and dilute to 100.0 ml with the medium. Dilute 2.0 ml of the solution to 100.0 ml with the same medium.

Measure the absorbance of the reference solution and test solution at 248 nm (2.4.7) against *0.1 M hydrochloric acid* as the blank. Calculate the content of quetiapine in the medium from the absorbance obtained from a solution of known concentration of *quetiapine fumarate RS* in the same medium.

D. Not less than 70 per cent of the stated amount of quetiapine.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 250 mg of quetiapine, disperse in 100.0 ml of the mobile phase, with the aid of ultrasound and dilute to 250.0 ml with the mobile phase. Dilute 5 ml of the solution to 50 ml with the mobile phase.

Reference solution. Weigh accurately about 58 mg of *quetiapine fumarate RS*, add 30 ml of the mobile phase and dissolve with aid of ultrasound. Cool and dilute to 50.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *water* and 60 volumes of *methanol.* Add 0.4 ml of *triethylamine* and adjust pH to 6.8 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 289 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

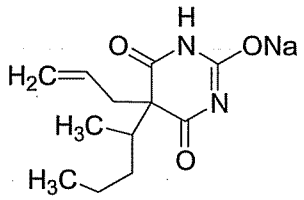
Calculate the content of $C_{21}H_{25}N_3O_2S$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of quetiapine.

Quinalbarbitone Sodium

Quinalbarbital Sodium; Secobarbital Sodium; Secobarbitone Sodium; Soluble Quinalbarbitone



$C_{12}H_{17}N_2NaO_3$ Mol.Wt.260.3

Quinalbarbitone Sodium is sodium (*RS*)-5-allyl-5-(1-methylbutyl)barbiturate.

Quinalbarbitone Sodium contains not less than 98.5 per cent and not more than 102.0 per cent of $C_{12}H_{17}N_2NaO_3$, calculated on the dried basis.

Category. Hypnotic.

Dose. 100 to 200 mg.

Description. A white powder; odourless; hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. To 10 ml of a 10.0 per cent w/v solution in *ethanol (95 per cent)* add 120 ml of *water* and 5 ml of *2 M acetic acid*, stir vigorously, add 200 ml of *water* and boil until the precipitate dissolves and no oily particles remain on the surface of the liquid. Allow to cool until a haziness begins to appear in the solution, induce crystallisation, if necessary and allow the solution to stand for 12 hours. Wash the crystals with three quantities, each of 10 ml, of *water* and dry the residue at 80°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *quinalbarbitone RS* treated in the same manner.

B. To 0.5 g, add 5 ml of *sodium carbonate solution* and 10 ml of *nitrobenzyl chloride solution*. Heat for 30 minutes on a water-bath under reflux and allow to stand for 1 hour. Filter, wash the precipitate successively with 10 ml of *dilute sodium hydroxide solution* and 50 ml of *water*; recrystallise from a mixture of equal volumes of *chloroform* and *ethanol (95 per cent)* and dry at 105°. The crystals melt at about 156° (2.4.21)

C. Complies with the test for identification of barbiturates (2.3.2).

D. Gives the reaction of non-nitrogen substituted barbiturates (2.3.1).

E. Ignite 1 g; the residue gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A freshly prepared 10.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). Not more than 11.0, determined on a 10.0 per cent w/v solution.

Heavy metals (2.3.13). 0.67 g dissolved in a mixture of 5 ml of *1 M sodium hydroxide* and 20 ml of *water* complies with the limit test for heavy metals, Method C (30 ppm).

Related substances. Complies with the test for related substances in barbiturates (2.3.4).

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 10 ml of *ethanol*, add 10 ml of *silver nitrate-pyridine reagent* and titrate with *0.1 M ethanolic sodium hydroxide* using 0.5 ml of *thymolphthalein solution* as indicator, until a pure blue colour is obtained. Carry out a blank titration.

1 ml of *0.1 M ethanolic sodium hydroxide* is equivalent to 0.02603 g of $C_{12}H_{17}N_2NaO_3$.

Storage. Store protected from moisture.

Quinalbarbitone Tablets

Quinalbarbital Sodium Tablets; Quinalbarbitone Sodium Tablets; Secobarbital Sodium Tablets; Secobarbitone Sodium Tablets

Quinalbarbitone Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of quinalbarbitone sodium, $C_{12}H_{17}N_2NaO_3$. The tablets are coated.

Usual strength. 100 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 0.5 g of Quinalbarbitone Sodium with 10 ml of *water* and filter. To 10 ml of the filtrate add 5 ml of *sodium carbonate solution* and 10 ml of *nitrobenzyl chloride solution*. Heat for 30 minutes on a water-bath under reflux and allow to stand for 1 hour. Filter, wash the precipitate successively with 10 ml of *dilute sodium hydroxide solution* and 50 ml of *water*; recrystallise from a mixture of equal volumes of *chloroform and ethanol (95 per cent)* and dry at 105°. The crystals melt at about 156° (2.4.21)

B. Triturate a quantity of the powdered tablets containing 0.5 g of Quinalbarbitone Sodium with 10 ml of *water*, filter, acidify the filtrate with *acetic acid*; oily drops are formed which may eventually crystallise.

C. The powdered tablets give the reactions of sodium salts (2.3.1).

Tests

Other tests. Comply with the tests stated under Tablets.

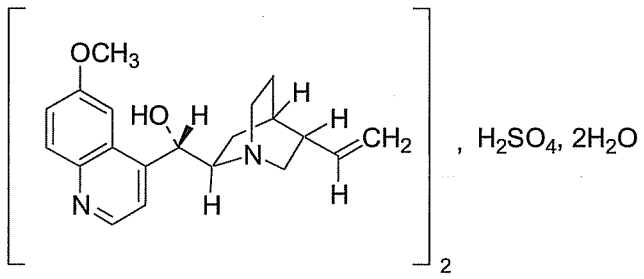
Assay. Weigh and digest 20 tablets with 50 ml of *water* until completely disintegrated and not more than a small residue remains. Add 5 ml of *1 M sodium hydroxide*, filter and wash the residue with sufficient *water* to produce 100.0 ml. Extract a volume of the solution containing 0.5 g of Quinalbarbitone Sodium with two quantities, each of 10 ml, of *ether*, washing each ether extract with the same 3 ml of *water*. Add the water to the aqueous liquid, acidify with *hydrochloric acid* and extract with successive quantities, each of 15 ml, of *ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 2 ml, of *water* and extract the combined washings with 10 ml of *ether*. Add the ether to the main ether layer, filter and wash the filter with *ether*. Evaporate the solvent and dry the residue to constant weight at 50°.

1 g of residue is equivalent to 1.092 g of $C_{12}H_{17}N_2NaO_3$.

Storage. Store protected from moisture.

Quinidine Sulphate

Quinidine Bisulphate



$(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$ Mol. Wt. 783.0

Quinidine Sulphate is (8*R*,9*S*)-6′ -methoxycinchonan-9-ol sulphate dihydrate. The alkaloid is obtained from the bark of various species of *Cinchona* and from *Remijia pedunculata* Fluckiger (Fam. *Rubiaceae*) or prepared from quinine.

Quinidine Sulphate contains not less than 99.0 per cent and not more than 101.5 per cent of alkaloid monosulphates, calculated as $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$ on the dried basis.

Category. Antiarrhythmic.

Dose. In the treatment of cardiac arrhythmias, 200 mg daily in three or four divided doses. In the treatment of atrial fibrillation, 200 to 400 mg every 2 to 4 hours, upto 3 g daily.

Description. A white or almost white, crystalline powder or needle-like crystals; odourless.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *toluene*, 36 volumes of *ether* and 15 volumes of *diethylamine*.

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 1 per cent w/v solution of *quinidine sulphate RS* in *methanol*.

Reference solution (b). A 1 per cent w/v solution of each of *quinidine sulphate RS* and *quinine sulphate RS* in *methanol*.

Apply to the plate 4 µl of each solution. After development, dry the plate in air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow to cool and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. To 5 ml of a 0.1 per cent w/v solution add 0.2 ml of *bromine solution* and 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

C. To a 0.5 per cent w/v solution add an equal volume of *dilute sulphuric acid*; a strong blue fluorescence is produced.

D. To 5 ml of a 1 per cent w/v solution add 1 ml of *silver nitrate solution* and stir with a glass rod; after a short interval, a white precipitate soluble in *nitric acid* is produced (distinction from many other alkaloids).

E. A 1 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *0.1 M hydrochloric acid* is clear (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

pH (2.4.24). 6.0 to 6.8, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +275° to +290°, determined in a 2.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Dihydroquinidine sulphate. Not more than 15.0 per cent, calculated on the dried basis and determined by the following method. Dissolve 0.2 g in 20 ml of *water*, add 0.5 g of *potassium bromide* and 15 ml of *2 M hydrochloric acid*. Titrate slowly with *0.0167 M potassium bromate* using *methyl red solution* as indicator until a yellow colour is obtained. Add a solution of 0.5 g of *potassium iodide* in 200 ml of *water* and stopper the flask immediately. Allow to stand in the dark for 5 minutes and titrate with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.01867 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

Calculate the content of dihydroquinidine sulphate by subtracting the result from the assay result.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of the mobile phase. Heat gently, if necessary to dissolve the powder as completely as possible, cool, dilute to 10 ml with the mobile phase and mix.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinidine is not greater than 15 per cent, the content of any related substance eluting before quinidine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 3.0 per cent to 5.0 per cent, determined on 1.0 g by drying in an oven at 130°.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 10 ml of *chloroform* and 20 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02490 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

Storage. Store protected from light.

Quinidine Tablets

Quinidine Sulphate Tablets

Quinidine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of quinidine sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Usual strength. 200 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene*, 20 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)* and filter.

Reference solution. 1.0 per cent w/v solution of *quinidine sulphate RS* in a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *0.05 M ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 0.1 g of Quinidine Sulphate with 20 ml of *water* and filter. The filtrate (solution A) is dextro-rotatory.

C. To 1 ml of solution A add 4 ml of *water*, 2 or 3 drops of *bromine solution* and 1 ml of *dilute ammonia solution*; an emerald green colour is produced.

D. Solution A gives the reactions of sulphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 248 nm (2.4.7). Calculate the content of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$ in the medium from the absorbance obtained from a solution of known concentration of *quinidine sulphate RS.*

D. Not less than 70 per cent of the stated amount of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Mix a quantity of the powdered tablets containing 50 mg of Quinidine Sulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of filtrate.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS.*

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water,* adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinidine is not greater than 15 per cent, the content of any related substance eluting before quinidine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Other tests. Comply with the tests stated under Tablets.

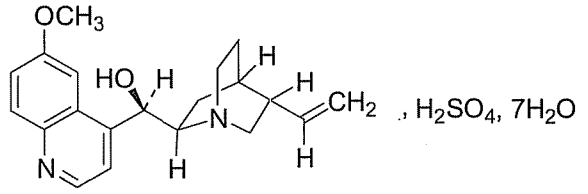
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Quinidine Sulphate, dissolve as completely as possible in 40 ml of *acetic anhydride* with the aid of heat and cool. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02610 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Storage. Store protected from light.

Quinine Bisulphate

Quinine Acid Sulphate



$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$ Mol. Wt. 548.6

Quinine Bisulphate is (8*S*,9*R*)-6′ -methoxycinchonan-9-ol hydrogen sulphate heptahydrate. The alkaloid is obtained from the bark of various species of *Cinchona*.

Quinine Bisulphate contains not less than 98.5 per cent and not more than 101.5 per cent of alkaloid hydrogen sulphates, calculated as $C_{20}H_{24}N_2O_2,H_2SO_4$ on the anhydrous basis.

Category. Antimalarial.

Dose. Suppressive, 300 to 600 mg daily; therapeutic, 1.2 to 2 g daily, in divided doses.

Description. Colourless or faintly yellow crystals or a white or faintly yellow, crystalline powder; efflorescent in dry air.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *toluene*, 36 volumes of *ether* and 15 volumes of *diethylamine*.

Test solution. Dissolve 1 g of the substance under examination in 100 ml *methanol*.

Reference solution (a). A 1 per cent w/v solution of *quinine sulphate RS* in *methanol*.

Reference solution (b). A 1 per cent w/v solution of each of *quinidine sulphate RS* and *quinine sulphate RS* in *methanol*.

Apply to the plate 4 µl of each solution. After development, dry the plate in air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow to cool and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. A 5 per cent w/v solution has a blue fluorescence.

C. A 5 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 2.8 to 3.4, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). –208° to –216°, determined at 20° in a 3.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of the mobile phase. Heat gently, if necessary to dissolve the powder as completely as possible, cool, dilute to 10 ml with the mobile phase and mix.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak

due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Titratable cation. 75.3 to 79.6 per cent, calculated on the anhydrous basis, determined by the following method. Titrate the combined aqueous solutions reserved in the Assay with *0.1 M hydrochloric acid* using 0.1 ml of *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01632 g of $[C_{20}H_{26}N_2O_2]^{2+}$.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 19.0 to 25.0 per cent, determined on 0.2 g.

Assay. Weigh accurately about 0.45 g, dissolve in 15 ml of *water*, add 25 ml of *0.1 M sodium hydroxide* and extract with three quantities, each of 25 ml, of *chloroform*. Wash each chloroform extract with the same 20 ml of *water*. Combine the aqueous solutions and reserve for the test for Titratable cation. Dry the chloroform extracts with *anhydrous sodium sulphate*, evaporate to dryness at a pressure of 2 kPa and dissolve the residue in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02113 g of $C_{20}H_{24}N_2O_2,H_2SO_4$.

Storage. Store protected from light.

Quinine Bisulphate Tablets

Quinine Acid Sulphate Tablets

Quinine Bisulphate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of quinine bisulphate, $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$. The tablets are coated.

Usual strength. 300 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene*, 20 volumes of *acetone* and 10 volumes of *diethylamine*.

Test solution. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Bisulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)* and filter.

Reference solution. A 1.0 per cent w/v solution of *quinine sulphate* in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *0.05 M ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Bisulphate with 20 ml of *water* and filter (solution A). To 5 ml of solution A add 0.2 ml of *bromine solution* and 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

C. Solution A is *levo-rotatory*.

D. Solution A gives the reactions of sulphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 348 nm (2.4.7). Calculate the content of

$C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$ in the medium taking 99 as the specific absorbance at 348 nm.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Bisulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of the filtrate.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Other tests. Comply with the tests stated under Tablets.

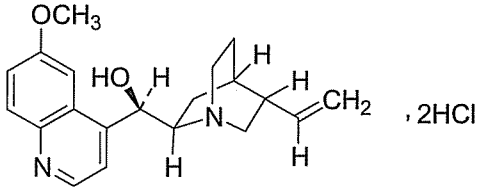
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.6 g of Quinine Bisulphate, dissolve as completely as possible in 40 ml of *acetic anhydride* with the aid of heat and cool. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05486 g of $C_{20}H_{24}N_2O_2,H_2SO_4,7H_2O$.

Storage. Store protected from light.

Quinine Dihydrochloride

Quinine Acid Hydrochloride



$C_{20}H_{24}N_2O_2,2HCl$ Mol. Wt. 397.3

Quinine Dihydrochloride is the (8*S*,9*R*)-6′–methoxycinchonan-9-ol dihydrochloride. The alkaloid is obtained from the bark of various species of *Cinchona*.

Quinine Dihydrochloride contains not less than 99.0 per cent and not more than 101.5 per cent of alkaloid dihydrochlorides, calculated as $C_{20}H_{24}N_2O_2,2HCl$ on the dried basis.

Category. Antimalarial.

Dose. Suppressive, 300 to 600 mg daily; therapeutic, 1.2 to 2 g daily, in divided doses. By slow intravenous injection, 300 to 600 mg.

Description. A white or almost white powder; odourless.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *toluene*, 36 volumes of *ether* and 15 volumes of *diethylamine*.

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 1 per cent w/v solution of *quinine sulphate RS* in *methanol*.

Reference solution (b). A 1 per cent w/v solution of each of *quinidine sulphate RS* and *quinine sulphate RS* in *methanol*.

Apply to the plate 4 µl of each solution. After development, dry the plate in air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow to cool and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. To a 0.5 per cent w/v solution add an equal volume of *dilute sulphuric acid*; a strong blue fluorescence is produced.

C. A 1 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 2.0 to 3.0, determined in a 3.0 per cent w/v solution.

Specific optical rotation (2.4.22). –223° to –229°, determined in a 3.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Barium. To 15 ml of a 2 per cent w/v solution add 1 ml of *dilute sulphuric acid*; the solution remains clear for at least 15 minutes.

Dihydroquinine dihydrochloride. Not more than 10.0 per cent, calculated on the dried basis and determined by the following method. Dissolve 0.2 g in 20 ml of *water*, add 0.5 g of *potassium bromide* and 15 ml of *2 M hydrochloric acid*. Titrate slowly with *0.0167 M potassium bromate* using *methyl red solution* as indicator until a yellow colour is obtained. Add a solution of 0.5 g of *potassium iodide* in 200 ml of *water* and stopper the flask immediately. Allow to stand in the dark for 5 minutes and titrate with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator. Repeat the operation without the substance under examination.

1 ml of *0.0167 M potassium bromate* is equivalent to 0.01987 g of $C_{20}H_{24}N_2O_2,2HCl$. Calculate the content of dihydroquinine dihydrochloride by subtracting the result from the assay result.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of the mobile phase. Heat gently, if necessary to dissolve the powder as completely as possible, cool, dilute to 10 ml with the mobile phase and mix.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0

(the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Titratable cation. 79.7 to 84.2 per cent, calculated on the dried basis, determined by the following method. Weigh accurately about 0.4 g, dissolve in 10 ml of *water*, add 40 ml of *methanol* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01632 g of $[C_{20}H_{26}N_2O_2]^{2+}$.

Sulphates (2.3.17). 0.125 g complies with the limit test for sulphates (0.12 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *anhydrous glacial acetic acid* and add 20 ml of *acetic anhydride* and 10 ml of *mercuric acetate* solution. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent of 0.01987 g of $C_{20}H_{24}N_2O_2,2HCl$.

Storage. Store protected from light.

Quinine Dihydrochloride Injection

Quinine Acid Hydrochloride Injection

Quinine Dihydrochloride Injection is a sterile solution of Quinine Dihydrochloride in Water for Injections.

Quinine Dihydrochloride Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of quinine dihydrochloride, $C_{20}H_{24}N_2O_2,2HCl$.

Usual strength. 300 mg per ml.

Description. A clear, almost colourless to light yellow solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *toluene*, 20 volumes of *acetone* and 10 volumes of *diethylamine*.

Solvent mixture. 2 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)*.

Test solution. Extract a volume of the injection containing 0.1 g of Quinine Dihydrochloride Bisulphate with 10 ml of the solvent mixture and filter.

Reference solution (a). A 1 per cent w/v solution of *quinine sulphate RS* in the solvent mixture.

Reference solution (b). A solution of 1 per cent w/v of each of *quinidine sulphate RS* and *quinine sulphate RS* in the solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *0.05 M ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows two clearly separated spots.

B. Dilute 1 ml to 20 ml with *water*, add *0.1 M sodium hydroxide* dropwise until the solution becomed turbid. Add 1 or 2 drops

of *0.05 M sulphuric acid* to obtain a clear solution. To this solution add an equal volume of *dilute sulphuric acid*; a strong blue fluorescence is produced.

C. It gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24).1.5 to 3.0.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Dilute, if necessary a suitable volume of the injection with the mobile phase to produce a solution containing 0.2 per cent w/v of Quinine Dihydrochloride.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution factor between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

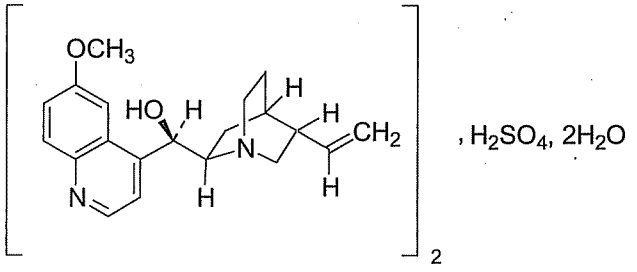
Assay. To an accurately measured volume containing about 0.5 g of Quinine Dihydrochloride add 20 ml of *water* and 5 ml of *sodium hydroxide solution*. Extract with successive quantities, each of 10 ml, of *chloroform* until complete extraction of the alkaloid is effected, washing each extract with the same two quantities, each of 5 ml, of *water*. Remove the chloroform from the combined extracts, dissolve the residue in 50 ml of *anhydrous glacial acetic acid* and add 20 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01987 g of $C_{20}H_{24}N_2O_2,2HCl$.

Storage. Store protected from light.

Labelling. The label states that the solution must be diluted to a strength not exceeding 30 mg per ml before administration and that care should be taken to ensure slow intravenous injection.

Quinine Sulphate



$(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$ Mol. Wt.783.0

Quinine Sulphate is (8*S*,9*R*)-6′ -methoxycinchonan-9-ol sulphate dihydrate. The alkaloid is obtained from the bark of various species of *Cinchona*.

Quinine Sulphate contains not less than 99.0 per cent and not more than 101.0 per cent of alkaloid monosulphates, calculated as $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$ on the dried basis.

Category. Antimalarial.

Dose. Suppressive, 300 to 600 mg daily. therapeutic, 1.2 to 2 g daily, in divided doses.

Description. White or almost white, needle-like crystals or a crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 40 volumes of *toluene*, 24 volumes of *ether* and 10 volumes of *diethylamine*.

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *methanol*.

Reference solution. A 1 per cent w/v solution of *quinine sulphate RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air for 15 minutes and repeat the development. Dry the plate at 105° for 30 minutes, allow to cool and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. To 5 ml of a 0.1 per cent w/v solution add 0.2 ml of *bromine solution* and 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

C. To a 0.5 per cent w/v solution add an equal volume of *dilute sulphuric acid*; a strong blue fluorescence is produced.

D. To 5 ml of a 1 per cent w/v solution add 1 ml of *silver nitrate solution* and stir with a glass rod; after a short interval, a white precipitate soluble in *nitric acid* is produced (distinction from many other alkaloids).

E. A 1 per cent w/v solution gives the reactions of sulphates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *0.1 M hydrochloric acid* is clear (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

pH (2.4.24). 5.7 to 6.6, determined in a 1.0 per cent w/v suspension in *water*.

Specific optical rotation (2.4.22). –237° to –245°, determined in a 2.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 5 ml of the mobile phase. Heat gently, if necessary to dissolve the powder as completely as possible, cool, dilute to 10 ml with the mobile phase and mix.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS*.

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water*, adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). 3.0 to 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 10 ml of *chloroform* and add 20 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02490 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4$.

Storage. Store protected from light.

Quinine Tablets

Quinine Sulphate Tablets

Quinine Sulphate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$. The tablets are coated.

Usual strengths. 100 mg; 300 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of toluene, *20 volumes of* acetone *and 10 volumes of* diethylamine.

Test solution. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Sulphate with 10 ml of a mixture of 2 volumes of *chloroform* and 1 volume of *ethanol (95 per cent)* and filter.

Reference solution. A 1 per cent w/v solution of *quinine sulphate* in the same solvent mixture.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with *0.05 M ethanolic sulphuric acid* and then with *dilute potassium iodobismuthate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Extract a quantity of the powdered tablets containing 0.1 g of Quinine Sulphate with 20 ml of *water* and filter (solution A). To 5 ml of solution A add 0.2 ml of *bromine solution* and 1 ml of *dilute ammonia solution*; an emerald-green colour is produced.

C. Solution A is *levo-rotatory*.

D. Solution A gives the reactions of sulphates (2.3.1).

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 348 nm (2.4.7). Calculate the content of $C_{20}H_{24}N_2O_2$, H_2SO_4, $2H_2O$ in the medium from a solution of known concentration of *quinine sulphate RS*.

D. Not less than 70 per cent of the stated amount of $C_{20}H_{24}N_2O_2$, H_2SO_4, $2H_2O$.

Other cinchona alkaloids. Determine by liquid chromatography (2.4.14).

Test solution. Remove any coating from the tablets and mix a quantity of the powdered tablet cores containing 50 mg of Quinine Sulphate with 20 ml of the mobile phase. Heat gently to dissolve the powder as completely as possible, cool, dilute to 25 ml with the mobile phase and filter, discarding the first few ml of the filtrate.

Reference solution (a). Dissolve 20 mg of *quinine sulphate RS*, with gentle heating if necessary, in 5 ml of the mobile phase and dilute to 10 ml with the mobile phase.

Reference solution (b). Prepare in the same manner as reference solution (a) but using *quinidine sulphate RS* in place of *quinine sulphate RS.*

Reference solution (c). Mix equal volumes of reference solutions (a) and (b).

Reference solution (d). Dilute 1 volume of reference solution (a) to 10 volumes with the mobile phase and dilute 1 volume of the resulting solution to 50 volumes with the mobile phase.

Reference solution (e). A solution containing 0.1 per cent w/v of *thiourea* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS 5 µm),
- mobile phase: a solution prepared by dissolving 6.8 g of *potassium dihydrogen orthophosphate* and 3.0 g of *hexylamine* in 700 ml of *water,* adjusting the pH to 2.8 with *1 M orthophosphoric acid*, adding 60 ml of *acetonitrile* and diluting to 1000 ml with *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 250 nm for reference solution (e) and 316 nm for the other solutions,
- injection volume. 10 µl.

Inject separately reference solutions (b) and (e). If necessary, adjust the concentration of acetonitrile in the mobile phase so that in the chromatogram obtained with reference solution (b) the capacity factor of the peak due to quinidine is 3.5 to 4.5, V_0 (the distance along the baseline between the point of injection and the perpendicular dropped from the maximum of the peak of an unretained component) being calculated from the peak due to thiourea in the chromatogram obtained with reference solution (e).

Inject reference solutions (a), (b), (c) and (d). The chromatogram obtained with reference solution (a) shows a principal peak due to quinine and a peak due to dihydroquinine with a retention time relative to quinine of about 1.4. The chromatogram obtained with reference solution (b) shows a principal peak due to quinidine and a peak due to dihydroquinidine, with a retention time relative to quinidine of about 1.2. The chromatogram obtained with reference solution (c) shows four peaks due to quinine, dihydroquinine, quinidine and dihydroquinidine which are identified by comparison of their retention times with those of the corresponding peaks in the chromatograms obtained with reference solutions (a) and (b).

The test is not valid unless (a) in the chromatogram obtained with reference solution (c) the resolution between the peaks due to quinine and quinidine is at least 1.5 and the resolution between the peaks due to dihydroquinidine and quinine is at least 1.0 and (b) the signal-to-noise ratio of the principal peak in the chromatogram obtained with reference solution (d) is at least 5.

Inject the test solution and allow the chromatography to proceed for 2.5 times the retention time of the principal peak. Calculate the percentage content of related substances by normalisation, ignoring any peaks the areas of which are less than that of the peak in the chromatogram obtained with reference solution (d) (0.2 per cent). The content of dihydroquinine is not greater than 10 per cent, the content of any related substance eluting before quinine is not greater than 5 per cent and the content of any other related substance is not greater than 2.5 per cent.

Other tests. Comply with the tests stated under Tablets.

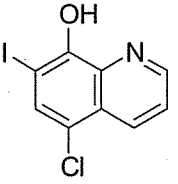
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.4 g of Quinine Sulphate, dissolve as completely as possible in 40 ml of *acetic anhydride* with the aid of heat and cool. Filter, if necessary through Whatman No.1 filter paper and rinse with an additional 40 ml of *acetic anhydride* in small volumes. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02610 g of $(C_{20}H_{24}N_2O_2)_2,H_2SO_4,2H_2O$.

Storage. Store protected from light.

Quiniodochlor

Clioquinol; Iodochlorhydroxyquinoline; Iodochlorhydroxyquin



C_9H_5ClINO Mol. Wt. 305.5

Quiniodochlor is 5-chloro-7-iodoquinolin-8-ol.

Quiniodochlor contains not less than 97.0 per cent and not more than 103.0 per cent of C_9H_5ClINO, calculated on the dried basis.

Category. Antiamoebic; topical and intestinal antiseptic.

Dose. 750 mg to 1.5 g daily, in divided doses.

Description. A yellowish white to brownish yellow powder; odour, faint and characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *quiniodochlor RS* or with the reference spectrum of quiniodochlor.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *3 M hydrochloric acid* shows an absorption maximum at about 267 nm.

C. Burn 20 mg by the oxygen-flask method (2.3.34), using 5 ml of *2 M sodium hydroxide* as the absorbing liquid and dilute to 25 ml with *water*. To 5 ml add 1 ml of *silver nitrate solution*; a yellow precipitate is produced. Add 5 ml of *5 M ammonia*, shake, filter and acidify the filtrate with *nitric acid*; a white precipitate is produced.

Tests

Acidity or alkalinity. Shake 0.5 g with 10 ml of *water* previously neutralised to *phenolphthalein solution*. The solution is colourless and not more than 0.05 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution to pink.

Free iodine. Shake 1.0 g with a solution of 1 g of *potassium iodide* in 20 ml of *water* for 30 seconds, allow to stand for 5 minutes and filter. To 10 ml of the filtrate add 1 ml of *1 M sulphuric acid* and 2 ml of *chloroform* and shake. Any colour in the chloroform layer is discharged on the addition of 0.1 ml of *0.005 M sodium thiosulphate*.

Halide ions. Shake 0.5 g with 25 ml of *water* for 1 minute and filter. To the filtrate add 0.5 ml of *2 M nitric acid* and 0.5 ml of *0.1 M silver nitrate* and allow to stand for 5 minutes. Any opalescence produced is not more intense than that obtained by adding 0.5 ml of *0.1 M silver nitrate* to 25 ml of *water* containing 0.5 ml of *2 M nitric acid* and 0.2 ml of *0.01 M hydrochloric acid* and allowing to stand for 5 minutes.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Add 0.5 ml of N,O-*bis (trimethylsilyl)acetamide* to 0.5 ml of a solution in *pyridine* containing 0.4 per cent w/v of each of *5-chloro-8- hydroxyquinoline, 5,7-dichloro-8-hydroxy- quinoline* and *5-chloro-7-iodo-8-hydroxyquinoline* and 0.04 per cent w/v of the substance under examination, mix, allow to stand for 15 minutes and add 5 ml of a 0.05 per cent w/v solution of *dibutylphthalate* (internal standard) in *hexane*.

Reference solution (a). Add 0.5 ml of N,O-*bis (trimethylsilyl)acetamide* to a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine*, mix, allow to stand for 15 minutes and add 5 ml of *hexane*.

Reference solution (b). Treat a mixture of 0.1 g of the substance under examination and 0.5 ml of *pyridine* as described for the test solution.

Chromatographic system

- a glass column 1.5 m x 4 mm, packed with silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of methyl silicone gum,
- temperature:
  column.190°,
  inlet port and detector. 240°,
- flame ionisation detector,
- nitrogen as carrier gas.

In the chromatogram obtained with the test solution the peaks following the solvent peak, in order of emergence, are due to (a) 5-chloro-8-hydroxyquinoline, (b) 5,7-dichloro-8-hydroxy-quinoline, (c) the internal standard, (d) quiniodochlor and (e) 5,7-diiodo-8-hydroxyquinoline.

In the chromatogram obtained with reference solution (b) calculate the content of 5-chloro-8-hydroxy-quinoline, 5,7-dichloro-8-hydroxyquinoline and 5,7-diiodo-8-hydroxy-quinoline by reference to the corresponding peaks in the chromatogram obtained with the test solution.

The total content of the named impurities does not exceed 3.0 per cent w/w, the content of any other impurity does not exceed 0.2 per cent w/w and the sum of the contents is not more than 4.0 per cent w/w.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh accurately about 0.3 g and dissolve in 25 ml of *anhydrous pyridine*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03055 g of C_9H_5ClINO.

Storage. Store protected from light.

Quiniodochlor Cream

Clioquinol Cream; Iodochlorhydroxyquinoline Cream; Iodochlorhydroxyquin Cream

Quiniodochlor Cream contains Quiniodochlor in a suitable base.

Quiniodochlor Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quiniodochlor, C_9H_5ClINO.

Usual strength. 4 per cent w/v.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution. The test solution and the reference solution prepared by using 1.0 ml of pyridine instead of the internal standard solution.

B. Weigh accurately a quantity containing about 25 mg of quiniodochlor, in a 100 ml volumetric flask, add 75 ml of 25 per cent v/v solution of *hydrochloric acid*, heat on a steam bath to melt the cream, shake vigorously to extract the quiniodochlor. Cool under running water, and dilute to the volume with the same solvent. Dilute 3.0 ml of the filtrate to 100.0 ml with the same solvent.

When examined in the range 200 nm to 400 nm (2.4.7), the solution shows an absorption maximum at about 267 nm.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.2 per cent w/v solution of *pyrene* in *pyridine*.

Solvent mixture. 80 volumes of *pyridine* and 20 volumes of *n-hexane* .

Test solution. Weigh accurately a quantity containing about 150 mg of quiniodochlor, in a 60-ml separator. Place the separator on its side in a vacuum oven at a pressure of about 10 mm of mercury at 45° for 4 hours. Remove the separator from the oven, allow to cool, add 15 ml of the solvent mixture, insert a polytef stopper, and mix. Transfer the mixture to a 50-ml volumetric flask, and rinse the separator with two 15 ml portions of the solvent mixture, shaking each time for 30 seconds. Transfer both rinsings to the volumetric flask, dilute with the solvent mixture to volume, and mix. Transfer 1.0 ml to a screw-capped glass vial fitted with a septum, add 1.0 ml of *bis(trimethylsilyl)acetamide* and 1.0 ml of internal standard solution, attach the cap, and mix. Heat in a water-bath at 50° for 15 minutes, and cool.

Reference solution. A 0.3 per cent w/v solution of *quiniodochlor RS* in the solvent mixture. Transfer 1.0 ml to a screw-capped glass vial fitted with a septum, add 1.0 ml of *bis(trimethylsilyl)acetamide* and 1.0 ml of internal standard solution, attach the cap, and mix. Heat in a water-bath at 50° for 15 minutes, and cool.

Chromatographic system

- a glass column 1.83 m x 2 mm, packed with 3 per cent liquid phase G3 on 80 to 100-mesh support S1AB,
- temperature:
  column. 165°,
  inlet port 170° and detector 250°,
- flame ionization detector,
- flow rate. 30 ml per minute of helium as the carrier gas.

Inject the reference solution. The test is not valid unless the resolution between the quiniodochlor and the internal standard peaks is not less than 3.0. The relative retention time with reference to pyrene for quiniodochlor is about 0.6.

Inject the test solution and the reference solution.

Calculate the content of C_9H_5ClINO in the Cream.

Storage. Store protected from light and moisture.

Quiniodochlor Ointment

Clioquinol Ointment; Iodochlorhydroxyquinoline Ointment; Iodochlorhydroxyquin Ointment

Quiniodochlor Ointment contains Quiniodochlor in a suitable base.

Quiniodochlor Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quiniodochlor, C_9H_5ClINO.

Usual strength. 4 per cent w/v.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution. The test solution and the reference solution prepared by using 1.0 ml of pyridine instead of the internal standard solution.

B. Weigh accurately a quantity containing about 25 mg of quiniodochlor in a 100-ml volumetric flask, add 75 ml of 25 per cent v/v solution of *hydrochloric acid*, heat on steam bath to melt the ointment, shake vigorously to extract the quiniodochlor. Cool under running water, dilute to volume with the same solvent. Filter and dilute 3.0 ml of this solution to 100.0 ml with the same solvent.

When examined in the range 200 nm to 400 nm (2.4.7), the solution shows an absorption maximum at about 267 nm.

Tests

Other tests. Complies with the tests stated under Ointments.

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.2 per cent w/v solution of *pyrene* in *pyridine*.

Solvent mixture. 80 volumes of *pyridine* and 20 volumes of *n-hexane* .

Test solution. Transfer an accurately weighed quantity of the ointment, containing about 150 mg of Quiniodochlor, to a suitable conical flask, add 75 ml of *n-hexane*, and mix. Add 15 ml of *dimethylformamide*, and mix for 1 minute. Allow the layers to separate, and transfer the lower layer to a 50-ml volumetric flask, repeat the extraction with separate 15 ml and 10 ml portions of *dimethylformamide*, and transfer the lower layers to the 50-ml volumetric flask. Dilute with *dimethylformamide* to volume, and mix. Transfer 1.0 ml of this solution to a screw-capped glass vial fitted with a septum, and evaporate at about 60° under a stream of nitrogen to dryness. Add 1.0 ml of a mixture of internal standard solution to the residue, add 1.0 ml of *bis(trimethylsilyl)acetamide* and 1.0 ml of internal standard solution, attach the cap, and mix. Heat in a water-bath at 50° for 15 minutes and cool.

Reference solution. A 0.3 per cent w/v solution of *quiniodochlor RS* in the solvent mixture. Transfer 1.0 ml to a screw-capped glass vial fitted with a septum, add 1.0 ml of *bis(trimethylsilyl)acetamide* and 1.0 ml of internal standard solution, attach the cap, and mix. Heat in a water-bath at 50° for 15 minutes, and cool.

Chromatographic system

- a glass column 1.83 m x 2 mm, packed with 3 per cent liquid phase G3 on 80 to 100-mesh support S1AB,
- temperature:
  column. 165°,
  inlet port 170° and detector 250°,
- flame ionization detector,
- flow rate. 30 ml per minute of helium as the carrier gas.

Inject the reference solution. The test is not valid unless the resolution between the quiniodochlor and the internal standard peaks is not less than 3. The relative retention time with reference to pyrene for quiniodochlor is about 0.6.

Inject the test solution and the reference solution.

Calculate the content of C_9H_5ClINO in the Ointment.

Storage. Store protected from light and moisture.

Quiniodochlor Tablets

Clioquinol Tablets; Iodochlorhydroxyquinoline Tablets; Iodochlorhydroxyquin Tablets

Quiniodochlor Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quiniodochlor, C_9H_5ClINO.

Usual strength. 250 mg.

Identification

Triturate a quantity of the powdered tablets containing about 250 mg of Quiniodochlor with 20 ml of *acetone*, filter and add 20 ml of *water* to the filtrate. Collect the precipitate formed on a filter and dry at 105°. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *quiniodochlor RS* or with the reference spectrum of quiniodochlor.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *3 M hydrochloric acid* shows an absorption maximum at about 267 nm.

Tests

Disintegration (2.5.1). Maximum time, 30 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing 0.125 g of Quiniodochlor, shake with 20 ml of hot *2-methoxyethanol*, decant the hot supernatant liquid through a fine filter. Repeat the extraction with two further quantities, of 20 ml and 10 ml, of *2-methoxyethanol*, combine the filtered extracts and dilute to 50.0 ml with *2-methoxyethanol*. To 5.0 ml of this solution add 1 ml of *water* and sufficient of a mixture of 24 volumes of *2-methoxyethanol* and 6 volumes of *water* to produce 50.0 ml. To 10.0 ml of the solution add 10 ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *ferric chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25.0 ml with *2-methoxyethanol* and measure the absorbance of the resulting solution at the maximum at about 650 nm (2.4.7), using as blank a solution prepared by treating 10 ml of the aqueous *2-methoxyethanol* in the same manner beginning at the words "add 10 ml of *2-methoxyethanol*.....".

Calculate the content of C_9H_5ClINO from the absorbance obtained using 10.0 ml of a solution prepared in the following manner. Dissolve 0.125 g of *quiniodochlor RS* in sufficient *2-methoxyethanol* to produce 50.0 ml, warming to effect solution; add 1 ml of *water* to 5.0 ml of the solution and add sufficient of the mixture of 24 volumes of *2-methoxyethanol* and 6 volumes of *water* to produce 50.0 ml. Using 10.0 ml of this solution repeat the operation beginning at the words "add 10 ml of *2-methoxyethanol*...."

Storage. Store protected from light.

Quiniodochlor and Hydrocortisone Cream

Clioquinol and Hydrocortisone Cream; Iodochlorhydroxyquinoline and Hydrocortisone Cream; Iodochlorhydroxyquin and Hydrocortisone Cream

Quiniodochlor and Hydrocortisone Cream contains Hydrocortisone and Quiniodochlor in a suitable base.

Quiniodochlor and Hydrocortisone Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quiniodochlor, C_9H_5ClINO and not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydrocortisone, $C_{21}H_{30}O_5$.

Usual Strength. Quiniodochlor 4 per cent w/w and Hydrocortisone 1 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel G*.

Mobile phase. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

Test solution. Disperse by warming and shaking, a quantity of the cream containing 2.5 g of Hydrocortisone in 10 ml of *ethanol (95 per cent)*, cool, allow to stand at 0° for 30 minutes, filter and use the filtrate.

Reference solution (a). A 0.25 per cent w/v solution of *hydrocortisone RS* in *ethanol (95 per cent)*.

Reference solution (b). Dissolve 12.5 mg of *hydrocortisone RS* in 5 ml of test solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with *alkaline tetrazolium blue solution.* The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the principal peak in the chromatogram due to hydrocortisone obtained with test solution (b) corresponds to the peak due to hydrocortisone in the chromatogram obtained with reference solution.

C. Fuse a quantity of the cream containing 0.1 g of quiniodochlor with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with *2 M nitric acid.* Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in *5 M ammonia.* Add *5 M ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with *2 M nitric acid*; a white precipitate is produced which darkens on exposure to light.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. *For hydrocortisone* — Determine by liquid chromatography (2.4.14).

Test solution (a). Add 30 ml of *2,2,4-trimethylpentane* to a quantity of the cream containing 10 mg of Hydrocortisone and warm on a water-bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 ml of *methanol (80 per cent)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 ml with the same solvent.

Test solution (b). Prepare in the same manner as test solution (a) but adding 10 ml of a 4 per cent v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract and dilute to 100.0 ml with methanol (80 per cent).

Reference solution. Dissolve 5 mg of *hydrocortisone RS* in 5 ml of a 4 per cent v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 ml with *methanol (80 per cent).*

Chromatographic system
- a stainless steel column 25 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- mobile phase: *methanol (65 per cent)*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume. 10 µl.

Calculate the content of $C_{21}H_{30}O_5$ in the cream.

For quiniodochlor — Weigh accurately a quantity of the cream containing about 25 mg of quiniodochlor, add 80 ml of a hot mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* and heat on a water-bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 ml with a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol*, mix and filter. To 10 ml of the filtrate, add 10 ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25 ml with *2-methoxyethanol* and measure the absorbance of the resulting solution at the maximum at about 650 nm (2.4.7), using in the reference cell a solution prepared by treating 10 ml of the mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* in the same manner beginning at the words "add 10 ml of *2-methoxyethanol*.....".

Repeat the operation beginning at the words 'add 10 ml of *2-methoxyethanol* using 10 ml of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol RS* in sufficient *2-methoxyethanol* to produce 50 ml; warming to effect solution; add 1 ml of *water* to 5 ml of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 ml.

Calculate the content of C_9H_5ClINO in the cream.

Storage. Store protected from light.

Quiniodochlor and Hydrocortisone Ointment

Clioquinol and Hydrocortisone Ointment; Iodochlorhydroxyquinoline and Hydrocortisone Ointment; Iodochlorhydroxyquin and Hydrocortisone Ointment

Quiniodochlor and Hydrocortisone Ointment contains Quiniodochlor and Hydrocortisone in a suitable base.

Quiniodochlor and Hydrocortisone Ointment contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of quiniodochlor, C_9H_5ClINO and not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of hydrocortisone, $C_{21}H_{30}O_5$.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel G.*

Mobile phase. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water*.

Test solution. Disperse by warming and shaking, a quantity of the ointment containing 2.5 g of Hydrocortisone in 10 ml of *ethanol (95 per cent)*, cool, and allow to stand at 0° for 30 minutes, filter and use the filtrate.

Reference solution (a). A 0.25 per cent w/v solution of *hydrocortisone RS* in *ethanol (95 per cent).*

Reference solution (b). Dissolve 12.5 mg of *hydrocortisone RS* in 5 ml of test solution.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with *alkaline tetrazolium blue solution.* The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. In the Assay, the principal peak in the chromatogram due to hydrocortisone obtained with test solution (b) corresponds to the peak due to hydrocortisone in the chromatogram obtained with reference solution.

C. Fuse a quantity of the ointment containing 0.1 g of Quiniodochlor with *anhydrous sodium carbonate*, dissolve the fused mass in *water* and acidify with *2 M nitric acid.* Add *silver nitrate solution*; a pale yellow precipitate is produced which is insoluble in *5 M ammonia.* Add *5 M ammonia* until the solution becomes alkaline, boil gently, filter and acidify the filtrate with *2 M nitric acid*; a white precipitate is produced which darkens on exposure to light.

Tests

Other tests. Complies with the tests stated under Ointments.

Assay. *For hydrocortisone* — Determine by liquid chromatography (2.4.14).

Test solution (a). Add 30 ml of *2,2,4-trimethylpentane* to a quantity of the ointment containing 10 mg of Hydrocortisone and warm on a water-bath until the preparation has melted. Extract the warm mixture with successive quantities of 30, 20 and 20 ml of *methanol (80 per cent)*, combine the aqueous methanolic layers, cool to about 20° and dilute to 100 ml with the same solvent.

Test solution (b). Prepare at the same manner as test solution (a) but adding 10 ml of a 4 per cent v/v solution of *bromobenzene* in *methanol* to the cooled methanolic extract and dilute to 100.0 ml with *methanol (80 per cent).*

Reference solution. Dissolve 5 mg of *hydrocortisone RS* in 5 ml of a 4 per cent v/v solution of *bromobenzene* (internal standard) in *methanol* and dilute to 50 ml with *methanol (80 per cent).*

Chromatographic system
- a stainless steel column 25 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Spherisorb ODS 1),
- mobile phase: *methanol (65 per cent)*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume. 10 µl.

Calculate the content of $C_{21}H_{30}O_5$ in the ointment.

For Quiniodochlor— Weigh accurately a quantity of the ointment containing 25 mg of Clioquinol, add 80 ml of a hot mixture of 24 volumes of *2-methoxy-ethanol* and 6 volumes of *water* and heat on a water-bath for 5 minutes. Cool in ice for 10 minutes, allow to warm to room temperature, dilute to 100 ml with a mixture of 24 volumes of *2-methoxy-ethanol* and 6 volumes of *water*, mix and filter. To 10 ml of the filtrate, add 10

ml of *2-methoxyethanol* and 2 ml of a solution prepared by dissolving 0.5 g of *iron(III) chloride hexahydrate* in 80 ml of *2-methoxyethanol* and adding 0.1 ml of *hydrochloric acid* and sufficient *2-methoxyethanol* to produce 100 ml. Dilute the solution to 25 ml with *2-methoxyethanol* and measure the absorbance of the resulting solution at the maximum at 650 nm (2.4.7), using in the reference cell a solution prepared by treating 10 ml of a mixture of 24 volumes of *2-methoxy-ethanol* and 6 volumes of *water* in the same manner beginning at the words "add 10 ml of *2-methoxyethanol.....*".

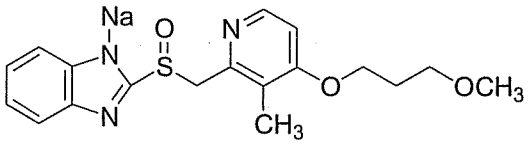
Repeat the operation beginning at the words 'add 10 ml of *2-methoxyethanol* using 10 ml of a solution prepared in the following manner. Dissolve 0.125 g of *clioquinol RS* in sufficient *2-methoxyethanol* to produce 50 ml, warming to effect solution; add 1 ml of *water* to 5 ml of the solution and add sufficient of a mixture of 6 volumes of *water* and 24 volumes of *2-methoxyethanol* to produce 50 ml.

Calculate the content of C_9H_5ClINO in the ointment.

Storage. Store protected from light.

R

Rabeprazole Sodium



$C_{18}H_{20}N_3O_3S,Na$ Mol. Wt. 381.4

Rabeprazole sodium is 2-({[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]methyl}sulphinyl)-1*H*-benzimidazole sodium.

Rabeprazole sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{20}N_3O_3S,Na$, calculated on the anhydrous basis.

Category. Antiulcer.

Description. A white to light yellow, crystalline powder, hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rabeprazole sodium RS.*

B. A 10 per cent w/v solution in *carbon dioxide-free water* gives reaction of sodium (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol*, 20 volumes of *water* and 0.1 volume of *diethylamine.*

Test solution. Dissolve 50 mg of the substance under examination in 100 ml with the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *rabeprazole sodium RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system as described under Assay.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). Run the chromatogram three times of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 7.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol*, 20 volumes of *water* and 0.1 volume of *diethylamine.*

Test solution. Dissolve 0.1 g of the substance under examination in 100.0 ml of solvent mixture. Dilute 5.0 ml of the solution to 100.0 ml with the same solvent.

Reference solution. A 0.005 per cent w/v solution of *rabeprazole sodium RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil keystone betabasic C_8),
- column temperature 40°,
- mobile phase: a mixture of 72 volumes of *0.1 M phosphate buffer pH 7.0* and 28 volumes of *acetonitrile,*
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the percentage content of $C_{18}H_{20}N_3O_3S,Na$.

Storage. Store protected from light and moisture.

Rabeprazole Tablets

Rabeprazole Sodium Tablets

Rabeprazole Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of rabeprazole sodium, $C_{18}H_{20}N_3O_3SNa$. The tablets are enteric coated.

Usual strength. 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 50 rpm and 120 minutes.

Replace the *0.1 M hydrochloric acid* with *phosphate buffer pH 7.4.* Run the apparatus at 75 rpm for 45 minutes. Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution immediately, suitably diluted with the dissolution medium, if necessary, at the maximum at about 291 nm (2.4.7). Calculate the content of $C_{18}H_{20}N_3O_3SNa$ in the medium from the absorbance obtained from a solution of known concentration of *rabeprazole sodium RS,* prepared by dissolving in minimum quantity of a mixture of 75 volumes of *acetonitrile* and 25 volumes of *methanol* and suitably diluted with the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{20}N_3O_3SNa$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol*, 20 volumes of *water* and 0.1 volume of *diethylamine.*

Test solution. Weigh accurately a quantity of the powdered tablet containing 50 mg of Rabeprazole Sodium, disperse in 100 ml of solvent mixture and filter.

Reference solution (a). A 0.05 per cent w/v solution of *rabeprazole sodium RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with solvent mixture.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). Run the chromatogram three times of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the peak in the chromatogram obtained with the reference solution (b) (6.0 per cent).

Uniformity of content *(For tablets containing 10 mg or less).* Comply with the tests stated under Tablets.

Disperse 1 tablet in sufficient *0.1 M sodium hydroxide* to produce 0.0015 per cent w/v solution. Measure the absorbance of the resulting solution at the maximum at about 292 nm (2.4.7). Calculate the content of $C_{18}H_{20}N_3O_3SNa$ from the absorbance obtained from same concentration of *rabeprazole sodium RS* in the same medium.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *methanol*, 20 volumes of *water* and 0.1 volume of *diethylamine.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing 50 mg of Rabeprazole Sodium, disperse in 20 ml of *0.1 M sodium hydroxide* and dilute to 100.0 ml with solvent mixture, filter.

Reference solution. Weigh accurately about 25 mg of *rabeprazole sodium RS*, dissolve in 10 ml of *0.1 M sodium hydroxide* and dilute to 50.0 ml with solvent mixture.

Chromatographic system

- a stainless steel column 25 cm ´ 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of 0.15 per cent w/v solution of *potassium dihydrogen phosphate* previously adjusted pH to 6.0 with *orthophosphoric acid* or *sodium hydroxide solution* and 35 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 10 µl.

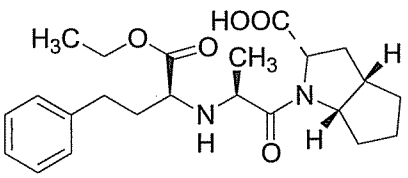
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{20}N_3O_3SNa$.

Storage. Store protected from light and moisture.

Ramipril



$C_{23}H_{32}N_2O_5$ Mol. Wt. 416.5

Ramipril is (2*S*,3a*S*,6a*S*)-1-[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid.

Ramipril contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{23}H_{32}N_2O_5$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ramipril RS.*

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). +32.0° to +38.0°, determined in 1.0 per cent w/v solution in *0.1 M methanolic hydrochloric acid.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25 ml of mobile phase B.

Reference solution (a). A 0.1 per cent w/v solution of *ramipril RS* in the mobile phase B.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase B.

Chromatographic system

- a stainless steel column 25 cm × 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 65°,
- mobile phase: A. dissolve 2.0 g of *sodium perchlorate* in a mixture of 0.5 ml of *triethylamine* and 800 ml of *water;* adjust pH to 3.6 with *orthophosphoric acid* and add 200 ml of *acetonitrile,*
  B. dissolve 2.0 g of *sodium perchlorate* in a mixture of 0.5 ml of *triethylamine* and 300 ml of *water;* adjust pH to 2.6 with *orthophosphoric acid* and add 700 ml of *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
6	90	10
7	75	25
20	65	35
30	25	75
40	25	75
45	90	10
55	90	10

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying in an oven at 60°, under vacuum, for 4 hours.

Assay. Weigh accurately about 0.3 gm, dissolve in 25 ml of *methanol* and add 25 ml of *water.* Titrate with *0.1 M sodium hydroxide.* Determine the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04165 gm of $C_{23}H_{32}N_2O_5$.

Storage. Store protected from light.

Ramipril Capsules

Ramipril Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ramipril, $C_{23}H_{32}N_2O_5$.

Usual strengths. 1.25 mg; 2.5 mg; 5 mg; 10 mg.

Identification

Shake a quantity of the content of the capsules containing 25 mg of Ramipril with 50 ml of *acetone,* centrifuge for 10 minutes, filter. Evaporate the filtrate to dryness at 60° for 3 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ramipril RS.*

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 500 ml of *0.1 M hydrochloric acid,*

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate to get 0.00025 per cent w/v solution of Ramipril with *0.1 M hydrochloric acid.*

Reference solution. A 0.00025 per cent w/v solution of *ramipril RS* in *0.1 M hydrochloric acid.*

Chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$.

D. Not less than 70 per cent of the stated amount of $C_{23}H_{32}N_2O_5$.

Uniformity of content *(For capsules containing 10 mg or less)*. Comply with the test stated under Capsules.

Determine by liquid chromatography (2.4.14).

Test solution. Disperse one capsule in 100 ml of *0.1 M hydrochloric acid*, sonicate for 15 minutes. Dilute if necessary, to produce 0.00025 per cent w/v solution of Ramipril in *0.1 M hydrochloric acid.*

Reference solution. A 0.00025 per cent w/v solution of *ramipril RS* in *0.1 M hydrochloric acid.*

Chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh a quantity of the content of capsules containing 25 mg of Ramipril, disperse in 100.0 ml of *0.1 M hydrochloric acid,* mix and centrifuge. Dilute 1.0 ml of the resulting solution to 100.0 ml with *0.1 M hydrochloric acid.*

Reference solution. A 0.00025 per cent w/v solution of *ramipril RS* in *0.1 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 12.5 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 42 volumes of *acetonitrile* and 58 volumes of a solution containing 1.4 per cent w/v solution of *sodium perchlorate* and 0.58 per cent w/v solution of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine*, adjust the pH of the mixture to 2.1 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$.

Ramipril Tablets

Ramipril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ramipril, $C_{23}H_{32}N_2O_5$.

Usual strengths. 1.25 mg; 2.5 mg; 5 mg; 10 mg.

Identification

Shake a quantity of the powdered tablets containing 25 mg of Ramipril with 50 ml of *acetone,* centrifuge for 10 minutes, filter. Evaporate the filtrate to dryness at 60° for 3 hours. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ramipril RS.*

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 500 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate to get 0.00025 per cent w/v solution of Ramipril with *0.1 M hydrochloric acid.*

Reference solution. A 0.00025 per cent w/v solution of *ramipril RS* in *0.1 M hydrochloric acid.*

Chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$.

D. Not less than 70 per cent of the stated amount of $C_{23}H_{32}N_2O_5$.

Uniformity of content *(For tablets containing 10 mg or less).* Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. Take one tablet, add 5 ml of *0.1 M hydrochloric acid*, sonicate for 10 minutes, dilute, if necessary, with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.00025 per cent w/v of Ramipril, centrifuge and use the supernatant liquid.

Calculate the content of $C_{23}H_{32}N_2O_5$.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh a quantity of powdered tablets containing 25 mg of Ramipril, disperse in 100.0 ml of *0.1 M hydrochloric acid* and centrifuge. Dilute 1.0 ml of the resulting solution to 100.0 ml with *0.1 M hydrochloric acid.*

Reference solution. A 0.00025 per cent w/v solution of *ramipril RS* in *0.1 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 12.5 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 42 volumes of *acetonitrile* and 58 volumes of a solution containing 1.4 per cent w/v solution of *sodium perchlorate* and 0.58 per cent w/v solution of *orthophosphoric acid* adjusted to pH 2.5 with *triethylamine*, adjust the pH of the mixture to

2.1 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$.

Storage. Store protected from moisture.

Ramipril and Hydrochlorothiazide Tablets

Ramipril and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ramipril, $C_{23}H_{32}N_2O_5$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual strength. Ramipril 2.5 mg and Hydrochlorothiazide 12.5 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution corresponds to the principal peaks in the chromatogram obtained with reference solution (b).

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium.750 ml of *0.1 M hydrochloric acid,*

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate, if necessary, with the dissolution medium.

Reference solution (a). Dissolve a quantity of *ramipril RS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Reference solution (b). Dissolve a quantity of *hydrochlorothiazide RS* in mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Thermo quest Hypersil),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *acetonitrile*, and 0.1 volume of *triethylamine*, adjusted to pH 3.0 with *orthophoshoric acid,*
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (a) and (b). The relative standard deviation for replicate injections for each peak is not more than 2.0 per cent.

Inject the test solution, reference solution (a) and (b).

Calculate the content of $C_{23}H_{32}N_2O_5$ and $C_7H_8ClN_3O_4S_2$.

D. Not less than 75 per cent of the stated amount of $C_{23}H_{32}N_2O_5$ and $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing about 10 mg of Ramipril in mobile phase A, sonicate for 15 minutes and dilute to 10.0 ml with the same solvent.

Reference solution (a). A solution containing 0.2 per cent w/v of *ramipril RS* and 1.0 per cent w/v of *hydrochlorothiazide RS* in mobile phase A.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50.0 ml with mobile phase A. Dilute 5.0 ml of this solution to 100.0 ml with the same solvent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 35°,
- mobile phase: A. a mixture of 60 volumes of buffer solution prepared by dissolving 4 g of *sodium perchlorate* in 600 ml of *water*, add 1.0 ml of *triethylamine*, adjusted to pH 2.6 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
  B. a mixture of 85 volumes of buffer solution and 15 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 13	0	100
13 – 17	0 →50	100 →50
17 – 20	50 →100	50 →0
20 – 80	100	0
80 – 82	100 →0	0 →100
82 – 87	0	100

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections for each peak is not more than 5.0 per cent. The relative retention time with reference to ramipril for ramipril impurity A is about 0.9, for ramipril impurity B is about 1.2, for ramipril impurity C is about 1.5 and for ramipril impurity D is about 1.7.

Inject the test solution and reference solution (b). The retention time of ramipril is about 30 minutes and of hydrochlorothiazide is about 10 minutes. In the chromatogram obtained with the test solution the area of each peak due to ramipril impurity A, B and C is not be more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent); the area of peak due to ramipril impurity D is not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (7.0 per cent); the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Uniformity of content. Comply with the test stated under Tablets.

For ramipril— Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 intact tablet in *water* and dilute to 50 ml with the mobile phase.

Reference solution. Dissolve a quantity of *ramipril RS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$ in the tablet.

For hydrochlorothiazide—Determine by liquid chromatography (2.4.14).

Test solution. Disperse 1 tablet with sufficient amount of the mobile phase, sonicate for 15 minutes and dilute to 100 ml with the mobile phase.

Reference solution. Dissolve a quantity of *hydrochlorothiazide RS* in the mobile phase and dilute with the mobile phase to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. *For ramipril*—Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing about 25 mg of Ramipril, disperse in *water* and dilute to 250 ml with the mobile phase, filter.

Reference solution. A 0.01 per cent w/v solution of *ramipril RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Thermo quest Hypersil),
- mobile phase: a mixture of 55 volumes of *water*, 45 volumes of *acetonitrile* and 0.1 volume of *triethylamine,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates of the principal peak is not less than 2000 theoretical plates and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{32}N_2O_5$ in the tablet.

For hydrochlorothiazide—

Test solution. Disperse a quantity of the powdered tablets containing about 125 mg of Hydrochlorothiazide with 50 ml of *acetonitrile*, sonicate for 15 minutes and dilute to 250 ml with the mobile phase. Dilute 5.0 ml of this solution to 20 ml with the mobile phase.

Reference solution. Dissolve 12.5 mg of *hydrochlorothiazide RS* with 10 ml of *acetonitrile*, sonicate and dilute to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 9 volumes of *0.1 M sodium dihydrogen orthophosphate* and 1 volume of *acetonitrile*, adjusted to pH 3.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the theoretical plates of hydrochlorothiazide peak is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

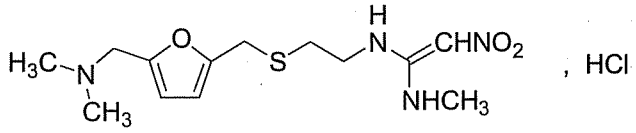
Inject the test solution and the reference solution.

Calculate the content of $C_7H_8ClN_3O_4S_2$ in the tablet.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of ramipril and hydrochlorothiazide.

Ranitidine Hydrochloride



$C_{13}H_{22}N_4O_3S,HCl$ Mol. Wt. 350.9

Ranitidine Hydrochloride is *N*-[2-[[[5-[(dimethylamino) methyl]furan-2-yl]methyl]thio]ethyl]-*N*-methyl-2-nitroethene-1,1-diamine hydrochloride.

Ranitidine Hydrochloride contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{13}H_{22}N_4O_3S$, HCl, calculated on the dried basis.

Category. Antiulcer (Histamine H_2-receptor antagonist).

Dose. Orally, the equivalent of 300 to 600 mg of ranitidine daily, in divided doses; by intramuscular or slow intravenous injection, the equivalent of 50 mg of ranitidine every 6 to 8 hours. (1.12 g of ranitidine hydrochloride is approximately equivalent to 1 g of ranitidine).

Description. A white to pale yellow, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ranitidine hydrochloride RS* or with the reference spectrum of ranitidine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 25 volumes of *ethyl acetate,* 15 volumes of *2-propanol,* 4 volumes of *strong ammonia solution* and 2 volumes of *water.*

Test solution (a). Dissolve 0.22 g of the substance under examination in 10 ml of *methanol.*

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with *methanol.*

Reference solution (a). Weigh accurately a quantity of *ranitidine hydrochloride RS* in *methanol,* and dilute with *methanol* to obtain a solution containing a known concentration of about 0.022 per cent w/v.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 20 ml with *methanol.*

Reference solution (c). Dilute 30.0 ml of reference solution (a) to 100 ml with *methanol.*

Reference solution (d). Dilute 5.0 ml of reference solution (a) to 100 ml with *methanol.*

Reference solution (e). Weigh accurately a quantity of *(N,N'-bis[2-[[[5-(dimethylamino) methyl]furan-2-yl]methyl] sulphanyl]ethyl]-2-nitroethene-1,1-diamine RS (ranitidine impurity A RS)* in *methanol,* and dilute with *methanol* to obtain a solution containing a known concentration of about 0.127 per cent w/v.

Reference solution (f). Weigh accurately a quantity of *2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl] sulphanyl]ethanamine RS (ranitidine impurity B RS)* in *methanol,* and dilute with *methanol* to obtain a solution containing a known concentration of about 0.1 per cent w/v.

Apply to the plate 10 µl of each solution except reference solution (e). Apply separately an additional 10 µl of the test solution and on top of this application, apply 10 µl of reference solution (e). After development, dry the plate in air and expose it to iodine vapours in a closed chamber until the spots are revealed. Any spot in the chromatogram obtained with the test solution (a) corresponding to the principal spot in the chromatogram obtained with reference solution (f) is not more intense than that of the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and no other spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (c) (0.3 per cent). The sum of the intensities of all the secondary spots in the chromatogram obtained with the test solution does not exceed 1.0 per cent.

The test is not valid unless the chromatogram obtained with the combined test solution and reference solution (e) shows two clearly separated principal spots and the chromatogram obtained with reference solution (d) shows a clearly visible spot.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.75 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 2.75 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. A 0.0112 per cent w/v of the substance under examination in the mobile phase.

Reference solution. 0.0112 per cent w/v of *ranitidine hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *methanol* and 15 volumes of *0.1 M ammonium acetate,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 322 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{13}H_{22}N_4O_3S$, HCl.

Storage. Store protected from light and moisture.

Ranitidine Injection

Ranitidine Hydrochloride Injection

Ranitidine Injection is a sterile solution of Ranitidine Hydrochloride in Water for Injections and may be suitably buffered.

Ranitidine Hydrochloride Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ranitidine, $C_{13}H_{22}N_4O_3S$.

Usual strength. The equivalent of 50 mg of ranitidine in 2 ml (1.12 g of ranitidine hydrochloride is approximately equivalent to 1 g of ranitidine).

Identification

A. To a volume of the injection containing 25 mg of ranitidine add 20 ml of *methanol*, mix and evaporate to dryness. Add 1 ml of *light petroleum (60° to 80°)* to the resulting residue, scratch the side of the vessel with a glass rod to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes.The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ranitidine hydrochloride RS* or with the reference spectrum of ranitidine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 6.7 to 7.3, if the preparation is buffered; 4.5 to 7.0, if the preparation is unbuffered.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 25 volumes of *ethyl acetate,* 15 volumes of *2-propanol,* 4 volumes of *strong ammonia solution* and 2 volumes of *water.*

Test solution. Dilute suitably a volume of the injection with *water* to produce a solution containing the equivalent of 2.5 per cent w/v of ranitidine in *water.*

Reference solution (a). Weigh accurately a quantity of *ranitidine hydrochloride RS* in *water,* and dilute with *water* to obtain a solution containing a known concentration of about 0.056 per cent w/v.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 20 ml with *water.*

Reference solution (c). Dilute 5.0 ml of reference solution (a) to 20 ml with *water.*

Reference solution (d). Dilute 6.0 ml of reference solution (c) to 10 ml with *water.*

Reference solution (e). Dilute 5.0 ml of reference solution (b) to 50 ml with *water.*

Reference solution (f). Dilute 5.0 ml of reference solution (e) to 10 ml with *water.*

Reference solution (g). A 0.127 per cent w/v of solution of *(N,N'-bis[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl]sulphanyl] ethyl]-2-nitroethene-1,1-diamine RS (ranitidine impurity A RS)* in *methanol.*

Apply to the plate 10 µl of each solution. Apply separately an additional 10 µl of the test solution and on top of this application, apply 10 µl of reference solution (g). After development, dry the plate in air and expose it to iodine vapours in a closed chamber until the spots are revealed. The major secondary spot in the chromatogram obtained with the test solution is not more intense than that of the principal spot in the chromatogram obtained with reference solution (a) (2.0 per cent) and no other secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of the intensities of all the secondary spots in the chromatogram obtained with the test solution does not exceed 5.0 per cent.

The test is not valid unless the chromatogram obtained with the combined test solution and reference solution (g) shows two clearly separated principal spots and the chromatogram obtained with reference solution (f) shows a clearly visible spot.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Dilute a volume of the injection containing 10.0 mg of ranitidine to 100.0 ml with the mobile phase.

Reference solution. A 0.0112 per cent w/v solution of *ranitidine hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *methanol* and 15 volumes of *0.1 M ammonium acetate,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 322 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the injection.

Storage. Store protected from light.

Labelling. The label states (1) the strength in terms of the equivalent amount of ranitidine; (2) where appropriate, that the injection is buffered.

Ranitidine Tablets

Ranitidine Hydrochloride Tablets

Ranitidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the ranitidine, $C_{13}H_{22}N_4O_3S$. The tablets are coated.

Usual strengths. The equivalent of 150 mg, 300 mg of ranitidine (1.12 g of ranitidine hydrochloride is approximately equivalent to 1 g of ranitidine).

Identification

A. Shake a quantity of the powdered tablets containing 25 mg of ranitidine with 5 ml of *methanol* for 5 minutes, filter and evaporate the filtrate to dryness. Add 1 ml of *light petroleum (60° to 80°)* to the resulting residue, scratch the side of the vessel with a glass rod to induce crystallisation, evaporate to dryness and dry the residue at 60° for 10 minutes. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ranitidine hydrochloride RS* or with the reference spectrum of ranitidine hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 25 volumes of *ethyl acetate,* 15 volumes of *2-propanol,* 4 volumes of *strong ammonia solution* and 2 volumes of *water.*

Test solution. Shake a quantity of the powdered tablets containing 0.45 g of ranitidine with 20 ml of *methanol* and filter.

Reference solution (a). Weigh accurately a quantity of *ranitidine hydrochloride RS* in *methanol,* and dilute with *methanol* to obtain a solution containing a known concentration of about 0.022 per cent w/v.

Reference solution (b). Dilute 10 ml of reference solution (a) to 20 ml with *methanol.*

Reference solution (c). Dilute 30 ml of reference solution (a) to 100 ml with *methanol.*

Reference solution (d). Dilute 5 ml of reference solution (a) to 50 ml with *methanol.*

Reference solution (e). Dilute 5 ml of reference solution (a) to 100 ml with *methanol.*

Reference solution (f). Weigh accurately a quantity of *(N,N'-bis[2-[[[5-[(dimethylamino)methyl]furan-2-yl]methyl] sulphanyl]ethyl]-2-nitroethene-1,1-diamine RS (ranitidine impurity A RS)* in *methanol,* and dilute with *methanol* to obtain a solution containing a known concentration of about 0.127 per cent w/v.

Apply to the plate 10 µl of each solution. Apply separately an additional 10 µl of the test solution and on top of this application, apply 10 µl of reference solution (f). After development, dry the plate in air and expose it to iodine vapours in a closed chamber until the spots are revealed. Any spot in the chromatogram obtained with the test solution corresponding to the principal spot in the chromatogram obtained with reference solution (f) is not more intense than that of the principal spot in the chromatogram obtained with reference solution (b) (0.5 per cent) and no other spot in the chromatogram obtained with the test solution is more intense

than the principal spot in the chromatogram obtained with reference solution (c) (0.3 per cent). The sum of the intensities of all the secondary spots in the chromatogram obtained with the test solution does not exceed 2.0 per cent.

The test is not valid unless the chromatogram obtained with the combined test solution and reference solution (f) shows two clearly separated principal spots and the chromatogram obtained with reference solution (e) shows a clearly visible spot.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh and powder 20 tablets. Shake 1.5 g of the powder with 400 ml of the mobile phase, dilute to 500.0 ml with the mobile phase, filter and dilute the filtrate with the mobile phase to obtain a solution containing the equivalent of 0.01 per cent w/v of ranitidine.

Reference solution. A 0.0112 per cent w/v solution of *ranitidine hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *methanol* and 15 volumes of *0.1 M ammonium acetate,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 322 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_{13}H_{22}N_4O_3S$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of ranitidine.

Purified Rayon

Viscose Fibre; absorbent viscose

Category. Regenerated cellulose used in surgical dressings.

Description. White or very slightly yellow, purified rayon is a fibrous form of bleached regenerated cellulose, which can be produced with a lustrous or matt appearance, and is soft to the touch. The fibres can be produced with average staple length between 32 mm to 80 mm, and are practically odourless.

Identification

A. When examined under a microscope in the dry state, or when mounted in *ethanol (95 per cent)* and *water*, the following characteristics are observed. They are usually of more or less uniform width. Many longitudinal parallel lines are distributed unequally over the width in the case of standard viscose fibres, but such lines are absent or very few in fibres produced through the zinc-free process. The ends are cut more or less straight. Matt fibres contain numerous granular particles of approximately 1µ average diameter.

B. Treat with *iodinated zinc chloride solution*; the fibres become violet.

C. To 0.1 g add 10 ml of *zinc chloride-formic acid solution,* heat to 40° and allow to stand for 2 hours 30 minutes, shaking occasionally. The fibres dissolve completely except for the matt variety where titanium dioxide particles remain.

D. Dissolve the residue obtained in the test for Sulphated ash in 5 ml of *sulphuric acid* with slight warming, allow to cool, and carefully add 0.2 ml of *hydrogen peroxide solution (10 volumes)*. The solution does not undergo colour change in case of lustrous variety of fibre, but for matt variety an orange-yellow colour is obtained, the intensity of which depends on the quantity of titanium dioxide present.

Tests

Colour of extract. Take 15 g of material under examination in a suitable vessel, add 150 ml of *water*, close the vessel and allow to macerate for 2 hours. Decant the solution, squeeze the residual liquid carefully from the sample with a glass rod, mix and filter. The filtered extract is colourless. Compare the colour of the extract with water using identical tubes of colourless, transparent, neutral glass 12 mm in diameter measuring 2 ml. Compare the colours in diffused daylight, viewing horizontally against a white background.

Acidity or alkalinity. To 25 ml of filtered extract obtained, add 0.1 ml of *dilute phenolphthalein solution*; to another 25 ml add 0.05 ml of *methyl orange solution*. Neither solution shows a pink colour.

Foreign fibres. When examined under a microscope, it is seen to consist exclusively of viscose fibres, except that occasionally a few isolated foreign fibres may be present.

Fluorescence. Examine a layer about 5 mm in thickness under ultraviolet light at 365 nm. It displays only a slight, brownish-violet fluorescence and a few yellow particles. Not more than a few isolated fibres show an intense blue fluorescence.

Absorbancy

A. Sinking time. Not more than 10 seconds, determined by the following method.

Apparatus

A dry, cylindrical copper wire basket, 80 mm high and 50 mm in diameter, fabricated from wire of diameter 0.4 mm and having a mesh aperture of 15 to 20 mm; the basket weighs 2.4 to 3.0 g.

Method

Weigh the basket to the nearest 10 mg. Take five samples, each of approximately 1 g, from different places in the material under examination, place loosely in the basket and weigh the packed basket to the nearest 10 mg. Hold the basket with its long axis in the horizontal position and drop it from a height of about 10 mm into *water* at 25° contained in a beaker at least 12 cm in diameter and filled to a depth of 10 cm. Measure with a stopwatch the time taken by the basket to sink below the surface of the water. Repeat the procedure on two further samples and calculate the average value.

B. Water-holding capacity. Not less than 18.0 g per g, determined by the following method.

After the sinking time has been recorded in test A, remove the basket from the *water,* allow it to drain for 30 seconds with its long axis in the horizontal position over the beaker, transfer it to a tared beaker and weigh to the nearest 10 mg. Calculate the weight of *water* retained by the sample. Repeat the procedure on two further samples and calculate the average value.

Colouring matter. Slowly extract 10 g in a narrow percolator with *ethanol (95 per cent)* until 50 ml of extract is obtained. The extract is not more intensely coloured than reference solution YS5 *or* GYS6, (2.4.1) or a solution prepared in the following manner. To 3.0 ml of CSS add 7.0 ml of a solution of *hydrochloric acid* containing 1 per cent w/v of *hydrochloric acid* and dilute 0.5 ml of the resulting solution to 10 ml with the same solution of *hydrochloric acid.*

Ether-soluble substances. Not more than 0.5 per cent, determined by the following method. Extract 5 g with *ether* in a continuous extraction apparatus such as a Soxhlet apparatus, for 4 hours in such a way that the rate is at least four extractions per hour. Evaporate the *ether* and dry the residue to constant weight at 105°.

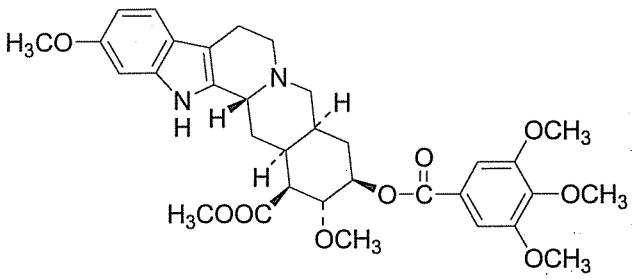
Water-soluble substances. Not more than 0.7 per cent, determined by the following method. Boil 5 g with 500 ml of *water* for 30 minutes, stirring frequently and replacing the *water* lost by evaporation. Decant the liquid into a beaker, squeeze the residual liquid from the material carefully with a glass rod, mix the liquids and filter the extract whilst hot. Evaporate 400 ml of the filtrate and dry the residue to constant weight at 105°.

Hydrogen sulphide. To 10 ml of the filtered extract obtained in the Colour of extract, add 1.9 ml of *water*, 0.15 ml of *dilute acetic acid* and 1 ml of *lead acetate solution.* After 2 minutes, the solution is not more intensely coloured than a reference solution prepared at the same time using 0.15 ml of *dilute acetic acid*, 1.2 ml of *thioacetamide reagent*, 1.7 ml of *lead standard solution (10 ppm Pb)* and 10 ml of filtered extract.

Sulphated ash (2.3.18). Not more than 1.5 per cent.

Loss on drying (2.4.19). Not more than 13.0 per cent, determined on 5 g by drying in an oven at 105°.

Reserpine



$C_{33}H_{40}N_2O_9$ Mol. Wt. 608.7

Reserpine is methyl 11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimbane-16β-carboxylate.

Reserpine contains not less than 99.0 per cent and not more than 101.0 per cent of total alkaloids and not less than 98.0 per cent and not more than 102.0 per cent of reserpine, $C_{33}H_{40}N_2O_9$, both calculated on the dried basis.

Category. Antihypertensive.

Dose. 500 μg daily.

Description. White to slightly yellow small crystals or a crystalline powder which darkens slowly on exposure to light.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *reserpine RS* or with the reference spectrum of reserpine.

B. Dilute 1 ml of a 0.2 per cent w/v solution in *chloroform* to 100.0 ml with *ethanol (95 per cent)*. When examined immediately after preparation, in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 268 nm; absorbance at about 268 nm, about 0.55. Over the range 288 nm to 295 nm, the spectrum exhibits a slight minimum and then a shoulder or a slight maximum; absorbance over this range, about 0.34.

C. To about 1 mg add 0.1 ml of a 0.1 per cent w/v solution of *sodium molybdate* in *sulphuric acid*; a yellow colour is produced which changes to blue within 2 minutes.

D. To 1 mg add 0.2 ml of a freshly prepared 1 per cent w/v solution of *vanillin* in *hydrochloric acid*; a pink colour develops within 2 minutes.

E. Mix about 0.5 mg with 5 mg of *4-dimethylaminobenzaldehyde* and 0.2 ml of *glacial acetic acid* and add 0.2 ml of *sulphuric acid*; a green colour is produced. Add 1 ml of *glacial acetic acid*; the colour changes to red.

Tests

Specific optical rotation (2.4.22). –116° to –128°, determined in a solution prepared immediately before use by dissolving 0.25 g in sufficient *chloroform* to produce 25 ml.

Oxidation products. Absorbance of a 0.02 per cent w/v solution in *glacial acetic acid* at about 388 nm, measured immediately after preparation, not more than 0.10 (2.4.7).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. *For total alkaloids* — Weigh accurately about 0.5 g and dissolve in a mixture of 40 ml of *anhydrous glacial acetic acid* and 6 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06087 g of total alkaloids.

For reserpine, $C_{33}H_{40}N_2O_9$ — Carry out the following procedure protected from light. Weigh accurately about 25.0 mg, moisten with 2 ml of *ethanol (95 per cent)*, add 2 ml of *0.25 M sulphuric acid* and 10 ml of *ethanol (95 per cent)* and warm gently to dissolve. Cool, dilute to 100.0 ml with *ethanol (95 per cent)* and dilute 5.0 ml to 50.0 ml with the same solvent (solution A). Transfer 10.0 ml to a boiling tube, add 2 ml of *0.25 M sulphuric acid* and 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix and heat in a water-bath at 55° for 35 minutes. Cool, add 1 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid* and dilute to 25.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 388 nm (2.4.7), using as the blank a solution prepared by treating a further 10.0 ml of solution A in the same manner and at the same time but omitting the sodium nitrite solution.

Calculate the content of $C_{33}H_{40}N_2O_9$ from the absorbance obtained by repeating the operation using *reserpine RS* in place of the substance under examination.

Storage. Store protected from light.

Reserpine Injection

Reserpine Injection is a sterile solution of Reserpine in Water for Injections prepared with the aid of a suitable acid. It may contain suitable antioxidants.

Reserpine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of reserpine, $C_{33}H_{40}N_2O_9$.

Usual strengths. 1 mg per ml; 2.5 mg per ml.

Identification

Extract a suitable volume of the injection containing 10 mg of Reserpine with 10 ml of *chloroform* and evaporate the chloroform layer to dryness. The residue complies with the following tests.

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C, D and E may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *reserpine RS* or with the reference spectrum of reserpine.

B. Dilute 1 ml of a 0.2 per cent w/v solution in *chloroform* to 100 ml with *ethanol (95 per cent)*. When examined immediately after preparation, in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 268 nm; absorbance at about 268 nm, about 0.55. Over the range 288 nm to 295 nm, the spectrum exhibits a slight minimum and then a shoulder or a slight maximum; absorbance over this range, about 0.34.

C. To about 1 mg add 0.1 ml of a 0.1 per cent w/v solution of *sodium molybdate* in *sulphuric acid*; a yellow colour is produced which changes to blue within 2 minutes.

D. To 1 mg add 0.2 ml of a freshly prepared 1 per cent w/v solution of *vanillin* in *hydrochloric acid*; a pink colour develops within 2 minutes.

E. Mix about 0.5 mg with 5 mg of *4-dimethylaminobenzaldehyde* and 0.2 ml of *glacial acetic acid* and add 0.2 ml of *sulphuric acid*; a green colour is produced. Add 1 ml of *glacial acetic acid*; the colour changes to red.

Tests

pH (2.4.24). 3.0 to 4.0.

Other tests. Comply with the tests stated under Parenteral Preparations (Injections).

Assay. *Protect the solutions from light throughout the assay.*

Measure accurately a volume of the injection containing about 10 mg of Reserpine and dilute with a 2 per cent w/v solution of *citric acid* to 100.0 ml. Extract 10.0 ml of this solution for

2 minutes with three quantities, each of 15 ml, of *chloroform*. Wash the combined extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate*, add sufficient *chloroform* to produce 50.0 ml, mix and evaporate 10.0 ml to dryness on a water-bath. Dissolve the residue in 10 ml of *ethanol (95 per cent)*, add 2 ml of *0.25 M sulphuric acid* and 10 ml of *ethanol (95 per cent)* and warm gently to dissolve. Cool, dilute to 100.0 ml with *ethanol (95 per cent)* and dilute 5.0 ml to 50.0 ml with the same solvent (solution A). Transfer 10.0 ml to a boiling tube, add 2 ml of *0.25 M sulphuric acid* and 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix and heat in a water-bath at 55° for 35 minutes. Cool, add 1 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid* and dilute to 25.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 388 nm (2.4.7), using as the blank a solution prepared by treating a further 10.0 ml of solution A in the same manner and at the same time but omitting the sodium nitrite solution.

Calculate the content of $C_{33}H_{40}N_2O_9$ from the absorbance obtained by repeating the operation using *reserpine RS* in place of the substance under examination.

Storage. Store protected from light in single dose (or if stabilising agents are present, in multiple dose) containers.

Reserpine Tablets

Reserpine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of reserpine, $C_{33}H_{40}N_2O_9$.

Usual strengths. 100 µg; 250 µg; 500 µg; 1 mg.

Identification

A. Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness, add 0.1 ml of a 0.1 per cent w/v solution of *sodium molybdate* in *sulphuric acid*; a yellow colour is produced which changes to blue within 2 minutes.

B. Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness, add 0.2 ml of a freshly prepared 1 per cent w/v solution of vanillin in *hydrochloric acid*; a pink colour develops within 2 minutes.

Tests

Uniformity of content. *Protect the solutions from light throughout the test.*

Comply with the test stated under Tablets.

Powder one tablet, disperse in 10 ml of a 2 per cent w/v solution of *citric acid* and extract for 2 minutes with three quantities, each of 5 ml, of *chloroform*, filter the extracts through a plug of cotton moistened with *chloroform*. Wash the chloroform extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate* and evaporate the chloroform extracts to dryness on a water-bath. For tablets containing upto 250 µg of Reserpine per tablet, dissolve the residue in 10.0 ml of *ethanol (95 per cent)*. For tablets containing more than 250 µg of Reserpine per tablet, dissolve the residue in a suitable volume of *ethanol (95 per cent)* to give a concentration of 250 µg of Reserpine per 10.0 ml; add 2 ml of *0.25 M sulphuric acid* and 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix and heat in a water-bath at 55° for 35 minutes. Cool, add 1 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid* and dilute to 25.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 388 nm (2.4.7), using as the blank a solution prepared by treating a further 10.0 ml of solution A in the same manner and at the same time but omitting the sodium nitrite solution.

Calculate the content of $C_{33}H_{40}N_2O_9$ in the tablet from the absorbance obtained by repeating the operation using *reserpine RS* in place of the substance under examination.

Other tests. Comply with the tests stated under Tablets.

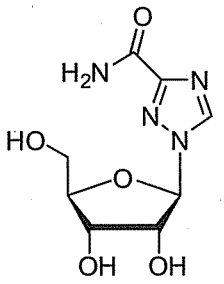
Assay. *Protect the solutions from light throughout the assay.*

Weigh accurately a quantity of the powdered tablets containing about 1 mg of Reserpine, add 10 ml of a 2 per cent w/v solution of *citric acid* and extract for 2 minutes with three quantities, each of 15 ml, of *chloroform*. Wash the combined extracts with 10 ml of a 1 per cent w/v solution of *sodium bicarbonate*, add sufficient *chloroform* to produce 50.0 ml and evaporate 10.0 ml to dryness on a water-bath. Dissolve the residue in 10 ml of *ethanol (95 per cent)* and add 2 ml of *0.25 M sulphuric acid* and 10 ml of *ethanol (95 per cent)* and warm gently to dissolve. Cool, dilute to 100.0 ml with *ethanol (95 per cent)* and dilute 5.0 ml to 50.0 ml with the same solvent (solution A). Transfer 10.0 ml to a boiling tube, add 2 ml of *0.25 M sulphuric acid* and 2 ml of a freshly prepared 0.3 per cent w/v solution of *sodium nitrite*, mix and heat in a water-bath at 55° for 35 minutes. Cool, add 1 ml of a freshly prepared 5 per cent w/v solution of *sulphamic acid* and dilute to 25.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 388 nm (2.4.7), using as the blank a solution prepared by treating a further 10.0 ml of solution A in the same manner and at the same time but omitting the sodium nitrite solution.

Calculate the content of $C_{33}H_{40}N_2O_9$ from the absorbance obtained by repeating the operation using *reserpine RS* in place of the substance under examination.

Storage. Store protected from light.

Ribavirin



$C_8H_{12}N_4O_5$ Mol.Wt. 244.2

Ribavirin is 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide.

Ribavirin contains not less than 98.0 per cent and not more than 102.0 per cent of $C_8H_{12}N_4O_5$, calculated on the dried basis.

Category. Antiviral.

Description. A white or almost white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ribavirin RS* or with the reference spectrum of ribavirin.

Tests

pH (2.4.24). 4.0 to 6.5, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water.*

Specific optical rotation (2.4.22). - 33° to - 37°, determined in a 1.0 per cent w/v solution, use freshly prepared solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution (a). Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Test solution (b). Dissolve 25 mg of the substance under examination in100 ml of the mobile phase. Dilute 10 ml of the solution to 100 ml with the mobile phase.

Reference solution (a). Dilute 1 ml of test solution (a) to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *ribavirin for system suitability RS* in 2 ml of the mobile phase.

Reference solution (c). Dissolve 25 mg of *ribavirin RS* in 100 ml of the mobile phase. Dilute 10 ml of the solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 7.8 mm, packed with strong cation-exchange resin (9 µm),
- column temperature. 40°,
- mobile phase. *water*, adjusted to pH 2.5 with *sulphuric acid,*
- flow rate. 1ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio is not less than 1.2, where H_p is the height above the baseline of the peak due to ribavirin impurity F and H_v is the height above the baseline of the lowest point of the curve separating this peak from the peak due to ribavirin.

Inject test solution (a), reference solution (a) and (b). Record the chromatogram eleven times the retention time of the principal peak. The relative retention time with reference to ribavirin for 1-(5-*O*-acetyl-β-D-ribofuranosyl) -1*H*-1,2,4-triazole-3-carboxamide (5'-*O*-acetylribavirin) (ribavirin impurity F) is about 1.2. The peak area corresponding to ribavirin impurity F is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The area of each secondary peak corresponding to 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxylic acid (ribavirin impurity A), 1-α-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (ribavirin impurity B), 1*H*-1,2,4-triazole-3-carboxylic acid (ribavirin impurity C), *H*-1,2,4-triazole-3-carboxamide (ribavirin impurity D), 1-(5-*O*-benzoyl-α -D-ribofuranosyl)-1*H*-1,2,4-triazole-3-carboxamide (5'-O-benzoylribavirin) (ribavirin impurity E), 1- β -D-ribofuranosyl-1*H*-1,2,4-triazole-5-carboxamide (N-isomer) (ribavirin impurity G) is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). Dissolve 4.0 g in 20 ml of *water* with heating if necessary and use 12 ml of the solution. The resulting solution complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 5 hour.

Assay. Determine by liquid chromatography (2.4.14) same as described under Related substances with the following modification.

Inject test solution (b) and reference solution (c).

Calculate the content of $C_8H_{12}N_4O_5$.

Storage. Store protected from light.

Ribavirin Inhalation Solution

Ribavirin Solution for Inhalation

Catogery. Antiviral.

Ribavirin Inhalation Solution is a sterile solution of Ribavirin in Water for Injections. It is prepared by dissolving Ribavirin for Inhalation in the requisite amount of Water for Injections.

Ribavirin for Inhalation

Ribavirin for Inhalation is a sterile ribavirin consisting of Ribavirin with or without excipients. It is filled in a sealed container.

The inhalation solution is constituted by dissolving the contents of the sealed container in the requisite amount of sterile water for injection, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and particulate matter stated under Inhalation Preparations.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Ribavirin Inhalation Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of $C_8H_{12}N_4O_5$.

The content of the sealed container complies with the requirements stated under Inhalation Preparations and with the following requirements.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 20 volumes of *0.1 M ammonium chloride* and 90 volumes of *acetonitrile.*

Test solution. Dissolve ribavirin inhalation solution containing about 0.1 g of Ribavirin in 10 ml of *water.*

Reference solution. A 1.0 per cent w/v solution of *ribavirin RS* in *water.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air for 15 minutes, and spray the plate with the mixture of 0.5 ml of *anisaldehyde*, 0.5 ml of *sulphuric acid,* 0.1 ml of *glacial acetic acid* and 9 ml of *ethanol (95 per cent)* and heat at 105° for 40 minutes and allow to cool.The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to that in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that of the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. A 0.05 per cent w/v solution of ribavirin in the mobile phase.

Reference solution (a). A 0.000125 per cent w/v solution of ribavirin in the mobile phase.

Reference solution (b). A 0.05 per cent w/v solution of *ribavirin RS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm x 7.8 mm, packed with strong cation-exchange resin of sulphonated, cross-linked *styrene-divinylbenzene co-polymer* in the hydrogen form (7 to 11 µm) (Such as Aminex HPAH),
- column temperature. 40°,
- mobile phase: *water*, adjusted to pH 2.5 with *sulphuric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the tailing factor for the principal peak is not less than 0.7 and not more than 1.5.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent) and the sum of the areas of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reconstitute the contents of one container as instructed on the label using an accurately measured volume of diluent, dilute a suitable volume of the solution to 200 volumes with the mobile phase to produce a solution containing 0.1 per cent w/v of Ribavirin, mix and dilute 5 volumes of the resulting solution to 20 volumes with the mobile phase.

Reference solution. A 0.025 per cent w/v solution of *ribavirin RS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 7.8 mm, packed with strong cation-exchange resin of sulphonated, cross-linked *styrene-divinylbenzene co-polymer* in the hydrogen form (7 to 11 µm) (Such as Aminex HPAH),
- column temperature. 40°,
- mobile phase: *water*, adjusted to pH 2.5 with *sulphuric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 207 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not less than 0.7 and not more than 1.5.

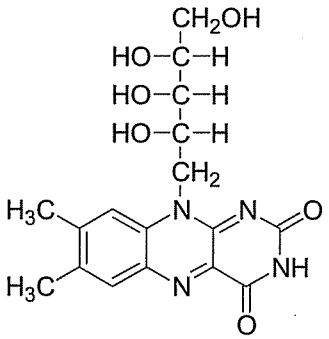
Inject the test solution and the reference solution.

Calculate the content of $C_8H_{12}N_4O_5$ in inhalation solution.

Labelling. The label of the sealed container states (1) the procedure for preparing the solution, (2) the delivery system to be used for the constituted solution (3) the conditions under which the constituted solution should be stored.

Riboflavin

Lactoflavin; Vitamin B_2



$C_{17}H_{20}N_4O_6$ Mol. Wt. 376.4

Riboflavin is 3,10-dihydro-7,8-dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4-dione.

Riboflavin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_4O_6$, calculated on the dried basis.

Category. B-group vitamin.

Dose. Prophylactic, 1 to 4 mg daily; therapeutic, 5 to 10 mg daily.

Description. A yellow to orange-yellow, crystalline powder; odour, slight.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *riboflavine RS* or with the reference spectrum of riboflavine.

B. Dissolve about 1 mg in 100 ml of *water*. The solution has a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence by reflected light, which disappears on addition of mineral acids or alkalis.

Tests

pH (2.4.24). 5.5 to 7.2, determined in a saturated solution.

Specific optical rotation (2.4.22). –115° to –135°, determined in a 0.5 per cent w/v solution in carbonate-free *0.05 M sodium hydroxide*. Measure the angle of rotation within 30 minutes of preparing the solution.

Light absorption (2.4.7). Dilute a suitable volume of the final solution obtained in the Assay with an equal volume of *water*.

When examined in the range 210 nm to 460 nm, the resulting solution exhibits maxima at about 223 nm, 267 nm, 373 nm and 444 nm; the ratio of the absorbance at the maximum at about 373 nm to that at about 267 nm, 0.31 to 0.33 and the ratio of the absorbance at the maximum at about 444 nm to that at about 267 nm, 0.36 to 0.39.

Lumiflavine. Shake 25 mg with 10 ml of *chloroform* for 5 minutes and filter. The filtrate is not more intensely coloured than reference solution BYS6 (2.4.1).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. *Carry out the procedure in subdued light.*

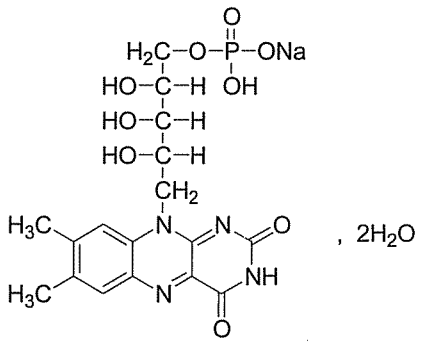
Weigh accurately about 65 mg and transfer to an amber-glass 500-ml volumetric flask, suspend in 5 ml of *water*, ensuring that it is completely wetted. Dissolve in 5 ml of *2 M sodium hydroxide*. As soon as dissolution is complete add 100 ml of *water* and 2.5 ml of *glacial acetic acid* and dilute to 500.0 ml with *water*. To 20.0 ml of this solution add 3.5 ml of a 1.4 per cent w/v solution of *sodium acetate* and dilute to 200.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 444 nm (2.4.7).

Calculate the content of $C_{17}H_{20}N_4O_6$ taking 328 as the specific absorbance at 444 nm.

Storage. Store protected from light.

Riboflavin Sodium Phosphate

Riboflavine-5-phosphate (Sodium Salt); Vitamin B_2 Sodium Phosphate



$C_{17}H_{20}N_4NaO_9P, 2H_2O$ Mol. Wt. 514.4

Riboflavin Sodium Phosphate is monosodium 3,10-dihydro-7,8-dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4-trihydroxypentyl] benzo[*g*]pteridine-2,4-dione 5-phosphate dihydrate.

Riboflavin Sodium Phosphate contains the equivalent of not less than 73.0 per cent and not more than 79.0 per cent of $C_{17}H_{20}N_4O_6$, calculated on the dried basis.

Category. B-group vitamin.

Dose. Prophylactic, the equivalent of 1 to 4 mg of riboflavine daily; therapeutic, the equivalent of 5 to 10 mg of riboflavine daily. (1.37 g of riboflavine sodium phosphate is approximately equivalent to 1 g of riboflavine).

Description. A yellow to orange-yellow, crystalline powder; hygroscopic.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *phosphate buffer pH 7.0* shows an absorption maximum at about 267 nm; absorbance at about 267 nm, 0.58 to 0.64.

B. Dissolve about 10 mg in sufficient *2 M sodium hydroxide* to produce 100 ml, expose 1 ml to ultraviolet light at 254 nm for 5 minutes, add sufficient *5 M acetic acid* to make the solution acidic to *litmus paper* and shake the mixture with 2 ml of *dichloromethane*; the lower layer exhibits a yellow fluorescence.

C. To 0.5 g add 10 ml of *nitric acid*, evaporate the mixture to dryness on a water-bath, ignite the residue until the carbon is removed, dissolve the final residue in 5 ml of *water* and filter. The filtrate gives the reactions of sodium salts and reaction B of phosphates (2.4.1).

Tests

pH (2.4.24). 4.0 to 6.3, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). +38.0° to +42.0°, determined in a 1.5 per cent w/v solution in *5 M hydrochloric acid.*

Heavy metals (2.3.13). To 2.0 g in a silica crucible add 2 ml of *nitric acid* dropwise followed by 0.25 ml of *sulphuric acid.* Heat cautiously until white fumes are evolved and ignite. Extract the cooled residue with two quantities, each of 2 ml, of *hydrochloric acid* and evaporate the extracts to dryness. Dissolve the residue in 2 ml of *2 M acetic acid* and dilute to 20 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (10 ppm). Use 1.0 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Lumiflavine. Shake 35 mg with 10 ml of *dichloromethane* for 5 minutes and filter. The filtrate is not more intensely coloured than reference solution BYS6 (2.4.1).

Inorganic phosphate. Not more than 1.5 per cent, determined by the following method. Dissolve 0.1 g in sufficient *water* to produce 100 ml. Dilute 5 ml with 10 ml of *water* and add 5 ml of *buffered cupric sulphate solution pH 4.0*, 2 ml of a 3 per cent w/v solution of *ammonium molybdate*, 1 ml of a freshly prepared solution containing 2 per cent w/v of *4-methyl-aminophenol sulphate* and 5 per cent w/v of *sodium meta-bisulphite* and 1 ml of a 3 per cent v/v solution of *perchloric acid.* Add sufficient *water* to produce 25 ml, mix and measure the absorbance of the resulting solution at the maximum at about 800 nm (2.4.7), within 15 minutes of its preparation, using as the blank a solution prepared in the same manner but omitting the substance under examination. The absorbance is not greater than that produced by repeating the operation using a solution prepared in the same manner using 15 ml of *phosphate standard solution (5 ppm PO_4)* and beginning at the words "add 5 ml of *buffered cupric sulphate solution pH 4.0*,"

Loss on drying (2.4.19). Not more than 8.0 per cent, determined on 0.5 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa for 5 hours.

Assay. *Carry out the procedure protected from light.*

Weigh accurately about 0.1 g, dissolve in 150 ml of *water*, add 2 ml of *glacial acetic acid* and dilute to 1000.0 ml with *water*. To 10.0 ml add 3.5 ml of a 1.4 per cent w/v solution of *sodium acetate*, dilute to 50.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 444 nm (2.4.7). Calculate the percentage content of $C_{17}H_{20}N_4O_6$ taking 328 as the specific absorbance at 444 nm.

Storage. Store protected from light.

Riboflavin Tablets

Lactoflavin Tablets; Vitamin B_2 Tablets

Riboflavin Tablets contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of riboflavine, $C_{17}H_{20}N_4O_6$.

Usual strengths. 2 mg; 5 mg.

Identification

Shake a quantity of the powdered tablets containing 1 mg of Riboflavine with 100 ml of *water* and filter; the filtrate has a pale greenish yellow colour by transmitted light and an intense yellowish green fluorescence by reflected light, which disappears on addition of mineral acids or alkalis.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, add a mixture of 2.5 ml of *glacial acetic acid* and 50 ml of *water* and heat on a water-bath for 1 hour with occasional stirring. Dilute with 50 ml of *water*, add 15 ml of *1 M sodium hydroxide* with continuous stirring and then sufficient *water* to produce a solution containing 10 µg of Riboflavine per ml. Filter and discard the first few ml of the filtrate. On the clear filtrate carry out the Assay beginning at the words "Measure the absorbance....".

Other tests. Comply with the tests stated under Tablets.

Assay. *Carry out the procedure in subdued light.*

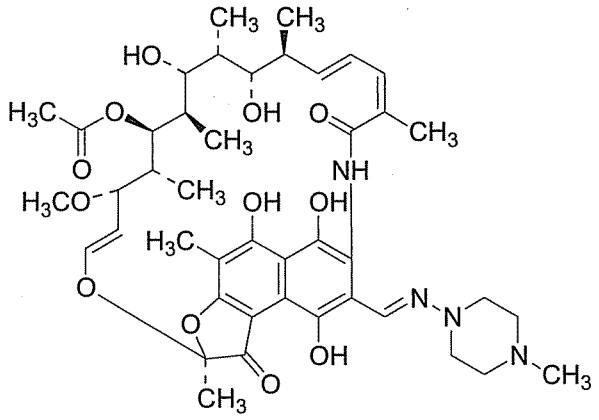
Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of Riboflavine, add a mixture of 5 ml of *glacial acetic acid* and 100 ml of *water* and heat on a water-bath for 1 hour with occasional shaking. Dilute with 50 ml of *water*, cool, add 30 ml of *1 M sodium hydroxide* with continuous stirring. Add sufficient *water* to produce 1000.0 ml, mix and filter, discarding the first few ml of the filtrate. Measure the absorbance of the filtrate at the maximum at about 444 nm (2.4.7).

Calculate the content of $C_{17}H_{20}N_4O_6$ taking 328 as the specific absorbance at 444 nm.

Storage. Store protected from light.

Rifampicin

Rifampin



$C_{43}H_{58}N_4O_{12}$ Mol. Wt. 823.0

Rifampicin is (12*Z*,14*E*,24*E*)-(2*S*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*)-1,2-dihydro-5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methyl-piperazin-1-yliminomethyl)-1,11-dioxo-2,7-(epoxypentadeca-1,11,13-trienimino)naphtho[2,1-*b*]furan-21-yl acetate.

Rifampicin contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{43}H_{58}N_4O_{12}$, calculated on the dried basis.

Category. Antitubercular.

Dose. For an adult, 450 to 600 mg (about 10 mg per kg) daily preferably before breakfast. For a child, upto 20 mg per kg daily to a maximum of 600 mg.

Description. A brick-red to reddish brown, crystalline powder; practically odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rifampicin RS* or with the reference spectrum of rifampicin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 6.5, determined in a 1.0 per cent w/v suspension.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of powder containing 20 mg of Rifampicin, add 10 ml of *acetonitrile*, shake and filter. Dilute 5 ml of the filtrate to 50 ml with the solvent mixture.

Reference solution. A solution containing 0.02 per cent w/v of *rifampicin quinone RS* in *acetonitrile*. To 1 ml of the solution, add 1 ml of the test solution and dilute to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone is not more than 1.5 times the area of the peak due to rifampicin quinone in the chromatogram obtained with the reference solution (1.5 per cent); the area of any peak, other than the peak due to rifampicin and rifampicin quinone, is not more than the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of any such peaks is not more than 3.5 times the area of the peak due to rifampicin in the chromatogram obtained with the reference solution (3.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Dissolve 20.0 mg of the substance under examination in 10.0 ml of *acetonitrile* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with the solvent mixture.

Reference solution. A solution containing 0.2 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture.

Use the chromatographic system and the system suitability parameters described under the test for Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{43}H_{58}N_4O_{12}$.

Storage. Store protected from light, in an atmosphere of nitrogen.

Rifampicin Capsules

Rifampin Capsules

Rifampicin Capsules contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of rifampicin, $C_{43}H_{58}N_4O_{12}$.

Usual strengths. 150 mg; 300 mg; 450 mg; 600 mg.

Identification

A. Shake a quantity of the contents of the capsules containing 0.15 g of Rifampicin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rifampicin RS* or with the reference spectrum of rifampicin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent

w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Shake a quantity of the contents of the capsules containing 200 mg of Rifampicin, with 100 ml of *acetonitrile* and filter. Dilute 5 ml of the filtrate to 50 ml with the solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide and 3-formylrifamycin SV respectively. The response factors are 1.00, 1.19, 1.03 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution, the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution and the area of any peak due to 3-formylrifamycin SV should not be more than the area of the principal peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution the area of any unknown peak should not be more than the area of the principal peak in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 475 nm. Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the medium from the absorbance obtained from a solution of known concentration of *rifampicin RS*.

D. Not less than 75 per cent of the stated amount of $C_{43}H_{58}N_4O_{12}$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the contents of the capsules containing about 300.0 mg of Rifampicin, with 200.0 ml of *acetonitrile*, filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.15 per cent w/v solution of *rifampicin RS* in *acetonitrile*. Dilute 10.0 ml of this solution to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of the resulting solution to 50.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5ml of this solution to 50 ml with the solvent mixture.

Use the chromatographic system and system suitability parameters, as described under the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the capsules.

Storage. Store protected from light and moisture.

Rifampicin Oral Suspension

Rifampicin oral suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of rifampicin, $C_{43}H_{58}N_4O_{12}$.

Usual strengths. 10 mg per ml; 20 mg per ml.

Identification

A. To a quantity containing 0.1 g of Rifampicin add 30 ml of *water* and shake with two quantities, each of 50 ml, of *chloroform*. Dry the combined extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness at a temperature not exceeding 70°. Wash the residue with 1 ml of *ether* and dry at 70°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rifampicin RS* or with the reference spectrum of rifampicin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.2 to 4.8.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Add 5 ml of *water* to a quantity of the oral suspension containing 20 mg of Rifampicin and extract with four quantities, each of 10 ml, of *dichloromethane*, filter the combined extracts and evaporate to dryness at a temperature not exceeding 40°. Dissolve the residue in 10 ml of *acetonitrile*. Dilute 5 ml of the resulting solution to 50 ml with the solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 1 ml of this solution to a 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4; the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide and 3-formylrifamycin SV respectively. Multiply the areas of each known impurity by their response factor. The response factors are 1.00, 1.19, 1.03, 1.22 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide, Isonicotinyl hydrazone and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution, the area of any peak due to rifampicin N-oxide should not be more than the area of the principal peak in the chromatogram obtained with the reference solution and the area of any peak due to 3-formylrifamycin SV should not be more than 5.0 times the area of the principal peak in the chromatogram obtained with the reference solution. In the chromatogram obtained with the test solution the area of any unknown peak should not be more than the area of the principal peak in the chromatogram obtained with the reference solution.

Other tests. Comply with the tests stated under Oral Suspensions.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the oral suspension containing about 150 mg of rifampicin and dilute to 100.0 ml with *acetonitrile*. Dilute 10.0 ml of this solution to 50.0 ml with *acetonitrile*. Dilute 10.0 ml of the resulting solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.15 per cent w/v solution of *rifampicin RS* in *acetonitrile*. Dilute 10.0 ml of the solution to 50.0 ml with *acetonitrile*. Dilute 10.0 ml of the resulting solution to 50.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Use the chromatographic system and the system suitability parameters as described under the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Determine the weight per ml of the suspension (2.4.29) and calculate the content of $C_{43}H_{58}N_4O_{12}$ weight in volume.

Storage. Store protected from light and moisture.

Rifampicin Tablets

Rifampin Tablets

Rifampicin Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of rifampicin, $C_{43}H_{58}N_4O_{12}$.

Usual strength. 150 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.15 g of Rifampicin with 5 ml of *chloroform*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rifampicin RS* or with the reference spectrum of rifampicin.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent solution w/v of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the powdered tablets containing 200 mg of Rifampicin, dissolve in 100 ml of *acetonitrile*, filter. Dilute 5 ml of the filtrate to 50 ml with the solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 1 ml of this solution to a 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v of each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column. temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide, and 3-formylrifamycin SV respectively. The response factors are 1.00, 1.19, 1.03 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4 per cent), the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent) and the area of any peak due to 3-formyl rifamycin SV should not be more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent). In the chromatogram obtained with the test solution the area of any unknown peak should not be more than the area of the principal peak in the chromatogram obtained with the reference solution.

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 475 nm. Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the medium from the absorbance obtained from a solution of known concentration of *rifampicin RS*.

D. Not less than 75 per cent of the stated amount of $C_{43}H_{58}N_4O_{12}$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 300.0 mg of Rifampicin, dissolve in 200.0 ml of *acetonitrile*, filter. Dilute 10.0 ml of the filtrate to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (a). A 0.15 per cent w/v solution of *rifampicin RS* in *acetonitrile*. Dilute 10.0 ml of this solution to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of the resulting solution to 50.0 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Use the chromatographic system and system suitability parameters described under the test for Related substances.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the tablets.

Storage. Store protected from light and moisture.

Rifampicin and Isoniazid Tablets

Rifampin and Isonicotinylhydrazid Tablets

Rifampicin and Isoniazid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of rifampicin, $C_{43}H_{58}N_4O_{12}$ and isoniazid, $C_6H_7N_3O$.

Usual strength. Rifampicin 50 mg and Isoniazid 150 mg.

Identification

A. In the Assay, the chromatogram obtained with the test solution shows peaks that correspond to the peaks due to *rifampicin RS* and *isoniazid RS* in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the powdered tablets containing 200 mg of Rifampicin in 100 ml of *acetonitrile* and filter. Dilute 5 ml of the filtrate to 50 ml with the solvent mixture.

Reference solution (a). Dissolve *rifampicin RS* in *acetonitrile* to obtain a solution containing 0.2 mg per ml. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25, 1.51 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide, 3-formylrifamycin SV isonicotinyl hydrazone and 3-formylrifamycin SV respectively. Multiply the area of each known impurity by its response factor. The response factors are 1.00, 1.19, 1.03, 1.22 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide, isonicotinyl hydrazone and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4 per cent), the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peaks due to 3-formylrifamycin SV and isonicotinyl hydrazone should not be more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (5 per cent) and the area of any peak due to 3-formylrifamycin SV should not be more than the area of the

principal peak in the chromatogram obtained with the reference solution (1.0 per cent). In the chromatogram obtained with the test solution the area of any unknown peak should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the medium.

Reference stock solution. A solution containing 0.0165 per cent of *rifampicin RS* and 0.00825 per cent of *isoniazid RS* in the dissolution medium.

For rifampicin — Measure the absorbance of the sample solution and the reference stock solution suitably diluted with the dissolution medium at 475 nm (2.4.7). Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the medium from the absorbance obtained from the reference stock solution.

For isoniazid — Determine by liquid chromatography (2.4.14).

Use the reference stock solution and sample solution suitably diluted with a solution of *0.05 M potassium dihydrogen orthophosphate*, adjust the pH to 6.2 with *0.1 M sodium hydroxide* to obtain the reference solution and the test solution, respectively.

Chromatographic system
- a stainless steel column 30 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 99 volumes of *0.05 M potassium dihydrogen phosphate* and 1 volume of *acetonitrile* with the pH adjusted to 4.0 ± 0.05 with 2 per cent w/v solution of *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_6H_7N_3O$ in the dissolution medium.

D. Not less than 75 per cent of the stated amounts of $C_{43}H_{58}N_4O_{12}$ and $C_6H_7N_3O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjust the pH to 6.8 with *dilute phosphoric acid.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 40 mg of Isoniazid, dissolve in 100.0 ml of *methanol* and dilute to 500.0 ml with the solvent mixture.

Reference solution. A solution containing 0.08 per cent w/v of *rifampicin RS* and 0.04 per cent w/v of *isoniazid RS* in *methanol.* Dilute 10.0 ml of this solution to 50.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature 30°,
- mobile phase: A. a mixture of 96 volumes of buffer solution pH 6.8 prepared by dissolving 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water* and adjusting the pH to 6.8 ± 0.05 with *dilute phosphoric acid*, and 4 volumes of *acetronitrile.*
  B. a mixture of 45 volumes of the buffer solution and 55 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
6	0	100
15	0	100
16	100	0
20	100	0

NOTE — Saturate the column with mobile phase B for about 1 hour before injection.

Inject the reference solution. The tailing factor is not more than 2.0 for rifampicin and isoniazid; the column efficiency for the isoniazid peak is not less than 3000 and for rifampicin not less than 25000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. The retention times are about 1.0 for rifampicin and about 0.3 for isoniazid.

Calculate the contents of $C_{43}H_{58}N_4O_{12}$ and $C_6H_7N_3O$ in the tablets.

Storage. Store protected from moisture.

Rifampicin, Isoniazid and Ethambutol Tablets

Rifampin, Isonicotinylhydrazid and Ethambutol Hydrochloride Tablets

Rifampicin, Isoniazid and Ethambutol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of rifampicin, $C_{43}H_{58}N_4O_{12}$, isoniazid, $C_6H_7N_3O$ and ethambutol hydrochloride $C_{10}H_{24}N_2O_2.2HCl$.

Usual strength. Rifampicin 300 mg, Isoniazid 200 mg and Ethambutol 200 mg.

Identification

A. In the Assay for rifampicin and isoniazid, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. In the Assay for ethambutol hydrochloride the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the powdered tablets containing 200 mg of Rifampicin, dissolve in 100 ml of *acetonitrile* and filter. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 1 ml of this solution to 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate* and 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25, 1.51 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide, 3-formylrifamycin SV Isonicotinyl hydrazone and 3-formylrifamycin SV respectively. Multiply the areas of each known impurity by its response factor. The response factors are 1.00, 1.19, 1.03, 1.22 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide, Isonicotinyl hydrazone and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4 per cent), the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peak due to 3-formylrifamycin SV Isonicotinyl hydrazone should not be more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (5 per cent) and the area of any peak due to 3-formylrifamycin SV should not be more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent). In the chromatogram obtained with the test solution the area of any unknown peak should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Dissolution (2.5.2).

Apparatus No. 2,

Medium. 900 ml of *0.1 M hydrochloric acid,*

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate.

Reference stock solution. A solution containing 0.0165 per cent w/v of *rifampicin RS*, 0.00825 per cent w/v of *isoniazid RS* and 0.031 per cent w/v of *ethambutol hydrochloride RS* in the dissolution medium. Keep this reference stock solution in the dissolution bath during the test run.

For rifampicin — Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 475 nm. Calculate the content

of $C_{43}H_{58}N_4O_{12}$ in the medium from the absorbance obtained from suitably diluted reference stock solution.

For isoniazid — Determine by liquid chromatography (2.4.14).

Test solution. Suitably dilute the filtered medium with *0.05 M potassium dihydrogen orthophosphate* with the pH adjusted to 6.2 with *0.1 M sodium hydroxide.*

Reference solution. Suitably dilute the reference stock solution with *0.05 M potassium dihydrogen orthophosphate* with the pH adjusted to 6.2 with *0.1 M sodium hydroxide.*

Chromatographic system

- a stainless steel column 30 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 99 volumes of *0.05 M potassium dihydrogen phosphate* and 1 volume of *acetonitrile* previously adjusted to pH 4.0 with a 2 per cent w/v solution of *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 1500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_6H_7N_3O$ in the dissolution medium.

For ethambutol hydrochloride — Determine by liquid chromatography (2.4.14).

Test solution. Suitably dilute the filtered medium with *0.05 M potassium dihydrogen orthophosphate* adjusted to pH 6.2 with *0.1 M sodium hydroxide.*

Reference solution. Suitably dilute the reference stock solution with *0.05 M potassium dihydrogen orthophosphate* with the pH adjusted to 6.2 with *0.1 M sodium hydroxide.*

Use the chromatographic system described under the Assay of ethambutol hydrochloride.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the dissolution medium.

D. Not less than 75 per cent of the stated amounts of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$ and $C_{10}H_{24}N_2O_2.2HCl$.

Other tests. Comply with the tests stated under Tablets.

Assay. *For rifampicin and isoniazid* — Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 40 mg of Isoniazid dissolve in 100.0 ml of *methanol*, dilute to 500.0 ml with the diluent and mix.

Reference solution. A solution containing 0.08 per cent w/v of *rifampicin RS* and *0.04* per cent w/v of *isoniazid RS* in *methanol.* Dilute 10.0 ml of this solution to 50.0 ml with the diluent.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: A. a mixture of 96 volumes of buffer solution pH 6.8 (diluent) prepared by dissolving 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water*, and adjusting the pH to 6.8 ± 0.05 with *dilute phosphoroic acid*, and 4 volumes of *acetonitrile*, B: a mixture of 45 volumes of the buffer solution and 55 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
6	0	100
15	0	100
16	100	0
22	100	0

NOTE — Saturate the column with mobile phase B for about 1 hour.

Inject the reference solution. The test is not valid unless the tailing factor is not less than 2.0 for rifampicin and isoniazid, the column efficiency determined from isoniazid peak is not less than 3000 and that from rifampicin is not less than 25000 theoretical plates respectively, and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention times are about 1.0 for rifampicin and about 0.3 for isoniazid.

Calculate the contents of $C_{43}H_{58}N_4O_{12}$ and $C_6H_7N_3O$ in the tablets.

For ethambutol hydrochloride — Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 60 mg of Ethambutol Hydrochloride and dissolve in 100.0 ml of the diluent.

Reference solution. A 0.06 per cent w/v solution of *ethambutol hydrochloride RS* in the diluent.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles 5 (µm) (such as Zorbax SB CN),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, and 50 volumes of a buffer solution pH 7.0 prepared by dissolving 1 ml of *triethylamine* in 1000 ml of *water* and adjusting the pH to 7.0 with *dilute phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 3.0 and the column efficiency determined from Ethambutol hydrochloride peak is not less than 1500 theoretical plates.

Inject the test solution and the reference solution.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the tablets.

Storage. Store protected from moisture.

Rifampicin, Isoniazid and Pyrazinamide Tablets

Rifampin, Isonicotinylhydrazid and Pyrazinamide Tablets

Rifampicin, Isoniazid and Pyrazinamide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of rifampicin, $C_{43}H_{58}N_4O_{12}$, isoniazid, $C_6H_7N_3O$ and pyrazinamide $C_5H_5N_3O$.

Usual strength. Rifampicin 120 mg, Isoniazid 50 mg and Pyrazinamide 300 mg.

Identification

In the Assay of rifampicin, isoniazid and pyrazinamide, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the powdered tablets containing about 200 mg of Rifampicin, dissolve in 100 ml of *acetonitrile* and filter. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Reference solution (a). A solution containing 0.02 per cent w/v of *rifampicin RS* in *acetonitrile*. Dilute 1ml of this solution to a 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate*, 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25, 1.51 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide, 3-formylrifamycin SV Isonicotinyl hydrazone and 3-formylrifamycin SV respectively. Multiply the area of each known impurity by its response factor. The response factors are 1.00, 1.19, 1.03, 1.22 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide, Isonicotinyl hydrazone and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4 per cent), the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peak due to 3-formylrifamycin SV Isonicotinyl hydrazone should not be more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (5 per cent) and the area of any peak due to 3-formylrifamycin SV should not be more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent). In the chromatogram obtained with the test solution the area of any unknown peak should not be more than

1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of a solution containing 2 g of *sodium chloride* and 7.0 ml of *hydrochloric acid* in 1000 ml of *water*, with a pH of about 1.2,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate.

Reference stock solution. A solution containing 0.0165 per cent w/v of *rifampicin RS*, 0.00825 per cent w/v of *isoniazid RS* and 0.04375 per cent w/v of *pyrazinamide RS* in the dissolution medium and filtered. Keep this reference stock solution in the dissolution bath during the test run.

For rifampicin — Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 475 nm. Calculate the content of $C_{43}H_{58}N_4O_{12}$ in the medium from the absorbance obtained from suitably diluted reference stock solution.

For isoniazid and pyrazinamide — Determine by liquid chromatography (2.4.14).

NOTE — Use this solution within one hour from preparation.

Test solution. To 15.0 ml of the filtered medium, add 15 ml of *1 M dibasic potassium phosphate*, dilute to 100.0 ml with the mobile phase and mix.

Reference solution (a). To 15.0 ml of the reference stock solution, add 15 ml of *1 M dibasic potassium phosphate*, dilute to 100.0 ml with the mobile phase and mix.

Reference solution (b). To 10.0 ml of a 0.0125 per cent w/v solution of *isonicotinic acid* in the dissolution medium, add 4.0 ml of the reference stock solution and 15 ml of *1 M dibasic potassium phosphate*, dilute to 100.0 ml with the mobile phase and mix.

Chromatographic system
- a stainless steel column 30 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 860 volumes of *water*, 100 volumes of *1 M monobasic potassium phosphate* and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 50 µl.

Inject the test solution and reference solutions (a) and (b). The relative retention times are about 0.7 for isonicotinic acid, 1.0 for pyrazinamide and 1.8 for isoniazid.

The test is not valid unless in the chromatogram obtained with reference solution (b) the resolution between isonicotinic acid and pyrazinamide is not more than 2.5 and between pyrazinamide and isoniazid is not more than 4.0.

Calculate the contents of $C_6H_7N_3O$ and $C_5H_5N_3O$ in the medium.

D. Not less than 75 per cent of the stated amounts of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$ and $C_5H_5N_3O$.

Other tests. Comply with the tests stated under Tablets.

Assay. *For rifampicin, isoniazid and pyrazinamide* — Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 40 mg of Isoniazid, dissolve in 100.0 ml of *methanol*, dilute to 500 ml with the buffer solution pH 6.8 and mix.

Reference solution. A solution containing 0.08 per cent w/v of *rifampicin RS*, 0.04 per cent w/v of *isoniazid RS* and 0.2 per cent w/v of *pyrazinamide RS* in *methanol*. Dilute 10.0 ml of this solution to 50.0 ml with the buffer solution pH 6.8.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature 30°,
- mobile phase: A. a mixture of 96 volumes of buffer solution pH 6.8 (diluent) prepared by dissolving 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusted to pH 6.8 ± 0.05 with *dilute phosphoroic acid* and 4 volumes of *acetonitrile*,
  B. a mixture of 45 volumes of the buffer solution and 55 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
5	100	0
6	0	100
15	0	100
16	100	0
22	100	0

NOTE — Saturate the column with mobile phase (B) for about 1 hour.

Inject the test solution and the reference solution. The test is not valid unless the tailing factor is not less than 2.0 for rifampicin, isoniazid and pyrazinamide, the column efficiencies determined from Isoniazid, pyrazinamide and that from rifampicin are not less than 3000, 5000 and 25000 theoretical plates respectively, and the relative standard deviation for

replicate injections is not more than 2.0 per cent. The relative retention times are about 1.8 for rifampicin, about 0.7 for isoniazid and about 1.0 for pyrazinamide.

Inject the test solution and the reference solution.

Calculate the contents of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$ and $C_5H_5N_3O$ in the tablets.

Storage. Store protected from moisture.

Rifampicin, Isoniazid, Pyrazinamide and Ethambutol Tablets

Rifampin, Isonicotinylhydrazid, Pyrazinamide and Ethambutol Hydrochloride Tablets

Rifampicin, Isoniazid, Pyrazinamide and Ethambutol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of rifampicin, $C_{43}H_{58}N_4O_{12}$, isoniazid, $C_6H_7N_3O$, pyrazinamide, $C_5H_5N_3O$ and ethambutol hydrochloride, $C_{10}H_{24}N_2O_2.2HCl$.

Usual strength. Rifampicin 150 mg, Isoniazid 75 mg, Pyrazinamide 400 mg and Ethambutol 275 mg.

Identification

A. In the Assay for rifampicin, isoniazid and pyrazinamide, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with the reference solution.

B. In the Assay for ethambutol hydrochloride, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. A mixture of 10 volumes of a 21.01 per cent w/v solution of *citric acid*, 23 volumes of a 13.61 per cent w/v solution of *potassium dihydrogen phosphate*, 77 volumes of a 17.42 per cent w/v solution of *dipotassium hydrogen phosphate*, 640 volumes of *water* and 250 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of powdered tablets containing 200 mg of Rifampicin, dissolve in 100 ml of *acetonitrile* and filter. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Reference solution (a). Dissolve 20 mg of *rifampicin RS* in 100 ml of *acetonitrile*. Dilute 1 ml of this solution to a 100 ml with the solvent mixture.

Reference solution (b). A solution containing 0.01 per cent w/v each of *rifampicin RS* and *rifampicin quinone RS* in *acetonitrile*. Dilute 5 ml of this solution to 50 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 65 volumes of a solution containing 0.1 per cent v/v of *orthophosphoric acid*, 0.19 per cent w/v of *sodium perchlorate*, 0.59 per cent w/v of *citric acid* and 2.09 per cent w/v of *potassium dihydrogen phosphate*, 35 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between rifampicin and rifampicin quinone is not less than 4, the tailing factor is not more than 2.0 and the column efficiency is not less than 2000 theoretical plates.

Inject the test solution and reference solution (a). The relative retention times are 1.0, 0.55, 1.25, 1.51 and 2.61 for rifampicin, rifampicin quinone, rifampicin N-oxide, 3-formylrifamycin SV Isonicotinyl hydrazone and 3-formylrifamycin SV respectively. Multiply the area of each known impurity by its response factor. The response factors are 1.00, 1.19, 1.03, 1.22 and 1.25 for rifampicin, rifampicin quinone, rifampicin N-oxide, Isonicotinyl hydrazone and 3-formylrifamycin SV respectively.

In the chromatogram obtained with the test solution the area of any peak due to rifampicin quinone should not be more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4 per cent), the area of any peak due to rifampicin N-oxide should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent), the area of any peak due to 3-formylrifamycin SV Isonicotinyl hydrazone should not be more than 5 times the area of the principal peak in the chromatogram obtained with the reference solution (5 per cent) and the area of any peak due to 3-formylrifamycin SV should not be more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent). In the chromatogram obtained with the test solution the area of any unknown peak should not be more than 1.5 times the area of the principal peak in the chromatogram obtained with the reference solution (1.5 per cent).

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *sodium phosphate buffer pH 6.8* prepared by dissolving 7 g of *dibasic sodium phosphate anhydrous* in 5000 ml of *water* and adjusting to pH 6.8 with *phosphoric acid*,

Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc with an average pore diameter not greater than 0.8 µm. Reject the first few ml of the filtrate. Determine the amounts of rifampicin, $C_{43}H_{58}N_4O_{12}$, isoniazid, $C_6H_7N_3O$, pyrazinamide, $C_5H_5N_3O$ and ethambutol hydrochloride, $C_{10}H_{24}N_2O_2.2HCl$ by using the methods described in the Assay for rifampicin, isoniazid, pyrazinamide and ethambutol hydrochloride.

D. Not less than 75 per cent of the stated amounts of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$, $C_5H_5N_3O$ and $C_{10}H_{24}N_2O_2.2HCl$.

Other tests. Comply with the tests stated under Tablets.

Assay. *For rifampicin, isoniazid and pyrazinamide* — Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablets containing about 40 mg of Isoniazid, dissolve in 100 ml of *methanol*, dilute to 500 ml with the buffer solution pH 6.8, mix and filter.

Reference solution. A solution containing 0.08 per cent w/v of *rifampicin RS*, 0.04 per cent w/v of *isoniazid RS* and 0.2 per cent w/v of pyrazinamide *RS* in *methanol*. Dilute 10.0 ml of this solution to 50.0 ml with the buffer solution pH 6.8.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column. temperature 30°,
- mobile phase: A. a mixture of 96 volumes of buffer solution pH 6.8 prepared by dissolving 1.4 g of *disodium hydrogen orthophosphate anhydrous* in 1000 ml of *water*, adjusting to pH 6.8 ± 0.05 with *dilute phosphoric acid* and 4 volumes of *acetonitrile*,

  B. a mixture of 45 volumes of the buffer solution and 55 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Time (in min.)	Solution A (per cent v/v)	Solution B (per cent v/v)
0	100	0
5	100	0
6	0	100
15	0	100
16	100	0
22	100	0

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 2.0 for rifampicin, isoniazid and pyrazinamide and the column efficiencies for isoniazid, pyrazinamide and rifampicin are not less than 3000, 5000 and 25000 theoretical plates respectively.

Inject the test solution and the reference solution. The relative retention time for rifampicin is 1.8, for isoniazid, 0.7 and for pyrazinamide, 1.0.

Calculate the contents of $C_{43}H_{58}N_4O_{12}$, $C_6H_7N_3O$ and $C_5H_5N_3O$ in the tablets.

For ethambutol hydrochloride — Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 60 mg of Ethambutol Hydrochloride and dissolve in 100 ml of the solvent mixture.

Reference solution. A 0.06 per cent w/v solution of *ethambutol hydrochloride RS* in the solvent mixture.

Chromatographic system

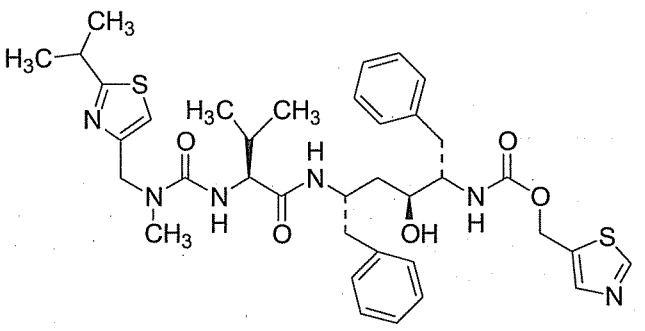
- a stainless steel column 15 cm × 4.6 mm, packed with nitrile groups chemically bonded to porous silica particles (5 µm),
- mobile phase: a mixture of 50 volumes of *acetonitrile*, and 50 volumes of buffer solution pH 7.0 (diluent) prepared by dissolving 1 ml of *triethylamine* in 1000 ml of *water* and adjusting the pH to 7.0 with *dilute phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 200 nm,
- injection volume. 50 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent, the tailing factor is not more than 3.0 and the column efficiency determined from ethambutol hydrochloride peak is not less than 1500 theoretical plates.

Calculate the content of $C_{10}H_{24}N_2O_2.2HCl$ in the tablets.

Storage. Store protected from moisture.

Ritonavir



$C_{37}H_{48}N_6O_5S_2$ Mol. Wt. 721.0

Ritonavir is (5*S*,8*S*,10*S*,11*S*)-10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester.

Ritonavir contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{37}H_{48}N_6O_5S_2$, calculated on the anhydrous basis.

Category. Antiretroviral.

Dose. 100 mg twice daily.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ritonavir RS* or with the reference spectrum of ritonavir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to ritonavir in the chromatogram obtained with the reference solution.

C. Melting point. 119° to 123° (2.4.21).

Tests

Specific optical rotation (2.4.22). +7.0° to +10.5°, determined in a 0.2 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14), as described in the Assay and calculate the percentage of each impurity peak in the chromatogram obtained with the test solution. Not more than 0.5 per cent of any individual impurity and not more than 1.0 per cent of total impurities is found.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent

Water (2.3.43). Not more than 0.5 per cent, determined on 2.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in sufficient of a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water* to produce 100.0 ml.

Reference solution. A 0.025 per cent w/v solution of *ritonavir RS* in a mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *acetonitrile* and 55 volumes of a buffer solution prepared by dissolving 3.4 g of *sodium acetate* and 0.94 g of *sodium hexanesulphonate* in 1000 ml of *water* and adjusting the pH to 4.0 with *hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the ritonavir peak is not less than 1000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{37}H_{48}N_6O_5S_2$.

Storage. Store protected from light.

Ritonavir Capsules

Ritonavir Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ritonavir, $C_{37}H_{48}N_6O_5S_2$.

Usual strength. 100 mg.

Identification

A. In the test for Assay, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima at the same wavelengths as the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid* with 25 mM polyoxyethylene 10 lauryl ether prepared by dissolving 15.65 g of *polyoxyethylene 10 lauryl ether* in 950 ml of *water*. Add 8.5 ml of *hydrochloric acid* and dilute to 1000 ml with *water*,
Speed and time. 50 rpm and 90 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate and if necessary, dilute with the dissolution medium.

Reference solution. A 0.1 per cent w/v solution of *ritonavir RS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the test solution and the reference solution.

D. Not less than 70 per cent of the stated amount of $C_{37}H_{48}N_6O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 40 volumes of *0.03M monobasic potassium phosphate buffer* and 60 volumes of *acetonitrile*.

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of Ritonavir, dissolve in 50 ml of the solvent mixture and filter.

Reference solution (a). A 0.05 per cent w/v solution of *ritonavir RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of the solution to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with butyl silane chemically bonded to porous silica (3 µm) (Such as ACE C4),
- mobile phase: A. a mixture of 69 volumes of *0.03 M monobasic potassium phosphate buffer* prepared by dissolving 4.1 g of *monobasic potassium phosphate* in 1000 ml of *water*, 18 volumes of acetonitrile, 8 volumes of *tetrahydrofuran* and 5 volumes of n-*butanol*,
  B. a mixture of 40 volumes of *0.03 M monobasic potassium phosphate buffer*, 47 volumes of acetonitrile, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-butanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- injection volume. 50 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	100	0
60	100	0
120	0	100
155	100	0

Inject the reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 5000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 25 mg of Ritonavir, disperse in 100.0 ml of *methanol* and filter.

Reference solution. A 0.025 per cent w/v solution of *ritonavir RS* in *methanol*.

Chromatographic system

- a stainless steel column 5 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm),
- column temperature 45°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 3.4 g of *sodium acetate trihydrate* and 0.94 g of *sodium 1-hexanesulphonate* in 1000 ml of *water* and adjusting the pH to 4.0 with *hydrochloric acid*, and 45 volumes of *acetonitrile*,
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Inject the reference solution. Run the chromatogram 1.5 times the retention time (about 3 minutes)of the principal peak The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{37}H_{48}N_6O_5S_2$ in the capsules.

Storage. Store protected from light in a refrigerator (2° to 8°).

Ritonavir Tablets

Ritonavir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ritonavir, $C_{37}H_{48}N_6O_5S_2$.

Usual strength. 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2)

Apparatus No. 1,

Medium. 900 ml of a solution prepared by dissolving 15.7 g of *polyoxyethylene 10-lauryl ether* in 1000 ml of a 0.85 per cent v/v solution of *hydrochloric acid*,
Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

Reference solution. A 0.1 per cent w/v solution of *ritonavir RS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system described under Assay.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{37}H_{48}N_6O_5S_2$.

D. Not less than 75 per cent of the stated amount of $C_{37}H_{48}N_6O_5S_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 40 volumes of *0.03 M monobasic potassium phosphate* and 60 volumes of *acetonitrile*.

Test solution: Shake a quantity of the powdered tablets containing 25 mg of Ritonavir with 50 ml of the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *ritonavir RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with silica gel consisting of porous spherical particles with chemically bonded with nitrile group (3µm) (such as YMC C4),
- mobile phase: A. a mixture of 69 volumes of *0.03 M monobasic potassium phosphate solution*, 18 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n- butanol*,
  B. a mixture of 40 volumes of *0.03 M monobasic potassium phosphate solution*, 47 volumes of *acetonitrile*, 8 volumes of *tetrahydrofuran* and 5 volumes of *n-butanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 240 nm,
- injection volume. 100 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
60	100	0
120	0	100
121	100	0
155	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (b) (5.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14)

Test solution. Weigh accurately a quantity of the powdered tablets containing 25 mg of Ritonavir and disperse in 100.0 ml of *methanol*.

Reference solution. A 0.025 per cent w/v solution of *ritonavir RS* in *methanol*.

Chromatographic system
- a stainless steel column 5 cm × 4.6 mm, packed with phenyl stationary phase bonded to porous silica (3µm),
- column temperature 45°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 3.4 g of *sodium acetate trihydrate* and 0.94 g of *sodium 1-hexanesulphonate* to 1000 ml with *water* and adjusting the pH to 4.0 with *dilute hydrochloric acid*, and 45 volumes of *acetonitrile*.
- flow rate. 2.5 ml per minute,
- spectrophotometer set at 239 nm,
- injection volume. 10 µl.

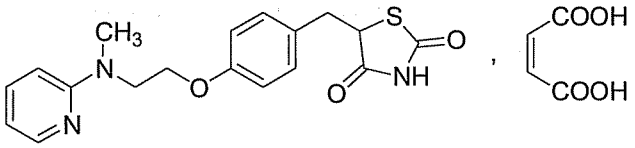
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{37}H_{48}N_6O_5S_2$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Rosiglitazone Maleate



$C_{18}H_{19}N_3O_3S,C_4H_4O_4$ Mol. Wt. 473.5

Rosiglitazone Maleate is (*RS*)-5-{*p*-[2-(methyl-2-pyridylamino) ethoxy]benzyl}-2,4-thiazolidinedione maleate (1:1).

Rosiglitazone Maleate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{18}H_{19}N_3O_3S,C_4H_4O_4$, calculated on the anhydrous basis.

Category. Antidibatic

Description. A white to off-white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rosiglitazone maleate RS*.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50 ml of mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *rosiglitazone maleate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent). Ignore any peak (due to maleic acid) with a relative retention time of about 0.4.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of mobile phase.

Reference solution. A 0.020 per cent w/v solution of *rosiglitazone maleate RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *buffer solution* prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water* and adjust the pH to 3.0 with *dilute phosphoric acid*, 25 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{19}N_3O_3S,C_4H_4O_4$.

Storage. Store protected from light.

Rosiglitazone Tablets

Rosiglitazone Maleate Tablets

Rosiglitazone Tablets contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of rosiglitazone, $C_{18}H_{19}N_3O_3S$.

Usual strengths. 1 mg; 2 mg; 4 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 75 rpm for 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure

the absorbance of the resulting solution at the maximum at about 318 nm (2.4.7). Similarly measure the absorbance of a standard solution of known concentration of *rosiglitazone maleate RS* and calculate the content of $C_{18}H_{19}N_3O_3S$.

D. Not less than 70 per cent of the stated amount of $C_{18}H_{19}N_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of powdered tablets containing 200 mg of Rosiglitazone, disperse in 100.0 ml of mobile phase. Centrifuge and use clear supernatant liquid.

Reference solution (a). A 0.2 per cent w/v solution of *rosiglitazone maleate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Use the chromatographic system described under Assay.

Inject reference solution (b). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent). Ignore any peak (due to maleic acid) with a relative retention time of about 0.4.

Uniformity of content. Comply with the tests stated under Tablets.

Disperse 1 tablet in *0.1 M hydrochloric acid* to produce 0.004 per cent w/v solution. Measure the absorbance of the resulting solution at the maximum at about 318 nm (2.4.7). Calculate the content of $C_{18}H_{19}N_3O_3S$ from the absorbance obtained from same concentration of *rosiglitazone maleate RS* in the same medium.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablets containing 20 mg of Rosiglitazone, disperse in 100.0 ml of mobile phase. Centrifuge and use clear supernatant liquid.

Reference solution. A 0.020 per cent w/v solution of *rosiglitazone maleate RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecysilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 65 volumes of *0.01 M potassium hydrogen phosphate* adjusted to pH 3.0 with *orthophosphoric acid*, 25 volumes of *acetonitrile* and 10 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

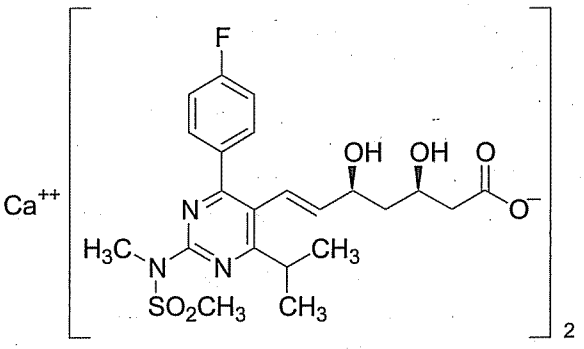
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 4000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{18}H_{19}N_3O_3S$.

Storage. Store protected from light and moisture.

Rosuvastatin Calcium



$(C_{22}H_{27}FN_3O_6S)_2.Ca$ Mol. Wt. 1001.1

Rosuvastatin Calcium is (*E*)-(3*R*,5*S*)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl(methylsulphonylamino)]pyrimidin-5-yl}-3,5-dihydroxyhepten-6-oic acid calcium.

Rosuvastatin Calcium contains not less than 98.0 per cent and not more than 102.0 per cent of $(C_{22}H_{27}FN_3O_6S)_2.Ca$. calculated on the anhydrous basis.

Category. Antihyperlipidaemic.

Description. An off- white to creamish white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rosuvastatin calcium RS*.

B. Dissolve 20 mg in 25 ml of *methanol*, add 2 drops of *methyl red indicator* neutralise with *6 M ammonium hydroxide*. Add *3 M hydrochloric acid* until the solution is acidic to the

indicator. Add *ammonium oxalate solution*, a white precipitate is obtained.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *rosuvastatin calcium RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of 0.2 per cent w/v *acetic acid* in *water*, 25 volumes of *acetonitrile* and 25 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Water (2.3.43). Not more than 8.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase, mix and filter.

Reference solution. A 0.05 per cent w/v of *rosuvastatin calcium RS* in mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of 0.2 per cent *acetic acid* in *water,* 25 volumes of *acetonitrile* and 25 volumes of *methanol*, filter and degas.
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $(C_{22}H_{27}FN_3O_6S)_2.Ca$.

Storage. Store protected from light and moisture.

Rosuvastatin Tablets

Rosuvastatin Calcium Tablets

Rosuvastatin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of rosuvastatin, $C_{44}H_{54}F_2N_6O_{12}S_2$.

Usual strengths. 5 mg; 10 mg; 20 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of phosphate buffer pH 6.8,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A 0.05 per cent w/v solution of *rosuvastatin calcium RS* in the mobile phase. Dilute 2 ml of the solution to 100 ml with Dissolution medium.

Chromatographic system as described under Assay.

Calculate the content of $C_{44}H_{54}F_2N_6O_{12}S_2$.

D. Not less than 70 per cent of the stated amount of $C_{44}H_{54}F_2N_6O_{12}S_2$.

Uniformity of content. Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. Disperse one tablet in 100 ml of the mobile phase. Dilute 5 ml of the solution to 10 ml with mobile phase and filter.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 25 mg of Rosuvastatin, disperse in 50 ml of mobile phase and filter.

Reference solution (a). A 0.05 per cent w/v solution of *rosuvastatin calcium RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3.0 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 25 mg of Rosuvastatin, disperse in 100.0 ml of mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase and filter.

Reference solution. A 0.025 per cent w/v solution of *rosuvastatin calcium RS* in mobile phase. Dilute 5.0 ml of the solution to 25.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 30°,
- mobile phase: a mixture of 585 volumes of a buffer solution prepared by dissolving 1.54 g of *ammonium acetate* in 900 ml *water*, adjust pH to 4.0 with *glacial acetic acid* and dilute to 1000 ml with *water*, 360 volumes of *acetonitrile* and 50 volumes of *tetrahydrofurane*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

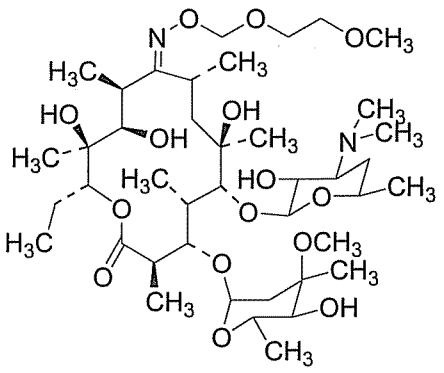
Inject the test solution and the reference solution.

Calculate the content of $C_{44}H_{54}F_2N_6O_{12}S_2$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of Rosuvastatin.

Roxithromycin



$C_{41}H_{76}N_2O_{15}$ Mol. Wt. 837.0

Roxithromycin is (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(*E*)-[(2-methoxyethoxy)methoxy]imino]-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecan-2-one; erythromycin 9-(*E*)-[*O*-[(2-methoxyethoxy)methyl]oxime].

Roxithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{41}H_{76}N_2O_{15}$, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. 150 mg twice a day.

Description. A white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *roxithromycin RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1) and colourless (2.4.1).

Specific optical rotation (2.4.22). – 93.0° to – 96.0°, determined in a 1.0 per cent w/v solution in *acetone*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 30 volumes of *acetonitrile* and 70 volumes of a 4.8 per cent w/v solution of *ammonium dihydrogen phosphate* and adjust the pH to 5.3 with *dilute sodium hydroxide solution*.

Test solution. Dissolve 50.0 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *roxithromycin RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the solvent mixture.

Reference solution (c). Dissolve 2 mg of *erythromycin 9-(E)-[O-[[(2-methoxyethoxy)methoxy]methyl]oxime] (impurity A)* in 10 ml of reference solution (a). Further dilute 1 ml of this solution to 10 ml with reference solution (a).

Reference solution (d). Dilute 1.0 ml of *toluene* to 100 ml with *acetonitrile*. Dilute 0.2 ml of this solution to 200 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm), with a 10 nm pore size and a carbon loading of about 19 per cent,
- column temperature. 15°,
- mobile phase: A. a mixture of 26 volumes of *acetonitrile* and 74 volumes of a 5.97 per cent w/v solution of *ammonium dihydrogen phosphate*, adjusted to pH 4.3 with *dilute sodium hydroxide solution*,
  B. a mixture of 30 volumes of *water* and 70 volumes of *acetonitrile*,
- flow rate.1.1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
50	100	0
80	90	10
100	100	0

Inject reference solution (c). Relative retention time between roxithromycin and erythromycin 9-(E)-[O-[[(2-methoxyethoxy) methoxy]methyl] oxime] (impurity A) is about 1.15. The test is not valid unless the peak-to-valley ratio H_p / H_v is not more than 2.0, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

Inject the test solution and reference solutions (b) and (d). In the chromatogram obtained with the test solution the area of the impurity A peak obtained immediately after the peak obtained with roxithromycin is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The area of any other individual impurity peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). The sum of the areas of all the peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent). Ignore any peak due to toluene identified using reference solution (d).

Heavy metals (2.3.13). Dissolve 2.0 g in a mixture of 15 volumes of *water* and 85 volumes of *acetone* and dilute to 20 ml with the same solvent mixture. 12 ml of this solution complies with the limit test for heavy metals, Method A (10 ppm). Use 1 ml of *lead standard solution (10 ppm lead)* prepared by diluting *lead standard solution (100 ppm lead)* with a mixture of 15 volumes of *water* and 85 volumes of *acetone*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.2 g.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 30 volumes of *acetonitrile* and 70 volumes of a 4.8 per cent w/v solution of *ammonium dihydrogen phosphate* and adjust the pH to 5.3 with *dilute sodium hydroxide solution*.

Test solution. Dissolve 50.0 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.2 per cent w/v solution of *roxithromycin RS* in the solvent mixture.

Reference solution (b). Dissolve 2 mg of *erythromycin 9-(E)-[O-[[(2-methoxyethoxy)methoxy]methyl]oxime] (impurity A)* in 10.0 ml of reference solution (a). Further dilute 1.0 ml of this solution to 10.0 ml with reference solution (a).

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm), with a 10 nm pore size and a carbon loading of about 19 per cent,
- column temperature 15°,
- mobile phase: mix 307 volumes of *acetonitrile* and 693 volumes of a 4.91 per cent w/v solution of *ammonium dihydrogen phosphate* adjusted to pH 5.3 with *dilute sodium hydroxide solution*,
- flow rate.1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the peak-to-valley ratio H_p / H_v is not less than 1.5.

Inject the test solution and reference solution (a)

Calculate the content of $C_{14}H_{26}N_2O_{15}$.

Storage. Store protected from light and moisture.

Roxithromycin Tablets

Roxithromycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of roxithromycin, $C_{41}H_{76}N_2O_{15}$.

Usual strengths. 150 mg; 300 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apprartus No. 1,
Medium. 900 ml of *phosphate buffer pH 6.0*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A 0.0166 per cent w/v solution of *roxithromycin RS* in the dissolution medium.

Chromatographic system as described under Assay.

Inject the test solution and the reference solution.

Calculate the content of $C_{41}H_{76}N_2O_{15}$ in the medium.

D. Not less than 70 per cent of the stated amount of $C_{41}H_{76}N_2O_{15}$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Mix 30 volumes of *acetonitrile* and 70 volumes of a 4.8 per cent w/v solution of *ammonium dihydrogen phosphate* and adjust the pH to 5.3 with *dilute sodium hydroxide solution.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Roxithromycin, add 80 ml of the solvent mixture and mix. Dilute to 100.0 ml with the solvent mixture and filter.

Reference solution (a). A 0.2 per cent w/v solution of *roxithromycin RS* in the solvent mixture.

Reference solution (b). Dissolve 2 mg of *erythromycin 9-(E)-[O-[[(2-methoxyethoxy)methoxy]methyl]oxime] (impurity A)* in 10 ml of reference solution (a). Further dilute 1 ml of this solution to 10 ml with reference solution (a).

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with spherical end-capped octadecylsilane bonded to porous silica (5 µm), with a 10 nm pore size and a carbon loading of about 19 per cent,
- column temperature. 15°,
- mobile phase: mix 307 volumes of *acetonitrile* and 693 volumes of a 4.91 per cent w/v solution of *ammonium dihydrogen phosphate* adjusted to pH 5.3 with *dilute sodium hydroxide solution*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time between roxithromycin and erythromycin-(E)-[O-[[(2-methoxyethoxy)methoxy]methyl]oxime] (impurity A) is about 1.15. The test is not valid unless the peak-to-valley ratio H_p / H_v is not more than 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to roxithromycin.

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

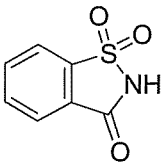
Calculate the content of $C_{41}H_{76}N_2O_{15}$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. If the tablets are dispersible, the label states that the tablets should be dispersed in water immediately before use.

S

Saccharin



$C_7H_5NO_3S$ Mol. Wt. 183.2

Saccharin is 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide.

Saccharin contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_5NO_3S$, calculated on the dried basis.

Category. Pharmaceutical aid (sweetening agent).

Description. White crystals or a white, crystalline powder; odourless or with a faint, aromatic odour.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *saccharin RS*.

B. Mix 20 mg with 20 mg of *resorcinol*, add 0.5 ml of *sulphuric acid* and heat over a small flame until a dark green colour is produced; allow to cool and add 10 ml of *water* and an excess of *2 M sodium hydroxide*; a fluorescent green liquid is produced.

C. Dissolve 0.1 g in 5 ml of a 10 per cent w/v solution of *sodium hydroxide*, evaporate to dryness and gently fuse the residue over a small flame until ammonia is no longer evolved. Allow to cool, dissolve in 20 ml of *water*, make the solution just acidic to litmus paper, filter and add 0.05 ml of *ferric chloride solution*; a violet colour is produced.

D. A saturated solution is acidic to *litmus paper*.

Tests

Appearance of solution. Dissolve 5.0 g in 20 ml of a 20 per cent w/v solution of *sodium acetate* and dilute to 25 ml with the same solution; the solution is clear (2.4.1), and colourless (2.4.1).

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 25 mg of *caffeine* in *dichloromethane* and dilute to 100 ml with the same solvent.

Test solution. Suspend 10.0 g of the substance under examination in 20 ml of *water* and dissolve using about 5 ml of *strong sodium hydroxide solution*. If necessary, adjust the pH of the solution to 7.8 with *1 M sodium hydroxide* or *1 M hydrochloric acid* and dilute to 50 ml with *water*. Shake the solution with 4 quantities, each of 50 ml, of *dichloromethane*. Combine the lower layers, dry over *anhydrous sodium sulphate* and filter. Wah the filter and the sodium sulphate with 10 ml of *dichloromethane*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a temperature not exceeding 40°. With a small quantity of *dichloromethane* transfer quantitatively the residue into a suitable 10-ml tube, evaporate to dryness in a current of *nitrogen* and dissolve the residue in 1.0 ml of the internal standard solution.

Blank solution. Evaporate 200 ml of *dichloromethane* to dryness in a water-bath at a temperature not exceeding 40°. Dissolve the residue in 1 ml of *dichloromethane*.

Reference solution. Dissolve 20 mg of *o-toluenesulphonamide* and 20 mg of *p-toluene sulphonamide* in *dichloromethane* and dilute to 100 ml with the same solvent. Dilute 5 ml of the solution to 50 ml with dichloromethane. Evaporate 5 ml of the final solution to dryness in a current of *nitrogen*. Dissolve the residue in 1 ml of the internal standard solution.

Chromatographic system
- a fused silica column 10 m × 0.53 mm packed with polymethylphenylsiloxane (film thickness 2 µm),
- temperature:
  column.180°,
  inlet port and detector. 250°,
- flame ionization detector,
- flow rate. 10 ml per minute of nitrogen (carrier gas),
- split ratio. 1:2.

Inject 1 µl of each solution. The order of elution is o-toluenesulphonamide, p-toluenesulphonamide, caffeine.

The test is not valid unless the resolution between the peaks due to o-toluenesulphonamide and p-toluenesulphonamide in the chromatogram obtained with the reference solution is not less than 1.5 and the chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, o-toluenesulphonamide and p-toluenesulphonamide.

In the chromatogram obtained with the test solution the ratio of the area of the peak due to o-toluenesulphonamide to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the rference solution (10 ppm).

In the chromatogram obtained with the test solution the ratio of the area of the peak due to p-toluenesulphonamide to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).

Arsenic (2.3.10). Mix 5.0 g with 3.0 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly.

Evaporate to dryness on a water-bath, gently ignite and add to the cooled residue a mixture of 16 ml of *brominated hydrochloric acid AsT* and 5 ml of *bromine solution*. Add 40 ml of *water* and boil gently, adding sufficient bromine solution from time to time to maintain a slight excess. Filter and remove the excess of bromine with a sufficient quantity of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 1.2 g complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb)*.

Readily carbonisable substances. Dissolve 0.2 g in 5 ml of *sulphuric acid (96 per cent w/w)* and maintain at about 50° for 10 minutes. The solution is not more intensely coloured than reference solution BYS6 (2.4.1).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

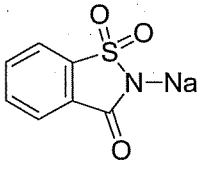
Assay. Weigh accurately about 0.5 g, dissolve in 75 ml of hot *water*, cool quickly and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01832 g of $C_7H_5NO_3S$.

Storage. Store protected from moisture.

Saccharin Sodium

Soluble Saccharin



$C_7H_4NNaO_3S$ Mol. Wt. 205.2

Saccharin Sodium is the sodium salt of 1,2-benzisothiazol-3(2*H*)-3-one 1,1-dioxide.

Saccharin Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_4NNaO_3S$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (sweetening agent).

Description. A white, crystalline powder or colourless crystals; efflorescent in dry air.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *saccharin sodium RS*.

B. Dissolve 0.1 g in 5 ml of a 10 per cent w/v solution of *sodium hydroxide*, evaporate to dryness and gently fuse the residue over a small flame until ammonia is no longer evolved. Allow to cool, dissolve in 20 ml of *water*, make the solution just acidic to *litmus paper*, filter and add 0.05 ml of *ferric chloride solution*; a violet colour is produced.

C. Mix 20 mg with 20 mg of *resorcinol*, add 0.5 ml of *sulphuric acid* and heat over a small flame until a dark green colour is produced; allow to cool and add 10 ml of *water* and an excess of *2 M sodium hydroxide*; a fluorescent green liquid is produced.

D. To 5 ml of a 10 per cent w/v solution add 3 ml of *2 M hydrochloric acid*; a white precipitate is produced. The precipitate, after washing with *water* and drying at 105° melts at 226° to 230° (2.4.21).

E. 0.5 ml of a 10 per cent w/v solution gives reaction B of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 5.0 g in sufficient *carbon dioxide-free water* to produce 50 ml (solution A). To 10 ml of solution A add 5 ml of *0.005 M sulphuric acid*, heat to boiling, cool and add 0.1 ml of *phenolphthalein solution*. Not less than 4.5 ml and not more than 5.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink.

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. Dissolve 25 mg of *caffeine* in *dichloromethane* and dilute to 100 ml with the same solvent.

Test solution. Suspend 10.0 g of the substance under examination in 50 ml of *water*. If necessary, adjust the pH of the solution to 7.8 with *1 M sodium hydroxide* or *1 M hydrochloric acid* and dilute to 50 ml with *water*. Shake the solution with 4 quantities, each of 50 ml, of *dichloromethane*. Combine the lower layers, dry over *anhydrous sodium sulphate* and filter. Wah the filter and the sodium sulphate with 10 ml of *dichloromethane*. Combine the solution and the washings and evaporate almost to dryness in a water-bath at a

temperature not exceeding 40°. With a small quantity of *dichloromethane* transfer quantitatively the residue into a suitable 10-ml tube, evaporate to dryness in a current of *nitrogen* and dissolve the residue in 1.0 ml of the internal standard solution.

Blank solution. Evaporate 200 ml of *dichloromethane* to dryness in a water-bath at a temperature not exceeding 40°. Dissolve the residue in 1 ml of *dichloromethane.*

Reference solution. Dissolve 20 mg of *o-toluenesulphonamide* and 20 mg of *p-toluene sulphonamide* in *dichloromethane* and dilute to 100 ml with the same solvent. Dilute 5 ml of this solution to 50 ml with *dichloromethane.* Evaporate 5 ml of the final solution to dryness in a current of *nitrogen.* Dissolve the residue in 1 ml of the internal standard solution.

Chromatographic system
- a fused silica column 10 m x 0.53 mm packed with polymethylphenylsiloxane (film thickness 2 µm),
- temperature:
  column.180°,
  inlet port and detector. 250°
  flame ionization detector,
- flow rate. 10 ml per minute of nitrogen (carrier gas),
- split ratio. 1:2.

Inject 1 µl of each solution. The order of elution is o-toluenesulphonamide, p-toluenesulphonamide, caffeine.

The test is not valid unless the resolution between the peaks due to o-toluenesulphonamide and p-toluenesulphonamide in the chromatogram obtained with the reference solution is not less than 1.5 and the chromatogram obtained with the blank solution does not show any peak with the same retention times as the internal standard, o-toluenesulphonamide and p-toluenesulphonamide.

In the chromatogram obtained with the test solution the ratio of the area of the peak due to o-toluenesulphonamide to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the reference solution (10 ppm).

In the chromatogram obtained with the test solution the ratio of the area of the peak due to p-toluenesulphonamide to that of the internal standard is not greater than the corresponding ratio in the chromatogram obtained with the rference solution (10 ppm).

Arsenic (2.3.10). Mix 5.0 g with 3 g of *anhydrous sodium carbonate,* add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite and add to the cooled residue a mixture of 16 ml of *brominated hydrochloric acid AsT* and 5 ml of *bromine solution.* Add 40 ml of water and boil gently, adding sufficient *bromine solution* from time to time to maintain a slight excess. Filter and remove the excess of bromine with a sufficient quantity of *stannous chloride solution AsT.* The resulting solution complies with the limit test for arsenic (2 ppm).

Readily carbonisable substances. Dissolve 0.2 g in 5 ml of *sulphuric acid (96 per cent w/w)* and maintain at about 50° for 10 minutes. The solution is not more intensely coloured than reference solution BS6 (2.4.1).

Heavy metals (2.3.13). 12 ml of solution A complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (2 ppm Pb).*

Water (2.3.43). Not more than 15.0 per cent, determined on 0.2 g.

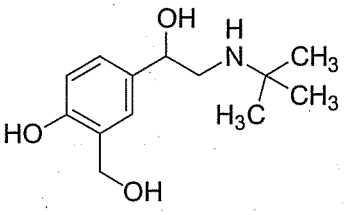
Assay. Weigh accurately about 0.15 g, dissolve in 50 ml of *anhydrous glacial acetic acid,* with slight heating if necessary. Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02052 g of $C_7H_4NNaO_3S$.

Storage. Store protected from moisture.

Salbutamol

Albuterol



$C_{13}H_{21}NO_3$ Mol. Wt. 239.3

Salbutamol is (*RS*)-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol.

Salbutamol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_{21}NO_3$, calculated on the dried basis.

Category. Beta-adrenoceptor agonist.

Dose. Orally, 6 to 16 mg daily, in divided doses; by inhalation, for chronic bronchial asthma or as a prophylactic, 2 inhalations of 100 µg, 3 or 4 times daily; for the relief of acute bronchospasm, 1 or 2 inhalations of 100 µg as a single dose when required, up to a maximum of 8 inhalations in 24 hours; by subcutaneous or intramuscular injection, 500 µg, repeated every 4 hours, if necessary; by slow intravenous injection, 250 µg, repeated if necessary.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *salbutamol RS* or with the reference spectrum of salbutamol.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.008 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 276 nm; absorbance at about 276 nm, about 0.53 to 0.60.

C. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve 10 mg in 50 ml of a 2 per cent w/v solution of *borax*, add 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide* and 10 ml of *chloroform*, shake and allow to separate; an orange-red colour is produced in the chloroform layer.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *methanol* is clear (2.4.1), and not more intensely coloured than reference solution BYS5 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. A solution containing 0.02 per cent w/v each of *salbutamol RS* and *(1RS)-2-[(1,1-dimethylethyl)amino]-1-(4- hydroxyphenyl)ethanol RS (salbutamol impurity A RS)* in the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.29 per cent w/v of *sodium heptanesulphonate* and 0.25 per cent w/v of *potassium dihydrogen phosphate,* adjusted to pH 3.7 with *orthophosphoric acid,*
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to due to salbutamol and salbutamol impurity A is not less than 3.0. The relative retention time with reference to salbutamol for salbutamol impurity A is about 1.3.

Inject the test solution and the reference solution. Run the chromatogram 25 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of areas of all the secondary peaks is not more than 3.3 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.05 per cent the area of the principal peak in the chromatogram obtained with the reference solution.

Boron. To 50 mg add 5 ml of a solution containing 1.3 per cent w/v of *anhydrous sodium carbonate* and 1.7 per cent w/v of *potassium carbonate*, evaporate to dryness on a water-bath and dry at 120°. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool, add 0.5 ml of *water* and 3.0 ml of a freshly prepared 0.125 per cent w/v solution of *curcumin* in *glacial acetic acid.* Evaporate to dryness and allow to cool. Add 3 ml of a mixture prepared by adding 5 ml of *sulphuric acid*, slowly and with stirring, to 5 ml of *glacial acetic acid.* Mix and allow to stand for 30 minutes. Add sufficient *ethanol (95 per cent)* to produce 100.0 ml, filter and measure the absorbance of the filtrate at 555 nm (2.4.7). Prepare a reference solution in the following manner. Dissolve 0.572 g of *boric acid* in 1000.0 ml of *water*. Dilute 1.0 ml to 100.0 ml with water. To 2.5 ml of this solution add 5 ml of a solution containing 1.3 per cent w/v of *anhydrous sodium carbonate* and 1.7 per cent w/v of *potassium carbonate* and treat this mixture in the same manner as described above beginning at the words "Evaporate to dryness...". The absorbance of the solution prepared from the substance under examination is not more than that of the reference solution (50 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in 30 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02393 g of $C_{13}H_{21}NO_3$.

Storage. Store protected from light.

Salbutamol Inhalation

Salbutamol Inhalation Aerosol; Albuterol Inhalation Aerosol

Salbutamol Inhalation is a suspension of microfine Salbutamol or Salbutamol Sulphate in a suitable liquid in a suitable

pressurised container. It may contain suitable pharmaceutical aids such as surfactants, stabilising agents etc.

Salbutamol Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amount of salbutamol, $C_{13}H_{21}NO_3$, per inhalation, by actuation of the valve.

Usual strength. 100 µg in each metered-dose.

Identification

A. Discharge the container a sufficient number of times into a mortar to obtain about 2 mg of salbutamol, grind the residue thoroughly with 0.1 g of potassium bromide, add a further 0.2 g of potassium bromide and mix thoroughly.

On the resultant dispersion determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *salbutamol RS* or with the reference spectrum of salbutamol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Discharge the inhaler a sufficient number of times into a small, dry beaker to obtain 10 mg of Salbutamol and dissolve the residue in 0.5 ml of *methanol*.

Reference solution (a). Dilute 1.0 volume of test solution (a) to 200 volumes with *methanol*.

Reference solution (b). A 0.010 per cent w/v solution of *salbutamol RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, place it for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised sulphanilic acid solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). Ignore any spot with an R_f value higher than 0.85.

Other tests. Complies with the tests stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Follow the procedure described under Assay wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Use 30 ml of *ethanol* for preparations containing salbutamol and 30 ml of a mixture of equal volumes of *ethanol* and *water* for preparations containing salbutamol sulphate and dilute the final solution and washings to 200.0 ml with *ethanol*. Dilute a suitable volume of this solution with *ethanol* to produce a solution containing 10 µg of salbutamol per ml. To 20 ml of the solution in a separating funnel add 180 ml of *water* and in the following order, 4 ml of *N,N-dimethyl-4-phenylenediamine sulphate solution* and 4 ml of a freshly prepared 8 per cent w/v solution of *potassium ferricyanide*. Mix, allow to stand for 15 minutes in subdued light and extract with two quantities, each of 10 ml, of *chloroform*. Filter the extracts through a plug of *cotton wool*, dilute to 25 ml with *chloroform* and measure the absorbance of the resulting solution at 605 nm (2.4.7). Calculate the content of $C_{13}H_{21}NO_3$ in the solution from the absorbance obtained by repeating the operation using a suitable quantity of a 0.001 per cent w/v solution of *salbutamol RS* in *ethanol*.

Calculate the amount of $C_{13}H_{21}NO_3$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $C_{13}H_{21}NO_3$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from light and moisture at a temperature not exceeding 30°.

Labelling. The label states whether the preparation contains Salbutamol or Salbutamol Sulphate.

When the active ingredient is Salbutamol Sulphate, the quantity is stated in terms of the equivalent amount of salbutamol.

Salbutamol Sulphate

Albuterol Sulphate

$(C_{13}H_{21}NO_3)_2,H_2SO_4$ Mol. Wt. 576.7

Salbutamol Sulphate is (*RS*)-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(*tert*-butylamino)ethanol sulphate.

Salbutamol Sulphate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{13}H_{21}NO_3)_2, H_2SO_4$, calculated on the dried basis.

Category. Beta-adrenoceptor agonist.

Dose. Orally, the equivalent of 6 to 16 mg of salbutamol daily, in divided doses; by slow intravenous injection, the equivalent of 250 µg of salbutamol or by intravenous infusion, the equivalent of 3 to 20 µg of salbutamol per minute. (1 mg of Salbutamol Sulphate is approximately equivalent to 830 µg of salbutamol).

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and C may be omitted if tests A, D and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *salbutamol sulphate RS* or with the reference spectrum of salbutamol sulphate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.008 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 276 nm; absorbance at about 276 nm, 0.44 to 0.51.

C. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with reference solution (b).

D. Dissolve 10 mg in 50 ml of a 2 per cent w/v solution of *borax*, add 1 ml of a 3 per cent w/v solution of *4-amino-phenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide* and 10 ml of *chloroform*, shake and allow to separate; an orange-red colour is produced in the chloroform layer.

E. Gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity or alkalinity. To 10 ml of a 1.0 per cent w/v solution in *carbon dioxide-free water* add 0.15 ml of *methyl red solution* and 0.2 ml of *0.01 M sodium hydroxide*. The solution is yellow and not more than 0.4 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. A solution containing 0.02 per cent w/v each of *salbutamol RS* and *(1RS)-2-[(1,1-dimethylethyl)amino]-1-(4- hydroxyphenyl)ethanol RS (salbutamol impurity A RS)* in the mobile phase. Dilute 2.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.29 per cent w/v of *sodium heptanesulphonate* and 0.25 per cent w/v of *potassium dihydrogen phosphate,* adjusted to pH 3.7 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to due to salbutamol and salbutamol impurity A is not less than 3.0. The relative retention time with reference to salbutamol for salbutamol impurity A is about 1.3.

Inject the test solution and the reference solution. Run the chromatogram 25 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.3 per cent) and the sum of areas of all the secondary peaks is not more than 3.3 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.05 per cent the area of the principal peak in the chromatogram obtained with the reference solution.

Boron. To 50 mg add 5 ml of a solution containing 1.3 per cent w/v of *anhydrous sodium carbonate* and 1.7 per cent w/v of *potassium carbonate*, evaporate to dryness on a water-bath and dry at 120°. Ignite the residue rapidly until the organic matter has been destroyed, allow to cool, add 0.5 ml of *water* and 3.0 ml of a freshly prepared 0.125 per cent w/v solution of *curcumin* in *glacial acetic acid*. Evaporate to dryness and allow to cool. Add 3 ml of a mixture prepared by adding 5 ml of *sulphuric acid*, slowly and with stirring, to 5 ml of *glacial acetic acid*. Mix and allow to stand for 30 minutes. Add sufficient *ethanol (95 per cent)* to produce 100.0 ml, filter and measure the absorbance of the filtrate at 555 nm (2.4.7). Prepare a reference solution in the following manner. Dissolve 0.572 g of *boric acid* in 1000.0 ml of *water*. Dilute 1.0 ml to 100.0 ml with *water*. To 2.5 ml of this solution add 5 ml of a solution containing 1.3 per cent w/v of *anhydrous sodium carbonate* and 1.7 per cent w/v of *potassium carbonate* and treat this mixture in the same manner as described above beginning at the words " Evaporate to dryness.....".The absorbance of the solution prepared from the substance under examination is not more than that of the reference solution (50 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g, dissolve in 5 ml of *anhydrous formic acid*, add 35 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05767 g of $(C_{13}H_{21}NO_3)_2, H_2SO_4$.

Storage. Store protected from light.

Salbutamol Injection

Albuterol Sulphate Injection; Salbutamol Sulphate Injection

Salbutamol Injection is a sterile solution of Salbutamol Sulphate in Water for Injections containing suitable stabilising agents.

Salbutamol Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of salbutamol, $C_{13}H_{21}NO_3$.

Usual strengths. The equivalent of 250 µg of salbutamol per ml; the equivalent of 500 µg of salbutamol per ml; the equivalent of 5 mg of salbutamol in 5 ml (for intravenous infusion). (1 mg of Salbutamol Sulphate is approximately equivalent to 830 µg of salbutamol).

Identification

A. Dilute a volume with sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.008 per cent w/v of salbutamol. When examined in the range 230nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 276 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Evaporate a suitable volume of the injection to dryness using a rotary evaporator, wash the residue with four quantities, each of 5 ml, of *ethanol*, filter, evaporate the filtrate to dryness and dissolve the residue in sufficient *water* to produce a solution containing the equivalent of 0.1 per cent w/v of salbutamol.

Reference solution. A 0.12 per cent w/v solution of *salbutamol sulphate RS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable, place for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Dilute a volume containing 0.5 mg of salbutamol to 50 ml with water, add 1 ml of *dilute ammonia solution*, 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide* and 10 ml of *chloroform*. Shake and allow to separate; an orange-red colour is produced in the chloroform layer.

D. A volume containing 1 mg of salbutamol gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 3.4 to 5.0.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of injection containing about 5 mg of Salbutamol in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.0004 per cent w/v of *(1RS)-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxyphenyl)ethanol RS (salbutamol impurity A RS)* and 0.0005 per cent w/v of *salbutamol sulphate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with endcapped octylsilane bonded to porous silica (5 µm) (such as Waters Symmetry C8),
- mobile phase: a mixture of 22 volumes of *acetonitrile* and 78 volumes of a solution containing 0.29 per cent w/v of *sodium heptanesulphonate* and 0.25 per cent w/v of *potassium dihydrogen phosphate*, adjusted to pH 3.7 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the two principal peaks is not less than 3.0.

Inject the test solution and reference solution (a). Run the chromatogram 25 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of all the secondary peaks is not more than twice the area of the principal peak in

the chromatogram obtained with reference solution (a) (2.0 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a volume, accurately measured, containing about 0.15 mg of salbutamol with sufficient *water* to produce 80 ml, add 4 ml of a 5 per cent w/v solution of *sodium bicarbonate*, 4 ml of *N,N-dimethyl-4-phenylenediamine sulphate solution* and 4 ml of a freshly prepared 8 per cent w/v solution of *potassium ferricyanide*. Mix, allow to stand for 15 minutes, protected from light. Extract with two quantities, each of 10 ml, of *chloroform*. Filter the extracts through a plug of cotton wool and dilute to 25.0 ml with *chloroform*. Measure the absorbance of the resulting solution at 605 nm (2.4.7).

Calculate the content of $C_{13}H_{21}NO_3$ from the absorbance obtained by repeating the operation using 10.0 ml of a 0.0018 per cent w/v solution of *salbutamol sulphate*.

Storage. Store protected from light, in single dose containers in which the air has been displaced by nitrogen or other suitable inert gas.

Labelling. The label states the strength in terms of the equivalent amount of salbutamol in a suitable dose-volume.

Salbutamol Syrup

Albuterol Sulphate Syrup; Salbutamol Sulphate Syrup

Salbutamol Syrup contains Salbutamol Sulphate equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of salbutamol, $C_{13}H_{21}NO_3$.

Usual strength. The equivalent of 2 mg of salbutamol in 5 ml. (1 mg of Salbutamol Sulphate is approximately equivalent to 830 µg of salbutamol).

Identification

A. To 5 ml add 50 ml of a 2 per cent w/v solution of *borax*, 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide* and 10 ml of *chloroform*. Shake and allow to separate; an orange-red colour is produced in the chloroform layer.

B. To 5 ml add sufficient *1 M sodium hydroxide* to make the solution alkaline, add 1 ml of *alkaline borate buffer pH 9.2* and 1 ml of a 0.04 per cent w/v solution of *2,6-dichloroquinone chlorimide* in *ethanol (95 per cent)*; a blue colour develops.

Tests

pH (2.4.24). 3.4 to 4.5.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. To an accurately measured volume containing about 4 mg of salbutamol add 25 ml of *0.05 M sulphuric acid* and extract with two quantities, each of 50 ml, of *ether*. Collect the aqueous layers into a 250-ml volumetric flask and combine the ether extracts. Wash the combined ether extracts with 50 ml of *water* and add the aqueous layer to the solution in the 250 ml volumetric flask. Discard the ether extracts and dilute the aqueous solution with sufficient *water* to produce 250.0 ml. To 10.0 ml of this solution add sufficient *water* to produce 80 ml and add 4 ml of a 5 per cent w/v solution of *sodium bicarbonate*, 4 ml of *N,N-dimethyl-4-phenylenediamine sulphate solution* and 4 ml of a freshly prepared 8 per cent w/v solution of *potassium ferricyanide*. Mix, allow to stand for 15 minutes, protected from light. Extract with two quantities, each of 10 ml, of *chloroform*. Filter the extracts through a plug of cotton wool and dilute to 25.0 ml with *chloroform*. Measure the absorbance of the resulting solution at 605 nm (2.4.7).

Calculate the content of $C_{13}H_{21}NO_3$ from the absorbance obtained by repeating the operation using 10.0 ml of a 0.0018 per cent w/v solution of *salbutamol sulphate*.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of salbutamol in a suitable dose-volume.

Salbutamol Tablets

Albuterol Sulphate Tablets; Salbutamol Sulphate Tablets

Salbutamol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of salbutamol, $C_{13}H_{21}NO_3$.

Usual strengths. The equivalent of 2 mg; 4 mg of salbutamol. (1 mg of Salbutamol Sulphate is approximately equivalent to 830 µg of salbutamol).

Identification

A. Carry out the method described under Related substances applying separately to the plate 2 µl of each of the following solutions. For the test solution shake a quantity of the powdered tablets containing 10 mg of salbutamol with 10 ml of *methanol (80 per cent)* and filter. The reference solution contains 0.12 per cent w/v of *salbutamol sulphate RS*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Shake a quantity of the powdered tablets containing 8 mg of salbutamol with 50 ml of a 2 per cent w/v solution of *borax*, add 1 ml of a 3 per cent w/v solution of *4-aminophenazone*, 10 ml of a 2 per cent w/v solution of *potassium ferricyanide*

and 10 ml of *chloroform*. Shake and allow to separate; an orange-red colour is produced in the chloroform layer.

C. Shake a quantity of the powdered tablets containing 4 mg of salbutamol with 10 ml of *water* and filter; the filtrate gives the reactions of sulphates (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ethyl acetate*, 30 volumes of *2-propanol*, 16 volumes of *water* and 4 volumes of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 10 mg of salbutamol with 1 ml of *water* for 15 minutes, centrifuge and use the supernatant liquid.

Reference solution. A 0.006 per cent w/v solution of *salbutamol sulphate RS* in *water*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, place it for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised sulphanilic acid solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution. Ignore any pink spot near the line of application.

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Add 50 ml of *water* to one tablet, shake for 1 hour, add sufficient *water* to produce 100.0 ml, mix and centrifuge. Dilute further with *water*, if necessary, to produce a solution containing 0.002 per cent w/v of salbutamol.

Reference solution (a). A 0.0024 per cent w/v of *salbutamol sulphate RS* in *water*.

Reference solution (b). A 0.048 per cent w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl) ethanol sulphate RS* and 0.048 per cent w/v of *salbutamol sulphate RS* in *methanol (10 per cent)*.

Chromatographic system
- a stainless steel column 20 cm x 5 mm, packed with spherical particles of silica, 5 µm in diameter, the surface of which has been modified with chemically-bonded nitrile groups (such as Spherisorb CN),
- mobile phase: a mixture of 65 volumes of *water*, 30 volumes of *0.05 M ammonium acetate* and 5 volumes of *2-propanol*, the pH of the mixture being adjusted to 4.5 with *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume. 20 µl.

The test is not valid unless resolution between two principal peaks in the chromatogram obtained with reference solution (b) is at least 1.5.

Calculate the content of $C_{13}H_{21}NO_3$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake 10 tablets or a sufficient number of tablets containing 5.0 mg of salbutamol with about 60 ml of *water* for 1 hour, add sufficient *water* to produce 250.0 ml, mix and centrifuge 10 ml of the mixture and use the supernatant liquid.

Reference solution (a). A 0.0024 per cent w/v of *salbutamol sulphate RS* in *water*..

Reference solution (b). A solution containing 0.0024 per cent w/v of *2-tert-butylamino-1-(4-hydroxy-3-methylphenyl) ethanol sulphate RS* and 0.0024 per cent w/v of *salbutamol sulphate RS in methanol (10 per cent)*.

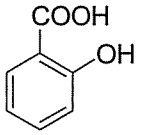
Follow the chromatographic procedure described under Uniformity of content. The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is at least 1.5.

Calculate the content of $C_{13}H_{21}NO_3$ in the tablets.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of salbutamol.

Salicylic Acid



$C_7H_6O_3$ Mol. Wt. 138.1

Salicylic Acid is 2-hydroxybenzoic acid.

Salicylic Acid contains not less than 99.0 per cent and not more than 100.5 per cent of $C_7H_6O_3$, calculated on the dried basis.

Category. Keratolytic.

Description. White or colourless, acicular crystals or a white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *salicylic acid RS* or with the reference spectrum of salicylic acid.

B. Dissolve about 30 mg in 5 ml of *0.05 M sodium hydroxide*, neutralise if necessary and dilute to 20 ml with *water*. 1 ml of the solution gives reaction A of salicylates (2.3.1).

C. Melting point. 158° to 161° (2.4.21).

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *ethanol (95 per cent)*. The resulting solution is clear, and colourless (2.4.1).

Heavy metals (2.3.13). Dissolve 2.0 g in 15 ml of *ethanol (95 per cent)* and add 5 ml of *water*. 12 ml of the resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Use *lead standard solution (100 ppm Pb)* diluted with a mixture of 3 volumes of *ethanol (95 per cent)* and 1 volume of *water* to contain 2 µg of Pb per ml to prepare the standard.

Iron (2.3.14). Boil 12.0 g with 14 ml of *dilute ammonia solution* and 35 ml of *water*. Cool and adjust the pH 5.0 to 6.0 by the dropwise addition of *dilute ammonia solution* or *dilute sulphuric acid* and dilute to 50 ml with *water*, if necessary. Any pink colour produced is not more intense than that obtained by boiling 2.0 g with 1 ml of *iron standard solution (20 ppm Fe)*, 2 ml of *dilute ammonia solution* and 45 ml of *water*, adjusting the pH 5.0 to 6.0 and diluting to 50 ml with *water* (2 ppm).

Chlorides (2.3.12). Dissolve 5.0 g in 50 ml of boiling *distilled water*, cool and filter (solution A). 20 ml of solution A complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 7.5 ml of solution A complies with the limit test for sulphates (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

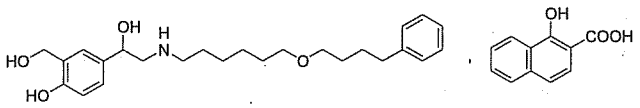
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* in a desiccator.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *ethanol (95 per cent)*, add 20 ml of *water* and titrate with *0.1 M sodium hydroxide*, using *phenol red solution* as indicator, until a reddish violet colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01381 g of $C_7H_6O_3$.

Storage. Store protected from light.

Salmeterol Xinafoate



$C_{25}H_{37}NO_4, C_{11}H_8O_3$ Mol. Wt. 603.7

Salmeterol Xinafoate is (*RS*)-4-hydroxy-α^1-[[[6-(4-phenylbutoxy)hexyl]amino]methyl]-1,3-benzenedimethanol 1-hydroxy-2-naphthoate.

Salmeterol Xinafoate contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{36}H_{45}NO_7$, calculated on the anhydrous basis.

Category. Bronchodilator.

Description. A white to off-white powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *salmeterol xinafoate RS* or with the reference spectrum of salmeterol xinafoate.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the mobile phase.

Reference solution. A 0.002 per cent w/v solution of *salmeterol xinafoate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *a* buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water* and adjusting the pH to 7.0 with *triethylamine* and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 4500 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities found is not more than 1.0 per cent.

Heavy metals (2.3.13). 1 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.2 g and dissolve in 50 ml of *glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.06038 g of $C_{36}H_{45}NO_7$.

Storage. Store protected from light.

Salmeterol and Fluticasone Propionate Inhalation

Salmeterol and Fluticasone Propionate Inhalation is a suspension of microfine Salmeterol Xinafoate and Fluticasone Propionate in a suitable liquid filled in a suitable pressurised container. It may contain suitable pharmaceutical aids such as surfactants, stabilizing agents.

Salmeterol and Fluticasone Propionate Inhalation delivers not less than 80.0 per cent and not more than 120.0 per cent of the stated amounts of salmeterol, $C_{25}H_{37}NO_4$ and fluticasone propionate, $C_{25}H_{31}F_3O_5S$, per inhalation by actuation of the valve.

Identification

In the Assay, the principal peaks in the chromatogram obtained with test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Other tests. Comply with the tests stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Determine by liquid chromatography (2.4.14).

Test solution. Prepare using the mobile phase as described under the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised Metered-dose Preparations).

Reference solution (a). A solution containing 0.5 mg of salmeterol per ml prepared by dissolving 10 mg of *salmeterol xinafoate RS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml.

Reference solution (b). A solution containing 0.5 mg of fluticasone propionate per ml prepared by dissolving 10 mg of *fluticasone propionate RS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml.

Reference solution (c). Dilute suitable volumes of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution containing 5 µg Salmeterol and 50 µg Fluticasone propionate per ml or quantities as per the label claim.

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octylsilyl silica gel (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 1.15 g *ammonium dihydrogen orthophosphate* to 1000 ml of *water* and adjusting the pH to 3.5 with *orthophosphoric acid*, 25 volumes of *acetonitrile* and 30 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- inject 200 µl.

Inject reference solution (c). The test is not valid unless the column efficiency for salmeterol and fluticasone propionate peak is not less than 1000 and 2500 theoretical plates respectively and the tailing factor is not more than 2.0 for each peak and the relative standard deviation for replicate injections for each component is not more than 2.0 per cent.

Inject the test solution and reference solution (c).

Calculate the contents of $C_{25}H_{37}NO_4$ and $C_{25}H_{31}F_3O_5S$ in the solution and the contents of $C_{25}H_{37}NO_4$ and $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve.

Determine the contents of the active ingredients a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average contents of $C_{25}H_{37}NO_4$ and $C_{25}H_{31}F_3O_5S$ delivered per actuation of the valve meet the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the amounts of active ingredients delivered per inhalation.

Salmeterol and Fluticasone Propionate Powder for Inhalation

Salmeterol and Fluticasone Propionate Powder for Inhalation consists of Fluticasone Propionate and Salmeterol Xinafoate in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Salmeterol and Fluticasone Propionate Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amounts of salmeterol $C_{25}H_{37}NO_4$ and fluticasone propionate, $C_{25}H_{31}F_3O_5S$ per unit dose.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the peaks in the chromatogram obtained with reference solution (c).

Tests

Other tests. Complies with the tests stated under the Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the mixed contents of 20 capsules in sufficient of the mobile phase to get a solution containing 5 µg per ml of Salmeterol.

Reference solution (a). A solution containing 0.5 mg of salmeterol per ml prepared by dissolving 10 mg of *salmeterol xinafoate RS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml.

Reference solution (b). A solution containing 0.5 mg of fluticasone propionate per ml prepared by dissolving 10 mg of *fluticasone propionate RS* in 10 ml *acetonitrile* and adding sufficient of the mobile phase to produce 20 ml.

Reference solution (c). Dilute suitable volumes of reference solution (a) and reference solution (b) with the mobile phase to obtain a solution containing 5 µg Salmeterol and 50 µg Fluticasone propionate per ml or quantities as per the label claim..

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octylsilyl silica gel (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 45 volumes of a buffer solution prepared by dissolving 1.15 g *ammonium dihydrogen orthophosphate* to 1000 ml of *water* and adjusting the pH to 3.5 with *orthophosphoric acid*, 25 volumes of acetonitrile and 30 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject reference solution(c). The test is not valid unless the column efficiency determined from the salmeterol and fluticasone propionate peak is not less than 1000 and 2500 theoretical plates respectively, the tailing factor for each of salmeterol and fluticasone propionate peaks is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

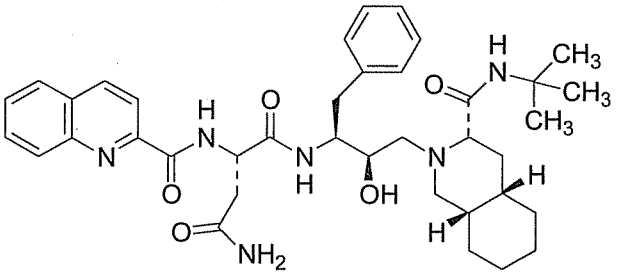
Inject the test solution and reference solution (c).

Calculate the contents of $C_{25}H_{37}NO_4$ and $C_{25}H_{31}F_3O_5S$ per unit.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Labelling. The label states the quantities of active ingredients per pre-metered unit.

Saquinavir



$C_{38}H_{50}N_6O_5$ Mol. Wt. 670.8

Saquinavir is (*S*)-*N*-[(α*S*)-α-{(1*R*)-2-[(3*S*,4a*S*,8a*S*)-3-(*tert*-butylcarbamoyl)octahydro-2(1*H*)-isoquinolyl]-1-hydroxyethyl}phenethyl]-2-quinaldamidosuccinamide.

Saquinavir contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{38}H_{50}N_6O_5$, calculated on the anhydrous basis.

Category. Antiretroviral.

Dose. 1 g twice daily.

Description. A white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *saquinavir RS* or with the reference spectrum of saquinavir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to saquinavir in the chromatogram obtained with reference solution (a).

C. When examined in the range 220 nm to 350 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows absorption maxima at about 239 nm and 290 nm.

Tests

Specific optical rotation (2.4.22). –50.0° to –55.0°, determined in a 0.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14), as described in the Assay using the test solution and reference solution (c).

Inject reference solution (c). Calculate the amount of related substance by area normalisation method. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than the area of the principal peak obtained with reference solution (c) (0.1 per cent) and the sum of the areas of all such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit tests for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *saquinavir RS* in the mobile phase.

Reference solution (b). Dissolve suitable quantities of *saquinavir-related compound A RS* and *saquinavir RS* in the mobile phase to obtain a solution containing 2 µg per ml of saquinavir-related compound A and 0.25 mg per ml of saquinavir.

Reference solution (c). A 0.000025 per cent w/v solution of *saquinavir RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol*, 50 volumes of *acetonitrile* and 30 volumes of a buffer prepared by dissolving 4 g of *sodium dihydrogen phosphate* in 1000.0 ml of *water* to which 1 ml of *diethylamine* and 1 g of *sodium octane sulphonate* has been added,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

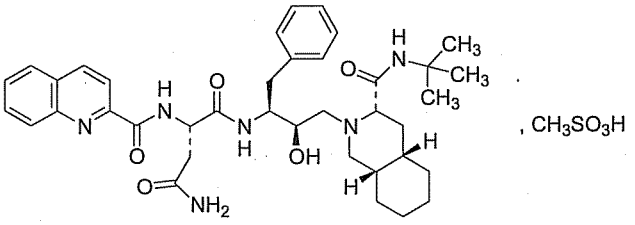
Inject reference solution (b). The relative retention times are about 0.89 for saquinavir-related compound A and about 1.0 for saquinavir. The test is not valid unless the resolution between the peaks due to saquinavir-related compound A and saquinavir is not less than 1.5, the column efficiency determined from the saquinavir peak is not less than 500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Separately inject the test solution and reference solution (a). Record the chromatograms up to three times the retention time of the principal peak and measure the responses for the principal peak.

Calculate the content of $C_{38}H_{50}N_6O_5$.

Storage. Store protected from light.

Saquinavir Mesylate



$C_{38}H_{50}N_6O_5,CH_4O_3S$ Mol. Wt. 767.0

Saquinavir mesylate is (*S*)-*N*-[(α*S*)-α-{(1*R*)-2-[(3*S*,4a*S*,8a*S*)-3-(*tert*-butylcarbamoyl)octahydro-2(1*H*)-isoquinolyl]-1-hydroxyethyl}phenethyl]-2-quinaldamidosuccinamide methanesulphonate.

Saquinavir Mesylate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{38}H_{50}N_6O_5$, CH_4O_3S, calculated on the anhydrous basis.

Category. Antiretroviral.

Dose. 1 g twice daily.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *saquinavir mesylate RS* or with the reference spectrum of saquinavir mesylate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to saquinavir mesylate in the chromatogram obtained with reference solution (a).

Tests

Specific optical rotation (2.4.22). –66.8° to –69.6°, determined in a 0.5 per cent w/v solution in *methanol* at 436 nm.

Related substances. Determine by liquid chromatography (2.4.14), as described in the Assay using the test solution and reference solution (c).

Inject reference solution (c). Calculate the amount of related substances by area normalisation method. In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent) and the sum of the areas of all such peaks is not greater than 5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Methanesulphonic acid. 11.9 to 13.1 per cent w/w, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 0.1 g of the substance under examination, dissolve in 50 ml of *methanol*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.00961 g of CH_3SO_3H.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25.0 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.025 per cent w/v solution of *saquinavir mesylate RS* in the mobile phase.

Reference solution (b). Dissolve suitable quantities of *saquinavir-related compound A RS* and *saquinavir mesylate RS* in the mobile phase to obtain a solution containing 2 µg per ml of saquinavir- related compound A and 0.25 mg per ml of saquinavir mesylate.

Reference solution (c). A 0.000025 per cent w/v solution of *saquinavir mesylate RS* in the mobile phase.

NOTE — Store the buffer solution protected from light. Make adjustments if necessary for system suitability.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *tetrahydrofuran*, 5 volumes of *acetonitrile* and 17 volumes of a buffer prepared by mixing 10 ml of *triethylamine* with *water* to make 1000 ml and adjusting the pH of the solution to 2.5 with *phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention times are about 0.89 for saquinavir-related compound A and about 1.0 for saquinavir mesylate. The test is not valid unless the resolution between the peaks due to saquinavir related compound A and saquinavir mesylate is not less than 1.5, the column efficiency determined from the saquinavir mesylate peak is not less than 500 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Separately inject the test solution and reference solution (a). Record the chromatograms upto three times the retention time of the principal peak and measure the responses for the principal peak.

Calculate the content of $C_{38}H_{50}N_6O_5$, CH_4O_3S.

Storage. Store protected from light.

Saquinavir Mesylate Tablets

Saquinavir Mesylate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of saquinavir $C_{38}H_{50}N_6O_5$.

NOTE —Perform the tests and assay using low-actinic glassware.

Usual strength. 200 mg.

Identification

A. In the Assay, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 400 nm (2.4.7), 1 ml of a 0.1 per cent w/v solution in *methanol* diluted to 100 ml with *citrate buffer pH 3.0* (see under Dissolution), shows absorption maxima at the same wavelengths as shown by the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No 1
Medium. 900 ml of *citrate buffer pH 3.0* prepared by dissolving 5.82 mg of *anhydrous dibasic sodium phosphate* and 16.7 mg of *citiric acid monohydrate* in 1000 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid*.

Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly. Dilute the filtrate, if necessary, with the medium. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 240 nm. Calculate the content of $C_{38}H_{50}N_6O_5$ in the medium from the absorbance obtained from a solution of known concentration of *saquinavir mesylate RS*.

D. Not less than 75 per cent of the stated amount of $C_{38}H_{50}N_6O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets Weigh accurately a quantity of the powder containing 100 mg of saquinavir in 100 ml of the mobile phase and filter.

Reference solution (a). A 0.1 per cent w/v solution of *saquinavir mesylate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14 volumes of *triethylamine phosphate solution* prepared by diluting 10 ml of *triethylamine* to 1000 ml with *water* and adjusting the pH to 2.5 with *orthophosphoric acid*, 5 volumes of tetrahydrofuran and 1 volume of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatogram for 5 times the retention time (about 12 minutes) of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with the reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. Disperse one tablet in 500 ml of the mobile phase and filter. Dilute 5 ml of the filtrate to 20 ml with the mobile phase.

Calculate the content of $C_{38}H_{50}N_6O_5$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 6.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of saquinavir, dissolve in 100.0 ml of mobile phase and filter. Dilute 5.0 ml of the filtrate to 20.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *saquinavir mesylate RS* in the mobile phase. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 14 volumes of a solution prepared by diluting 10.0 ml of *triethylamine* to 1000 ml with *water*, adjusting the pH to 2.5 with *orthophosphoric acid* and filtering, 5 volumes of *tetrahydrofuran* and 1 volume of *acetonitrile*.
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

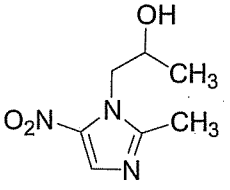
Inject the test solution and the reference solution.

Calculate the content of $C_{38}H_{50}N_6O_5$ in the tablets.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of saquinavir.

Secnidazole



$C_7H_{11}N_3O_3$ Mol. Wt. 185.2

Secnidazole is (*RS*)-1-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol.

Secnidazole contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_{11}N_3O_3$, calculated on the anhydrous basis.

Category. Antiamoebic.

Dose. Adults - 2g; Children - 30 mg per kg as single dose.

Description. A white to yellowish white, crystalline powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 277 nm, 0.325 to 0.355.

B. Heat about 10 mg in a water-bath with 10 mg of *zinc powder*, 1 ml of *water* and 0.25 ml of *2 M hydrochloric acid* for 5 minutes and cool. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. A 5 per cent w/v solution in *1 M hydrochloric acid* is not more opalescent than opalescence standard OS2 (2.4.1) and not more intensely coloured than reference solution GYS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with mobile phase. Dilute 1.0 ml of the solution to 10.0 ml with the same solvent.

Reference solution (b). A solution containing 0.005 per cent w/v each of *2-methyl-5-nitroimidazole RS* and *secnidazole RS* in the mobile phase. Dilute 1.0 ml of the solution to 100.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of 0.14 per cent w/v solution of *potassium dihydrogen phosphate,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 318 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between 2-methyl-4-nitroimidazole and secnidazole is not less than 1.5.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2 times the area of the peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of the peak in the chromatogram obtained with the reference solution (a) (0.5 per cent). Disregard any peak which is 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.01 per cent).

Heavy metals (2.3.13).1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.0 to 5.0 per cent, determined on 0.4 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 100.0 ml with the same solvent.

Reference solution. A 0.005 per cent w/v solution of *secnidazole RS* in mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of 0.14 per cent w/v solution of *potassium dihydrogen phosphate,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 318 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_7H_{11}N_3O_3$.

Storage. Store protected from light and moisture.

Secnidazole Tablets

Secnidazole Tablets contain not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of secnidazole, $C_7H_{11}N_3O_3$.

Usual strengths. 1 g; 2 g.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus. No. 1,

Medium. 900 ml of *0.1 M hydrochloric acid.*
Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first 1 ml of the filtrate. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance of the resulting solution at the maximum at about 277 nm (2.4.7). Calculate the content of $C_7H_{11}N_3O_3$ in the medium from the absorbance obtained from a solution of known concentration of *secnidazole RS* in the same medium.

D. Not less than 80 per cent of the stated amount of $C_7H_{11}N_3O_3$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of powdered tablets containing 50 mg of Secnidazole, disperse in 100 ml of mobile phase and filter.

Reference solution (a). A 0.05 per cent w/v solution of *secnidazole RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.0 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.0 per cent).

Water (2.3.43). Not more than 6.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powdered tablet containing 50 mg of Secnidazole, disperse in 100.0 ml of mobile phase and filter.. Dilute 5.0 ml of the filtrate to 50.0 ml with mobile phase.

Reference solution. A 0.005 per cent w/v solution of *secnidazole RS* in mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *0.01 M potassium dihydrogen orthophosphate* and 15 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 228 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation of replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_7H_{11}N_3O_3$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength of secnidazole.

Serratiopeptidase

Serratiopeptidase is a proteolytic enzyme for oral use which is produced by bacteria of genus Serratia.

Serratiopeptidase contains not less than 2000 units and not more than 2600 units of serratiopeptidase activity per mg.

Category. Prolytic enzyme.

Description. A grayish white to pale brown powder with characteristic odour.

Identification

Dissolve 0.4 g of serratiopeptidase in 100 ml of *acetic acid sodium acetate buffer solution pH 5.0*, transfer exactly 1 ml of this solution into 3 test tubes, refer them as A, B and C. Add 1 ml of *water* in tube A. In tube B and C add 1 ml of 0.04 ml of *disodium edetate solution.* Mix gently and allow them to stand at 4±1°, for 1 hour. Add 2 ml of *water* in tube A and C. Add 2 ml of *0.04 M zinc chloride* solution in tube B. Mix gently and allow them to stand at 4±1°, for 1 hour. Pipette 1 ml of A and B and make up the volume to 200 ml with *borate buffer pH 9.0.* Pipette 1 ml of C and make up the volume to 50 ml with *borate buffer pH 9.0.* Proceed with these solutions as sample solutions as directed in the assay. The activities of solution A and B are almost same and the activity of C is not more than 5 per cent of that of activity of A.

Tests

Arsenic (2.3.10). Dissolve 0.4 g in 50 ml of *water*, and add 10 ml of *stannated hydrochloric acid.* The resulting solution complies with the limit test for arsenic (5 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (50 ppm).

Sulphated Ash (2.3.18). Not more than 1.5 per cent.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1 g by drying in an oven at 105° for 4 hours.

Microbial contamination (2.2.9). Total viable aerobic count, not more than 100 micro-organisms per ml, determined by plate count. 1 ml is free from *Escherichia coli.*

Assay. Unit definition: one serratiopeptidase unit is defined as the amount of enzyme required to liberate 1 µm of free tyrosine per minute under the specified assay conditions.

sodium borate hydrochloric acid buffer pH 9.0. Dissolve 19 g *sodium borate* in 900 ml of *water.* Adjust the pH 9.0 with *1 M hydrochloric acid* and dilute with *water* to 1000 ml.

Substrate solution. Dissolve 1.2 g dried casein in 100 ml of *sodium borate hydrochloric acid buffer pH 9.0.* Keep it on boiling water-bath for 1-2 minutes to get clear solution. Cool and filter through cotton and dilute with *sodium borate hydrochloric acid buffer pH 9.0* to 200 ml.

Protein precipitating solution. To 18 g of *trichloroacetic acid,* add 30 g of *sodium acetate* and 20 ml of *glacial acetic acid,* and dilute with *water* to 100 ml.

Dilute folin's reagent. 1 ml of *folin's reagent,* add 2 ml of *water.*

Reference tyrosine solution. Dissolve 10 mg of *tyrosine* in 1 ml of *1 M hydrochloric acid* and dilute to 100 ml with *sodium borate hydrochloric acid buffer pH 9.0.*

Reference tyrosine curve. To 1, 2, 3, 4 and 5 ml of *reference tyrosine solution,* add 5, 4, 3, 2 and 1 ml of *sodium borate hydrochloric acid buffer pH 9.0* respectively. Add 5 ml of *protein precipitating reagent* in each tube. To 2 ml of these solutions, add 5 ml of *sodium carbonate solution.* Add 1 ml of diluted *folin's reagent* mix and allow to stand at 37° for 30 minutes, measure the absorbance at 660 nm (2.4.7). Plot a graph of µm of tyrosine per system against the absorbance.

Stock test solution. Weigh about 0.1 g of the substance under examination, dissolve in 100 ml of *sodium borate hydrochloric acid buffer pH 9.0* (solution A). Mix and keep it for 5 minutes. Take 1 ml of solution A and dilute to 200 ml with *sodium borate hydrochloric acid buffer pH 9.0* (solution B).

Test solution. Pipet 1.0 ml of *stock test solution,* allow to stand in a water-bath at 37° for 5 minutes. Add 5 ml of substrate solution shake immediately and allow to stand in water-bath at 37° for 20 minutes. Add 5 ml of *protein precipitating solution.* Mix and allow to stand in water-bath at 37° for 30 minutes and filter. Take 2 ml of the filtrate, add 5 ml of 0.6 per cent w/v *sodium carbonate solution.* Add 1 ml of *diluted folin's reagent* mix and allow to stand at 37° for 30 minutes. Measure the absorbance at 660 nm (2.4.7).

Blank solution. Pipet 1.0 ml of *stock test solution,* allow to stand in a water-bath at 37° for 5 minutes, add 5 ml of *protein precipitating solution,* shake immediately and allow to stand in water-bath at 37° for 20 minutes. Add 5 ml of substrate solution. Mix and allow to stand in water-bath at 37° for 30 minutes and filter. Take 2 ml of the filtrate, add 50 ml of 0.6 per cent w/v *sodium carbonate solution.* Add 1 ml of *diluted folin's reagent* mix and allow stand at 37° for 30 minutes. Measure the absorbance at 660 nm (2.4.7).

Calculate the serratiopeptidase IU/mg by using concentration of tyrosine from graph considering the reaction time and dilution.

Storage. Store protected from light and moisture, at temperature not exceeding 30°.

Serratiopeptidase Tablets

Serratiopeptidase Tablets contain not less than 90.0 per cent of the stated amount of Serratiopeptidase.

Usual strengths. 5 mg; 10 mg.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. The tyrosine units are dissolved in *sodium carbonate solution,* which is alkaline in nature. The *Folin's ciocalteau reagent* helps in colour development where the tyrosine units bind to the copper molecule in the reagent and causes the reduction of phosphomolybdate which is present in the reagent. There is formation of tyrosine-copper molybdate coloured complex. The intensity of colour depends upon the tyrosine units present which is read at 660 nm.

Disodium tetraborate buffer. Dissolve 19.0 gm of *disodium tetraborate* in 900 ml of *water,* adjusted to pH 9.0 with *1 M hydrochloric acid* and dilute to 1000 ml with *water.*

Casein hammerstein substrate. Dissolve 1.2 gm of *Casein Hammerstein* to 100 ml of the sodium tetraborate buffer. Allow the casein to dissolve and form homogeneous solution. Boil the solution in boiling water-bath for 2 minutes. After removing from boiling water-bath, cool the casein substrate immediately in ice cold water. Filter this solution through cotton plug to get clear solution and dilute to 200 ml with *water.*

NOTE—Always add casein to the buffer while stirring. Do not add buffer to casein as this will cause lumps and not go into solution completely and giving incorrect values.

Trichloroacetic acid reagent. Dissolve 18 gm of *trichloroacetic Acid,* 30 gm of *anhydrous sodium acetate* and 20 ml of *glacial acetic acid* in 1000 ml of *water.*

NOTE—TCA should be in the crystal form and not in liquid form as this will effect the quantity weighed and thereby interfere with the complete precipitation of casein. Use Sodium acetate anhydrous because if, sodium acetate trihydrate should be used, the trihydrate forms gives lower results.

Diluted Folin's Ciocalteau Reagent. Dilute 1 ml of Folin's Ciocalteau reagent with 2 ml of *water* to get 3 ml of the reagent.

NOTE—Folin's Ciocalteau reagent should be of the protein estimation grade and not the indicator grade. Folin's reagent should be diluted to just before addition to the tubes since it is sensitive to light. Do not prepare this solution at the beginning of the assay.

Tyrosine reference solution. Dissolve 160 mg of *tyrosine* in 100 ml of *0.2 M hydrochloric acid.*

Dilute 1 ml of this solution to 100 ml with *0.2 M hydrochloric acid.* Use 2 ml of this solution for colour development.

Trisodium phosphate buffer pH 6.8. Weigh 19 g of *trisodium phosphate* and 6.4 ml of *hydrochloric acid* in 1000 ml of *water*, adjusted to pH 6.8.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 120 mg of Serratiopeptidase with 100 ml of *trisodium phosphate buffer pH 6.8.* Stir on magnetic stirrer for 1 hour. Further dilute 5.0 ml to 25 ml with *5.0 per cent ammonium sulphate solution,* stir for 5 minutes and filter. Dilute 2 ml of the filtrate to 100 ml with *sodium tetraborate buffer.* 1 ml of resulting solution is used for analysis. Set the water bath to 37°.

For each sample, keep four test tubes. Mark two test tubes as TEST and two as BLANK. Add 5 ml of Casein Substrate to the tubes marked as TEST and 5 ml. of TCA to the test tube marked as BLANK. Prewarm these tubes for five minutes along with the tubes containing the sample dilutions. Add 1 ml of enzyme solution to all these tubes and vertex the tubes for exactly 10 seconds. Keep the test tubes in the water-bath at 37° for 20 minutes for the reaction. After exactly 20 minutes, add 5 ml of TCA to tubes marked as TEST and 5 ml of Casein substrate to the tube marked as BLANK. Vertex all the tubes exactly for 30 seconds. Keep the tubes in the water-bath at 37° for 30 minutes for precipitation, filter.

NOTE— For tablets, the settlement may take a longer time than 30 minutes. So filter the solution after the settlement is clearly seen (Vertex in between for better precipitation).

Mark tubes as TEST, BLANK, TYROSINE and TYROSINE BLANK.

Add the following respectively;

TEST – 2 ml of the TEST filtrate.

BLANK – 2 ml of BLANK filtrate.

TYROSINE – 2 ml of TYROSINE standard solution.

TYROSINE BLANK – 0.2 M hydrochloric acid.

Add 5 ml of 6 per cent sodium carbonate solution to all tubes.

Add 1 ml of diluted Folin's reagent to all the tubes. Keep the tubes at 37° for 30 minutes for colour development. Measure the absorbance at about 660 nm (2.4.7).

$$\text{Serratiopeptidase Units/Tablets} = \frac{A_1 - A_2}{A_3 - A_4} \times 176 \times \frac{1}{20} \times \frac{100}{\text{Spl.Wt.(g)}} \times \frac{25}{5} \times \frac{100}{2} \times \text{Avg.wt.in gm}$$

$$\% \text{ of L.A.} = \frac{\text{Serratiopeptidase Units/Tablet}}{\text{L.A.}} \times 100$$

Where, 176 = Conversion Co-efficient of Tyrosine to Serratiopeptidase
20 = Reaction time [minutes]
A_1 = Absorbance of test solution
A_2 = Absorbance of blank solution
A_3 = Absorbance of tyrosine reference solution.
A_4 = Absorbance of 0.2M hydrochloric acid
L.A. = 20000 Units/tablet.

Storage. Store protected from moisture, at a temperature not exceeding 25°.

Labeling. The label states the strength in terms of the equivalent to Enzyme activity units of Serratiopeptidase.

Colloidal Silicon Dioxide

Colloidal Anhydrous Silica

SiO_2 Mol. Wt. 60.1

Colloidal Silicon Dioxide is a submicroscopic fumed silica prepared by the vapour-phase hydrolysis of a silicon compound.

Colloidal Silicon Dioxide contains not less than 99.0 per cent and not more than 100.5 per cent of SiO_2, calculated on the ignite basis.

Category. Pharmaceutical aid (tablet excipient).

Description. A light, fine, white, amorphous powder. It has a particle size of about 15 nm.

Identification

About 20 mg gives the reaction of silicates (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.5, determined in a suspension of 1.0 g in 30 ml of *carbon dioxide-free water.*

Arsenic (2.3.10). To 2.5 g contained in a round-bottomed flask add 50 ml of *3 M hydrochloric acid* and heat under a reflux condenser for 30 minutes. Cool, filter with the aid of suction and transfer the filtrate to a 100-ml volumetric flask. Wash the filter with several portions of hot water and add the washings to the volumetric flask. Cool, dilute to volume with *water* and

mix. To 50.0 ml of the solution add 3 ml of *hydrochloric acid;* the resulting solution complies with the limit test for arsenic (8 ppm).

Heavy metals (2.3.13). Suspend 2.5 g in sufficient *water* to produce a semi-fluid slurry and dry at 140°. When the dried substance is white, break up the mass using a glass rod, add 25 ml of *1 M hydrochloric acid*, boil gently for 5 minutes, stirring frequently with the glass rod, centrifuge for 20 minutes and filter the supernatant liquid through a membrane filter. To the residue in the centrifuge tube add 3 ml of *2 M hydrochloric acid* and 9 ml of *water*, boil, centrifuge for 20 minutes and filter the supernatant liquid through the same membrane filter. Wash the residue with small quantities of *water*, combine the filtrates and washings and dilute to 50.0 ml with *water*. To 20.0 ml of the solution add 50 mg of *L-ascorbic acid* and 1 ml of *strong ammonia solution*, neutralise with *2 M ammonia* and dilute to 25 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (25 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

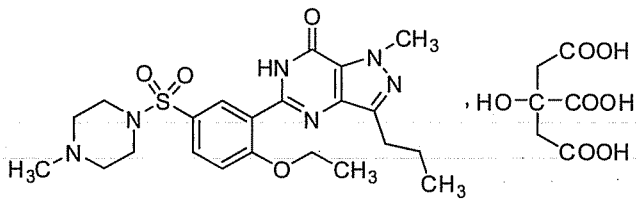
Chlorides (2.3.12). To 1.0 g add a mixture of 20 ml of *2 M nitric acid* and 30 ml of *water*, heat on a water-bath for 15 minutes, shaking frequently, dilute to 50 ml with *water* if necessary, filter and cool. The filtrate complies with the limit test for chlorides (250 ppm).

Loss on ignition (2.4.20). Not more than 5.0 per cent, determined on 0.2 g by igniting at 900° in a platinum crucible for 2 hours.

Assay. To the residue obtained in the test for Loss on ignition add 0.2 ml of *sulphuric acid* and sufficient *ethanol (95 per cent)* to moisten the residue completely, add 6 ml of *hydrofluoric acid* and evaporate to dryness on a hot plate at 95° to 105°, avoiding loss from sputtering. Wash the sides of the dish with 6 ml of *hydrofluoric acid*, evaporate to dryness in a well-ventilated hood, ignite at 1000°, allow to cool in a desiccator and weigh. The difference between the weight of the final residue and that of the residue obtained in the test for Loss on ignition represents the amount of SiO_2 in the amount of the substance taken for the test for Loss on ignition.

Storage. Store protected from light.

Sildenafil Citrate



$C_{28}H_{38}N_6O_{11}S$ Mol. Wt. 666.7

Sildenafil Citrate is 5-[2-ethoxy-5-(4-methylpiperazinylsulphonyl)phenyl]-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one citrate.

Sildenafil Citrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{28}H_{38}N_6O_{11}S$, calculated on the dried basis.

Category. Used in erectyle dysfunction.

Description. A white to off white powder.

Dose. 50 mg per day.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum that obtained with *sildenafil citrate RS* or with the reference spectrum of sildenafil citrate.

B. Complies with the test for Citrate (2.3.1).

Tests

Citric acid. 28.0 per cent to 34.0 per cent.

Accurately weigh about 0.2 g of the substance under examination and transfer into a 250-ml conical flask, add 50 ml of *methanol* and sonicate to dissolve, then add 50 ml of *water*. Titrate with *0.1 M sodium hydroxide solution* using *phenolphthalein* indicator until the colour changes from colourless to pink.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006403 g of $C_6H_8O_7$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution.

Solvent mixture. 30 volumes of *water* and 70 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.05 per cent w/v solution of *sildenafil citrate RS* in the solvent mixture.

Reference solution (b). Dilute 10.0 ml of reference solution (a) to 100.0 ml with the solvent mixture. Further dilute 3.0 ml of this solution to 100.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS C18),
- mobile phase: A. dissolve 3.85 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 7.5 with *ammonia solution*,

  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,

- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cen v/v)	Mobile phase B (per cent v/v)
0	65	35
15	65	35
30	20	80
40	20	80
45	65	35
50	65	35

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent. The relative retention time with reference to sildenafil for N-oxide is about 0.29 and for chlorosulphonyl is about 1.6.

Inject the test solution and reference solution (b). In the chromatogram obtained with test solution the area of the peak due to N-oxide impurity is not more than 1.67 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of peak due to chlorosulphonyl is not more than 0.67 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). The sum of areas of all the secondary peaks is not more than 3.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak corresponding to citric acid.

Heavy Metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solution.

Solvent mixture. 30 volumes of *water* and 70 volumes of *acetonitrile*.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *sildenafil citrate RS* in the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS C18),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water*, adjusted to pH 7.5 with *ammonia solution* and 50 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{28}H_{38}N_6O_{11}S$.

Storage. Store protected from light and moisture.

Sildenafil Tablets

Sildenafil Citrate Tablets

Sildenafil Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of sildenafil citrate, $C_{28}H_{38}N_6O_{11}S$.

Usual Strengths . 25 mg; 50 mg; 100 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.01 M Hydrochloric acid,*

Speed and time. 100 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 294 nm (2.4.7). Calculate the content of $C_{28}H_{38}N_6O_{11}S$ in the medium from the absorbance obtained from a solution of known concentration of *sildenafil citrate RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_{28}H_{38}N_6O_{11}S$.

Related substances. Determine by liquid chromatography (2.4.14)

Solvent mixture. 50 volumes of mobile phase A and 50 volumes of *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of sildenafil with 35 ml of

the solvent mixture, sonicate for 20 minutes and dilute to 50 ml with the solvent mixture, filter.

Reference solution (a). A 0.028 per cent w/v solution of *sildenafil citrate RS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm) (Such as Zorbax Eclipse XDB C18),
- column temperature. 40°,
- mobile phase: A. a solution containing 3.85 g of *ammonium acetate* and 1.0 ml of *triethylamine* in 1000 of *water*, adjusted to pH 5.5 with *acetic acid*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume.10 µl.

Time (in min.)	Mobile Phase A (per cent /v/v)	Mobile Phase B (per cent /v/v)
0	47	53
20	47	53
35	10	90
45	10	90
50	47	53
60	47	53

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.8 per cent), the area of peak corresponding to N-oxide impurity at relative retention time 0.54 is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.0 times the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent). Ignore the peak due to citric acid at retention time about 2.4 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of mobile phase and 50 volumes of *methanol*.

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 50 mg of sildenafil with 35 ml of the solvent mixture, sonicate for 20 minutes and dilute to 50.0 ml with the solvent mixture, filter.

Reference solution. A 0.14 per cent w/v solution of *sildenafil citrate RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica (5 µm) (Such as Zorbax Eclipse XDB C18),
- mobile phase: a mixture of 30 volumes of solution containing 3.85 g of *ammonium acetate* and 1.0 ml of *triethylamine* in 1000 ml of *water*, adjusted to pH 5.5 with *acetic acid* and 70 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 294 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution the test solution.

Calculate the content of $C_{28}H_{38}N_6O_{11}S$ in the tablet.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of sildenafil.

Silver Nitrate

$AgNO_3$ Mol. Wt. 169.9

Silver Nitrate contains not less than 99.0 per cent and not more than 100.5 per cent of $AgNO_3$.

Category. Local anti-infective.

Description. Colourless crystals or a white, crystalline powder.

Identification

A. Gives the reactions of silver salts (2.3.1).

B. 10 mg gives reaction A of nitrates (2.3.1).

Tests

Appearance of solution. A 4.0 per cent w/v solution (solution A) is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 2 ml of solution A add 0.1 ml of *bromocresol green solution*; the solution is blue. To 2 ml of this solution add 0.1 ml of *phenol red solution*; the solution is yellow.

Aluminium, bismuth, copper and lead. Dissolve 1.0 g in a mixture of 4 ml of *strong ammonia solution* and 6 ml of *water*; the resulting solution is clear (2.4.1), and colourless (2.4.1).

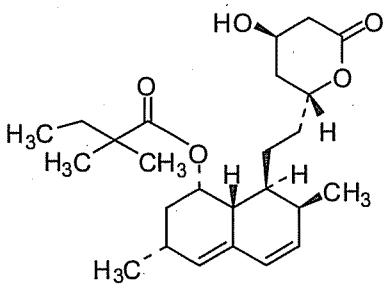
Foreign salts. Not more than 0.3 per cent, determined by the following method. To 30 ml of solution A add 7.5 ml of *2 M hydrochloric acid*, shake vigorously, heat for 5 minutes on a water-bath, filter and evaporate 20 ml of the filtrate to dryness on a water-bath. Dry the residue at 105° and weigh.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *water*, add 2 ml of *2 M nitric acid* and 2 ml of *ferric ammonium sulphate solution* and titrate with *0.1 M ammonium thiocyanate* until a reddish yellow colour is produced.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 0.01699 g of $AgNO_3$.

Storage. Store protected from light and moisture, in non-metallic containers.

Simvastatin



$C_{25}H_{38}O_5$ Mol.Wt. 418.6

Simvastatin is (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronapthalen-1-yl 2,2-dimethylbutyrate.

Simvastatin contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{25}H_{38}O_5$, calculated on the dried basis. A suitable antioxidant may be added.

Category. Antihyperlipidaemic.

Dose. 5 mg to 10 mg per day.

Description. A white or almost white, crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *simvastatin RS* or with the reference spectrum of simvastatin.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *methanol* is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Specific optical rotation (2.4.22). +285° to +300°, determined in a 0.5 per cent w/v solution in *acetonitrile*.

Related substances. Determine by liquid chromatography (2.4.14), as described under the Assay.

Inject test solution (a). Run the chromatogram 5 times the retention time of the principal peak. The relative retention time with reference to simvastatin for (3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid (hydroxy acid) (simvastatin impurity A) is about 0.45, for lovastatin (simvastatin impurity E) and epilovastatin (simvastatin impurity F) is about 0.6, for (1*S*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-7-methyl-3-methylene-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate) (simvastatin impurity G) is about 0.8, for (1*S*,3*R*,7*S*,8*S*,8a*R*)-8-[2-[(2*R*,4*R*)-4-(acetyloxy)-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (acetate ester) (simvastatin impurity B) is about 2.38, for (1*S*,3*R*,7*S*,8*S*,8a*R*)-3,7-dimethyl-8-[2-[(2*R*)-6-oxo-3,6-dihydro-2*H*-pyran-2-yl]ethyl]-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate (anhydrosimvastatin) (simvastatin impurity C) is about 2.42 and for (2*R*,4*R*)-2-[[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2, 6,7,8,8a-hexahydronaphthalen-1-yl]ethyl]-6-oxotetrahydro-2*H*-pyran-4-yl(3*R*,5*R*)-7-[(1*S*,2*S*,6*R*,8*S*,8a*R*)-8-[(2,2-dimethylbutanoyl)oxy]-2,6-dimethyl-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoate (dimer) (simvastatin impurity D) is about 3.8.

Inject test solution (a) and reference solution (b). In the chromatogram obtained with test solution (a) the area of the secondary peaks corresponding to lovastatin and epilovastatin is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent), the area of any secondary peak other than lovastatin and epilovastatin is not more than 0.8 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). The sum of the areas of all the secondary peaks other than lovastatin and epilovastatin is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13).). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° for 3 hours under high vacuum.

Assay. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Solvent mixture. 40 volumes of a 0.14 per cent w/v solution of *potassium dihydrogen phosphate*, adjusted to pH 4.0 with *orthophosphoric acid* and 60 volumes of *acetonitrile*.

Test solution (a). Dissolve 75 mg of the substance under examination in 50.0 ml of the solvent mixture.

Test solution (b). Dissolve 40 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution (a). Dissolve 1 mg each of *simvastatin RS* and *lovastatin RS* in 50.0 ml of the solvent mixture.

Reference solution (b). Dilute 0.5 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). Dissolve 40 mg of *simvastatin RS* in 50.0 ml of the solvent mixture.

Chromatographic system
- a stainless steel column 3.3 cm x 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a mixture 50 volumes of *acetonitrile* and 50 volumes of a 0.1 per cent v/v solution of *orthophosphoric acid,*
  B. 0.1 per cent v/v solution of *orthophosphoric acid* in *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 3.0 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 5 µl.

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0–4.5	100	0
4.5–4.6	100 →95	0 →5
4.6–8.0	95 →25	5 →75
8.0–11.5	25	75
11.5–11.6	25 →100	75 →0
11.6–13	100	0

Inject reference solution (a). The test is not valid unless resolution between the peaks due to lovastatin and epilovastatin and simvastatin is not less than 5.0.

Inject test solution (b) and reference solution (c).

Calculate the content of simvastatin.

Storage. Store protected from light and moisture, under nitrogen.

Simvastatin Tablets

Simvastatin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of simvastatin, $C_{25}H_{38}O_5$.

Usual strengths. 10 mg; 20 mg; 40 mg.

Identification

Shake a quantity of the powdered tablets containing about 50 mg of Simvastatin with 20 ml of *dichloromethane*, filter and evaporate the filtrate to dryness using a rotary evaporator and a water-bath at 40°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *simvastatin RS* or with the reference spectrum of simvastatin.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M sodium dihydrogen orthophosphate* containing 0.5 per cent w/v of *sodium dodecyl sulphate*, adjusted to pH 7.0 with *1 M sodium hydroxide,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter and transfer 10 ml of the filtrate into a centrifuge tube containing 0.1 g of *pre-washed manganese (IV) oxide*. Shake the tube for 30 minutes, or until the manganese (IV) oxide is completely dispersed, centrifuge and measure the absorbance (2.4.7) of the clear supernatant liquid, suitably diluted if necessary with dissolution medium at the maximum at about 257 nm and the minimum at about 257 nm. Calculate the content of simvastatin, $C_{25}H_{38}O_5$.in the medium from the absorbance obtained from a solution of known concentration of *simvastatin RS,* using the differences in absorbance at 247 nm and at 257 nm.

D. Not less than 70 per cent of the stated amount of $C_{25}H_{38}O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Prepare the solutions immediately before use.

Buffer solution. A 0.51 per cent w/v solution of *sodium dihydrogen orthophosphate*, adjusted to pH 4.5 with 25 per cent v/v solution of *orthophosphoric acid.*

Solution A. A mixture of 1 volume of 0.3 per cent v/v solution of *glacial acetic acid*, adjusted to pH 4.0 with *1 M sodium hydroxide* and 4 volumes of *acetonitrile.*

Test solution. Shake a quantity of the powdered tablets containing about 25 mg of Simvastatin with 20 ml of solution A for 15 minutes, dilute to 100 ml and filter.

Reference solution (a). Dilute 1 ml of the test solution to 500 ml with solution A.

Reference solution (b). A solution of 0.002 per cent w/v each of *simvastatin RS* and *lovastatin RS* in solution A.

Chromatographic system

- a stainless steel column 10 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (3µm) (such as Hypersil ODS),
- mobile phase: A. a mixture of 40 volumes of *acetonitrile* and 60 volumes of buffer solution,
  B. a mixture of 20 volumes of buffer solution and 80 volumes of *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 5	85	15
5 - 30	85 →0	15 →100
30 - 45	0	100
45 - 46	0 →85	100 →15
46 - 50	85	15

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to simvastatin and lovastatin is not less than 3.0. The relative retention time with reference to simvastatin for simvastatin hydroxy acid (simvastatin impurity A) is about 0.52, for lovastatin (simvastatin impurity E) is about 0.71, for epilovastatin (simvastatin impurity F) is about 0.74, for anhydrosimvastatin (simvastatin impurity C) is about 1.74 and for simvastatin dimer (simvastatin impurity D) is about 2.42.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution the area of peaks corresponding to lovastatin and epilovastatin is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent), the area of any secondary peak other than lovastatin and epilovastatin is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). The sum of the areas of all the secondary peaks other than lovastatin and epilovastatin is not more than five times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.25 times of the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solution A. Add 3 ml of *glacial acetic acid* to 900 ml of *water*, adjust the pH to 4.0 with *1 M sodium hydroxide* and add sufficient *water* to produce 1000 ml. Mix 1 volume of this solution with 4 volumes of *acetonitrile.*

Test solution. Disperse a quantity of whole tablets containing about 0.16 g of Simvastatin in *water* and mix with the aid of ultrasound and shaking. Add sufficient of solution A to produce about 75 ml, mix with the aid of ultrasound and shaking for 15 minutes, dilute to 100 ml with solution A and centrifuge. Dilute 1 ml of the clear supernatant solution with sufficient of solution A to produce a solution containing 0.01 per cent w/v of Simvastatin.

Reference solution. A 0.01 per cent w/v solution of *simvastatin RS* in solution A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- column temperature. 45°,
- mobile phase: a mixture of 7 volumes of a buffer solution prepared by dissolving 5.1 g of *sodium dihydrogen orthophosphate* in 900 ml of *water*, adjusted to pH 4.5 with *orthophosphoric acid* or *1 M sodium hydroxide* and add sufficient *water* to produce 1000 ml and 13 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor of the principal peak is not more than 2.0.

Inject the test solution and the reference solution.

Calculate the content of $C_{25}H_{38}O_5$ in the tablets.

Sodium Acetate

$CH_3COONa,3H_2O$

$C_2H_3NaO_2, 3H_2O$ Mol. Wt. 136.1

Sodium Acetate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_2H_3NaO_2$, calculated on the dried basis.

Category. Pharmaceutical aid (for peritoneal dialysis fluids).

Description. Colourless crystals or a white, crystalline powder.

Identification

Dissolve 10.0 g in sufficient *carbon dioxide-free water* to produce 100.0 ml (solution A). 1 ml of solution A gives reaction B of acetates and reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 7.5 to 9.0, determined in a 5.0 per cent w/v solution.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 15 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Calcium and magnesium. Not more than 50 ppm, calculated as Ca, determined by the following method. Mix 200 ml of *water* with 10 ml of *ammonia buffer pH 10.0*, 0.1 g of *mordant black 11 mixture* and 2 ml of *0.05 M zinc chloride*. Add dropwise *0.02 M disodium edetate* until the colour changes from violet to blue. To this solution add 10 g of the substance under examination, shake to dissolve and titrate with *0.02 M disodium edetate* until the blue colour is restored. Not more than 0.65 ml of *0.02 M disodium edetate* is required.

Heavy metals (2.3.13).12 ml of solution A complies with the limit test for heavy metals, Method D (10 ppm).

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Chlorides (2.3.12). 10 ml of solution A complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (225 ppm).

Reducing substances. Dissolve 1.0 g in 100 ml of boiling *water*, add 5 ml of *1 M sulphuric acid* and 0.5 ml of *0.002 M potassium permanganate*, mix and boil gently for 5 minutes; the pink colour is not completely discharged.

Loss on drying (2.4.19). 39.0 to 40.5 per cent, determined on 0.2 g by drying in an oven at 130°. Place the substance under examination in the oven while the oven is still cold.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, add 5 ml of *acetic anhydride*, mix and allow to stand for 30 minutes. Titrate with *0.1 M perchloric acid*, using 0.3 ml of *1-naphtholbenzein solution* as indicator, until a green colour is produced. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.00820 g of $C_2H_3NaO_2$.

Sodium Acetate intended for use in the preparation of dialysis solutions complies with the following additional requirement.

Aluminium. Dissolve 20 g in 100 ml of *water* and adjust to pH 6.0 by the addition of about 10 ml of *1 M hydrochloric acid*. Extract with successive quantities of 20, 20 and 10 ml of a 0.5 per cent w/v solution of *8-hydroxyquinoline* in *chloroform* and dilute the combined extracts to 50 ml with *chloroform*. Use as the standard solution a mixture of 0.4 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer pH 6.0* and 98 ml of *water* treated in the same manner and as the blank solution a mixture of 10 ml of *acetate buffer pH 6.0* and 100 ml of *water* treated in the same manner. Measure the fluorescence (2.4.5) of the test solution and the standard solution, using an excitation wavelength of about 392 nm and emission wavelength of about 518 nm, and setting the instrument to zero with the blank solution in each case. The fluorescence of the test solution is not greater than that of the standard solution (0.2 ppm).

Storage. Store protected from light.

Labelling. The label states whether or not the material is intended for use in the manufacture of dialysis solutions.

Sodium Alginate

Sodium Polymannuronate

Sodium Alginate consists mainly of the sodium salt of alginic acid which is a mixture of polyuronic acids $[(C_6H_8O_6)_n]$ composed of residues of D-mannuronic acid and L-guluronic acids and is obtained mainly from algae belonging to the order Phaeophyceae.

Category. Pharmaceutical aid (viscosity-increasing agent).

Description. A cream-coloured to pale yellowish brown powder; almost odourless.

Identification

A. Dissolve 0.2 g with shaking in 20 ml of *water* and to 5 ml of the resulting solution add 1 ml of *calcium chloride solution*; a voluminous gelatinous precipitate is produced.

B. To 10 ml of the solution obtained in test A add 1 ml of *1 M sulphuric acid*; a gelatinous mass is produced.

C. To 5 mg add 5 ml of *water*, 1 ml of a freshly prepared 1 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and 5 ml of *hydrochloric acid*. Boil for 3 minutes, cool, add 5 ml of *water* and shake with 15 ml of *di-isopropyl ether*. The upper layer exhibits a deeper bluish red colour than the upper layer obtained by repeating the procedure without the substance under examination.

D. The residue obtained in the test for Sulphated ash gives reaction A of sodium salts (2.3.1).

Tests

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm), using *nitric acid* instead of *sulphuric acid* for wetting the sample.

Chloride. Not more than 1.0 per cent, determined by the following method. Shake 2.5 g with 50 ml of *2 M nitric acid* for 1 hour, dilute to 100 ml with 2M nitric acid and filter. To 50 ml of the filtrate add 10.0 ml of *0.1 M silver nitrate* and 5 ml of *toluene*. Titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution* as indicator and shaking vigorously towards the end-point.

1 ml of *0.1 M silver nitrate* is equivalent to 0.00355 g of Cl.

Microbial contamination (2.2.9). Not more than 10^3 micro-organisms per gram. It complies with the test for *Escherichia coli* and *Salmonella.*

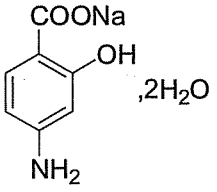
Sulphated ash (2.3.18). 30.0 to 36.0 per cent, determined on 0.1 g and calculated on the dried basis.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.2 g by drying in an oven at 105° for 4 hours.

Storage. Store protected from light.

Sodium Aminosalicylate

Sodium PAS



$C_7H_6NNaO_3,2H_2O$. Mol. Wt. 211.2

Sodium Aminosalicylate is sodium 4-amino-2-hydroxy-benzoate dihydrate.

Sodium Aminosalicylate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_6NNaO_3$, calculated on the anhydrous basis.

Category. Antitubercular.

Dose. 10 to 15 g daily, in divided doses.

Description. A white to cream coloured crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sodium aminosalicylate RS* or with the reference spectrum of sodium aminosalicylate.

B. A 5 per cent w/v solution complies with the tests for sodium salts (2.3.1).

Tests

pH (2.4.24). 6.5 to 8.5, determined in a 2.0 per cent w/v solution.

3-aminophenol. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dissolve 25.0 mg of *3-aminophenol RS* in 100.0 ml of the mobile phase (solution A). Dilute 5.0 ml of the solution to 100.0 ml with the mobile phase. Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

Reference solution (b). Dissolve 25.0 mg of *sodium aminosalicylate RS* in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution and 5 ml of solution A to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous base deactivated silica (5 µm) (such as Hypersil BDS),
- mobile phase: dissolve 6.0 g of *disodium hydrogen orthophosphate* and 6.6 g of *sodium dihydrogen orthophosphate dihydrate* in 1600 ml with *water*, add 19 ml of *tetra n-butyl ammonium hydroxide (20 per cent solution)* and make the volume to 1700 ml with *water* and add 300 ml of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 5.0 per cent

Inject reference solution (b). The test is not valid unless the resolution between m-aminophenol and sodium aminosalicylate is at least 5.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of the peak corresponding to 3-aminophenol is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.25 per cent).

Hydrogen Sulphide and Sulphur dioxide. Dissolve 0.5 g in 5 ml of *1 M sodium hydroxide*, add 6 ml of *3 M hydrochloric acid* and stir vigorously. No odour of hydrogen sulphide or sulpher dioxide is perceptible, and not more than a faint odour of Amyl Alcohol is perceptible. A piece of moistened *lead acetate paper* held over the mixture does not become discoloured.

Arsenic (2.3.10). Mix 5.0 g with 10 ml of *bromine solution* and evaporate to dryness on a water-bath, ignite gently, dissolve the cooled residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT* and remove the

excess bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 0.66 g complies with the limit test for heavy metals, Method B (30 ppm).

Chlorides (2.3.12). Dissolve 1.0 g in 10 ml of *water*, add 3 ml of *acetic acid* and filter, dilute the filtrate to 50 ml with *water*. 10 ml of the solution complies with the limit test for chlorides (0.25 per cent).

Sulphates (2.3.17). 0.1 g complies with the limit test for sulphates (0.15 per cent).

Loss on drying (2.4.19). 16.0 to 17.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (a). Dissolve 50.0 mg of *sodium aminosalicylate RS* in 100.0 ml of the mobile phase (solution A). Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 25.0 mg of *3-aminophenol RS* in 100.0 ml of the mobile phase. Dilute 5.0 ml of this solution and 5 ml of solution A to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 6.0 g of *disodium hydrogen orthophosphate* and 6.6 g of *sodium dihydrogen orthophosphate dihydrate* in 1600 ml with *water*, add 19 ml of *tetra n-butyl ammonium hydroxide (20 per cent solution)* and make the volume to 1700 ml with *water* and add 300 ml of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The tailing factor is not more than 2.0. The column efficiency in not less than 3000 theoretical plates. The relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (b). The test is not valid unless the resolution between 3-aminophenol and sodium aminosalicylate is at least 5.0.

Inject alternately the test solution and the reference solution.

Calculate the content of $C_7H_6NNaO_3$.

Storage. Store protected from light and moisture.

Sodium Aminosalicylate Tablets

Sodium PAS Tablets

Sodium aminosalicylate tablet contains not less than 95.0 per cent not more than 105.0 per cent of $C_7H_6NNaO_3.2H_2O$.

Usual strengths. 500 mg; 1 g.

Identification

Digest a quantity of powdered tablets containing about 3.0 g of sodium aminosalicylate, with 40 ml of *water*, and filter. Add to the filtrate 15 ml of *1M acetic acid*, and allow it to stand until precipitation has occurred. Collect the precipitate on a filter, wash well with *water* and dry at 105°C for 30 min. The residue complies with the following tests.

A. Place about 1 g of the residue in a small, round-bottom flask, and add 10 ml of *acetic anhydride*. Heat the flask on a steam bath for 30 minutes, and add 40 ml of *water*, mix, filter, cool, and allow to stand until diacetyl derivative crystallizes, wash well with *water*, and dry at 105° for 1 hour. The diacetyl derivative so obtained melts at 191° to 197°.

B. Shake 0.1 g of the residue with 10 ml of *water*, and filter. To 5 ml of the filtrate add 1 drop of *ferric chloride solution*. A violet colour is produced.

C. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

3-aminophenol. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh a quantity of the powder containing about 50 mg of Sodium Aminosalicylate and dissolve in 100 ml of the mobile phase.

Reference solution (a). A 0.024 per cent w/v solution of *m-aminophenol RS* in the mobile phase. Dilute 5 ml to 100 ml with the mobile phase. Dilute 5 ml of the resulting solution to 50 ml with the mobile phase.

Reference solution (b). A 0.024 per cent w/v solution of *sodium aminosalicylate RS* in the mobile phase.

Reference solution (c). Mix 5 ml each of reference solution (a) and reference solution (b) and dilute to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous base deactivated silica (5 µm) (such as Hypersil BDS),
- mobile phase: dissolve 6.0 g of *disodium hydrogen orthophosphate* and 6.6 g of *sodium dihydrogen orthophosphate dihydrate* in 1600 ml with *water*, add

19 ml of *tetra n-butyl ammonium hydroxide (20 per cent aqueous solution)* and make the volume to 1700 ml with *water*, add 300 ml of *methanol*, mix well and degas,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (c). The resolution between 3-aminophenol and sodium aminosalicylate is not less than 5.0.

Inject the test solution and reference solution (a). In the chromatogram obtained with the test solution, the area of the peak corresponding to 3-aminophenol is not more than the area of the peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Dissolution (2.5.2).

Apparatus. No. 2,
Medium. 900 ml *water*,
Speed and time.100 rpm for 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate diluted with the mobile phase to produce a 0.055 per cent w/v solution.

Reference solution. A 0.055 per cent w/v solution of *sodium aminosalicylate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 6.0 g of *disodium hydrogen orthophosphate* and 6.6 g of *sodium dihydrogen orthophosphate dihydrate* in 1600 ml with *water*, add 19 ml of *tetra n-butyl ammonium hydroxide (20 per cent aqueous solution)* and make the volume to 1700 ml with *water*, add 300 ml of *methanol*, mix well and degas,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject alternatively the test solution and the reference solution.

Calculate the content of $C_7H_6NNaO_3.2H_2O$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_7H_6NNaO_3.2H_2O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of sodium aminosalicylate, add sufficient mobile phase to produce 100.0 ml and filter. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution. A 0.05 per cent w/v solution of *sodium aminosalicylate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 6.0 g of *disodium hydrogen orthophosphate* and 6.6 g of *sodium dihydrogen orthophosphate dihydrate* in 1600 ml with *water*, add 19 ml of *tetra n-butyl ammonium hydroxide (20 per cent aqueous solution)* and make the volume to 1700 ml with *water*, add 300 ml of *methanol*, mix well and degas,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

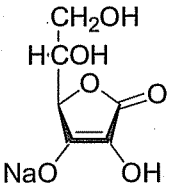
Inject the reference solution. The tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent

Inject alternately the test solution and the reference solution.

Calculate the content of $C_7H_6NNaO_3.2H_2O$ in the tablets.

Storage. Store protected from moisture.

Sodium Ascorbate



$C_6H_7NaO_6$ Mol. Wt. 198.1

Sodium Ascorbate is L-ascorbic acid monosodium salt.

Sodium Ascorbate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_7NaO_6$, calculated on the dried basis.

Category. Vitamin C.

Dose. The equivalent of up to 1 g of ascorbic acid daily.

Description. White or faintly yellow crystals or a crystalline powder; odourless or almost odourless. It darkens gradually on exposure to light.

Identification

Test A may be omitted if tests B, C and D are carried out. Test B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), Compare the spectrum with that obtained with *sodium ascorbate RS.*

B. To 4 ml of a 2 per cent w/v solution add 1 ml of *0.1 M hydrochloric acid*, add a few ml of *2,6-dichlorophenol-indophenol solution*; the solution is decolorised.

C. To 4 ml of a 2 per cent w/v solution add 1 ml of *0.1 M hydrochloric acid*, add 1 drop of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside* and 2 ml of *dilute sodium hydroxide solution*. Add 0.6 ml of *hydrochloric acid* dropwise and stir; the yellow colour turns blue.

D. A 2 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1) and not more intensely coloured than reference solution BYS7 (2.4.1).

pH (2.4.24). 7.0 to 8.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +103° to +108°, determined in a 10.0 per cent w/v solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

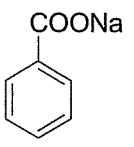
Loss on drying (2.4.19). Not more than 0.25 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 100 ml of *carbon dioxide-free water* and 25 ml of *1 M sulphuric acid.* Titrate immediately with *0.05 M iodine*, using 1 ml of *starch solution* as indicator, until a persistent violet-blue colour is obtained.

1 ml of *0.05 M iodine* is equivalent to 0.009905 g of $C_6H_7NaO_6$.

Storage. Store protected from light.

Sodium Benzoate



$C_7H_5NaO_2$ Mol. Wt. 144.1

Sodium Benzoate contains not less than 99.0 per cent and not more than 100.5 per cent of $C_7H_5NaO_2$, calculated on the dried basis.

Category. Pharmaceutical aid (preservative).

Description. A white, crystalline or granular powder or flakes; odourless or with a faint odour; hygroscopic.

Identification

A. To a 10 per cent w/v solution add *ferric chloride test solution*; a buff coloured precipitate is formed. Add *dilute hydrochloric acid*; a white crystalline precipitate is produced.

B. Gives the reactions of sodium salts and reactions B and C of benzoates (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Acidity or alkalinity. To 20 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* add 0.2 ml of *phenolphthalein solution*. Not more than 0.2 ml of *0.1 M hydrochloric acid* or 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Arsenic (2.3.10). Mix 5.0 g with 10 ml of *bromine solution* and evaporate to dryness on a water-bath. Ignite gently, dissolve the cooled residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT*, and remove the excess of *bromine* with 2 ml of *stannous* Chloride *solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 45 ml of *water* and 5 ml of *hydrochloric acid.* 25 ml of the solution complies with the limit test for heavy metals, Method B (20 ppm).

Chlorinated compounds. Dissolve 0.33 g in 5 ml of *0.5 M sodium carbonate*, evaporate to dryness and heat the residue until completely charred, keeping the temperature below 400°. Extract the residue with a mixture of 10 ml of *water* and 12 ml of *dilute nitric acid* and filter; the filtrate complies with the limit test for chlorides (2.3.12).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 20 ml of *anhydrous glacial acetic acid*, warming to 50° if necessary, cool. Titrate with *0.1 M perchloric acid*, using 0.05 ml of *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01441 g of $C_7H_5NaO_2$.

Storage. Store protected from light.

Sodium Bicarbonate

Sodium Hydrogen Carbonate

$NaHCO_3$ Mol. Wt. 84.0

Sodium Bicarbonate contains not less than 99.0 per cent and not more than 101.0 per cent of $NaHCO_3$.

Category. Electrolyte replenisher; systemic alkaliser.

Dose. 1 to 4 g.

Description. A white, crystalline powder or small, opaque, monoclinic crystals. It gradually forms sodium carbonate on heating in the dry state or in solution.

Identification

A. To 5 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) add 0.1 ml of *phenolphthalein solution*; a pale pink colour is produced. On heating, a gas is evolved and the solution becomes red.

B. Gives reaction A of bicarbonates (2.3.1).

C. Solution A gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water*, add 15 ml of *brominated hydrochloric acid AsT* and remove the excess of *bromine* with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Calcium. A 2.0 per cent w/v solution, when boiled for 5 minutes, is clear.

Heavy metals (2.3.13). Mix 4.0 g with 5 ml of *water* and 18 ml of *dilute hydrochloric acid*, heat to boiling and maintain that temperature for 1 minute. Add 0.05 ml of *phenolphthalein solution* and sufficient *5 M ammonia* dropwise to give the solution a faint pink colour, cool and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Iron (2.3.14). Dissolve 2.0 g in 20 ml of *water* and 4 ml of *hydrochloric acid* and dilute to 40 ml with *water*; the resulting solution complies with the limit test for iron (20 ppm).

Carbonate. pH of solution A, when freshly prepared, not more than 8.6.

Chlorides (2.3.12). 1.25 g dissolved in 15 ml of *water* and 2 ml of *nitric acid* complies with the limit test for chlorides (200 ppm).

Sulphates (2.3.17). Suspend 1.0 g in 10 ml of *distilled water*, neutralise with *hydrochloric acid* and dilute to 15 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (150 ppm).

Assay. Weigh accurately about 1.5 g, dissolve in 50 ml of *carbon dioxide-free water* and titrate with *1 M hydrochloric acid* using 0.2 ml of *methyl orange solution* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08401 g of $NaHCO_3$.

Storage. Store protected from moisture.

Sodium Bicarbonate Injection

Sodium Bicarbonate Intravenous Infusion

Sodium Bicarbonate Injection is a sterile solution of Sodium Bicarbonate in Water for Injections.

Sodium Bicarbonate Injection contains not less than 94.0 per cent and not more than 106.0 per cent of the stated amount of sodium bicarbonate, $NaHCO_3$.

Category. Electrolyte replenisher; systemic alkaliser.

Usual strengths. 1.4, 4.2, 5.0, 7.5 and 8.4 per cent w/v.

Description. A clear, colourless solution.

Identification

A. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

B. Gives reaction A of sodium salts and the reactions of bicarbonates (2.3.1).

Tests

pH (2.4.24). 7.0 to 8.5.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Titrate a volume containing 1.5 g of Sodium Bicarbonate with *1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 0.08401 g of $NaHCO_3$.

Storage. Store in single dose containers.

Labelling. The label states (1) the strength as the percentage w/v of Sodium Bicarbonate; (2) the approximate concentrations, in millimoles per litre, of the sodium ions and the bicarbonate ions; (3) that an injection containing visible particles in the solution should not be used.

Sodium Carbonate

Na_2CO_3 Mol. Wt. 106.0 (anhydrous)

Na_2CO_3, H_2O Mol.Wt.124.0 (monohydrate)

Sodium Carbonate is anhydrous or contains one molecule of water of hydration.

Sodium Carbonate contains not less than 99.5 per cent and not more than 100.5 per cent of Na_2CO_3, calculated on the dried basis.

Category. Pharmaceutical aid (alkalising agent)

Description. *Anhydrous* — A white or almost white, slightly granular powder, hygroscopic.

Monohydrate — A white, crystalline powder or colourless crystals.

Identification

A. Dissolve 1 g in *water* and dilute to 10 ml with *water*; the solution is strongly alkaline.

B. The solution prepared for test A gives reaction A of carbonates and reactions of sodium salts (2.3.1)

Tests

Appearance of solution. Dissolve 2.0 g in 10 ml of *water*. The resulting solution is clear and not more intensely coloured than reference solution YS6, (2.4.1).

Alkali hydroxides and bicarbonates. Dissolve 0.4 g in 20 ml of *water*, add 20 ml of *barium chloride solution* and filter. To 10 ml of the filtrate add 0.1 ml of *phenolphthalein solution*. The solution does not become red. Heat the remainder of the filtrate to boiling for 2 minutes. The solution remains clear.

Heavy metals (2.3.13). Dissolve 2.0 g in portions in a mixture of 5 ml of hydrochloric acid and 25 ml of water. Heat the solution to boiling and cool. Add dilute sodium hydroxide solution until the solution is neutral. Dilute to 50 ml with water (solution A). 12 ml of the resulting solution complies with the limit test for heavy metals, Method A (50 ppm). Use lead standard solution (2 ppm Pb) for the standard.

Iron (2.3.14). Dilute 5 ml of solution A to 10 ml with water. The solution complies with the limit test for iron (50 ppm).

Chlorides (2.3.12). Dissolve 0.4 g in water, add 4 ml of dilute nitric acid and dilute to 15 ml with water. The solution complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates, (250 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent (for anhydrous form) or between 12.0 per cent and 15.0 per cent (for monohydrate form), determined on 2.0 g by drying in an oven at 300°.

Assay. Weigh accurately about 1.0 g, dissolve in 25 ml of *water* and titrate with *1 M hydrochloric acid* using *methyl orange solution* as indicator.

1 ml of *1 M hydrochloric acid* is equivalent to 0.05299 g of Na_2CO_3.

Storage. Store in tightly-closed, non-metallic containers.

Labelling. The label states whether the material is anhydrous or monohydrate.

Sodium Chloride

NaCl Mol. Wt. 58.4

Sodium Chloride contains not less than 99.0 per cent and not more than 100.5 per cent of NaCl, calculated on the dried basis.

Category. Pharmaceutical aid (tonicity agent); fluid and electrolyte replenisher.

Description. White or colourless crystals or a white crystalline powder.

Identification

A. Gives the reactions of chlorides (2.3.1).

B. A 20 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* (solution A) gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 20 ml of solution A add 0.1 ml of *bromothymol blue solution*; not more than 0.5 ml of *0.01 M hydrochloric acid* or of *0.01 M sodium hydroxide* is required to change the colour of the solution.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and 12 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Barium. Dissolve 2 g in 10 ml of *water*, and add 2 ml of *dilute sulphuric acid*; no turbidity is produced within 2 hours.

Bromide. To 0.5 ml of solution A add 4.0 ml of *water*, 2.0 ml of *phenol red reagent* and 1.0 ml of 0.01 per cent w/v solution of *chloramine T* and mix immediately. After exactly 2 minutes, add 0.15 ml of *0.1 M sodium thiosulphate*, mix and dilute to 10.0 ml with *water*. The absorbance of the solution measured at about 590 nm (2.4.7), using *water* as the blank, is not more than that of the standard solution prepared at the same time and in the same manner, using 5.0 ml of a 0.0003 per cent w/v solution of *potassium bromide* (100 ppm).

Calcium and magnesium. Not more than 50 ppm, calculated as Ca, determined by the following method. Dissolve 20.0 g in 200 ml of *water*, and add 0.1 ml of *hydrochloric acid*, 5 ml of *strong ammonia-ammonium chloride solution*, 5 drops of *eriochrome black T solution* and titrate with *0.005 M disodium edetate* to a blue end-point.

1 ml of *0.005 M disodium edetate* is equivalent to 0.0002004 g of Ca.

Ferrocyanide. Dissolve 2.0 g in 6 ml of *water* and add 0.5 ml of a mixture of 5 ml of a 1 per cent w/v solution of *ferric ammonium sulphate* in a 0.25 per cent w/v solution of *sulphuric acid*, and 95 ml of a 1 per cent w/v solution of *ferrous sulphate*; no blue colour is produced within 10 minutes.

Heavy metals (2.3.13). 4.0 g in 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Iodide. Moisten 5 g by adding dropwise, a solution freshly prepared by mixing 25 ml of *iodide-free starch solution* 2 ml of *0.5 M sulphuric acid*, 0.15 ml of *sodium nitrite solution* and 25 ml of *water* and examine the mixture in daylight; the substance shows no blue colour after 5 minutes.

Iron (2.3.14). 2.0 g dissolved in 20 ml of *water* complies with the limit test for iron (20 ppm).

Sulphates (2.3.17). 2.5 ml of solution A diluted to 15 ml with *water* complies with the limit test for sulphates (300 ppm).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.1 g and dissolve in 50 ml of *water* in a glass-stoppered flask. Add 50.0 ml of *0.1 M silver nitrate*, 5 ml of *2 M nitric acid* and 2 ml of *dibutyl phthalate*, shake well and titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution* as indicator, until the colour becomes reddish yellow.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Sodium *Chloride* intended for use in the manufacture of parenteral preparations or in the manufacture of dialysis solutions complies with the following additional requirement.

Potassium. Not more than 0.1 per cent, determined by flame photometry (2.4.4), using a 1.0 per cent w/v solution and measuring at 768 nm. Use suitable dilutions in *water* of *potassium solution FP* for the standard solution.

Sodium *Chloride* intended for use in the preparation of dialysis solutions complies with the following additional requirement.

Aluminium. Not more than 0.2 ppm, determined by the following method. Dissolve 20 g in 100 ml of *water* and add 10 ml of *acetate buffer pH 6.0*. Extract the resulting solution with successive quantities of 20, 20 and 10 ml of a 0.5 per cent w/v solution of *8-hydroxyquinoline* in *chloroform* and dilute the combined extracts to 50 ml with *chloroform*. Use as the blank solution a mixture of 10 ml of *acetate buffer pH 6.0* and 100 ml of *water* treated in the same manner and as the standard solution a mixture of 2 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer pH 6.0* and 90 ml of *water* treated in the same manner. Measure the fluorescence of the test solution and of the standard solution (2.4.5), using an excitation wavelength of 392 nm and a secondary filter with a transmission band centered at 518 nm, or a monochromator set to transmit at this wavelength, and setting the instrument to zero with the blank solution in each case. The fluorescence of the test solution is not greater than that of the standard solution.

Storage. Store protected from light.

Labelling. The label states whether or not the material is suitable for use in the manufacture of parenteral preparations or for the preparation of dialysis solutions.

Sodium Chloride and Dextrose Injection

Sodium Chloride and Dextrose Intravenous Infusion; Sodium Chloride and Glucose Injection; Sodium Chloride and Glucose Intravenous Infusion

Sodium Chloride and Dextrose Injection is a sterile solution of Sodium Chloride and Dextrose in Water for Injections.

Sodium Chloride and Dextrose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium chloride, NaCl, and dextrose, $C_6H_{12}O_6$.

Usual strengths. Injections containing the following amounts of Sodium Chloride, NaCl and Dextrose, $C_6H_{12}O_6$.

Per centage w/v of sodium chloride (NaCl)	Percentage w/v of dextrose ($C_6H_{12}O_6$)	Percentage w/v of sodium chloride (NaCl)	Percentage w/v of dextrose ($C_6H_{12}O_6$)
0.11	5		
0.18	5	0.45	5
0.2	5	0.45	10
0.225	5	0.9	2.5
0.3	5	0.9	5
0.33	5	0.9	10
0.45	2.5	0.9	25

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction B of sodium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 6.5.

5-Hydroxymethylfurfural and related substances. Dilute a volume containing 1.0 g of dextrose, $C_6H_{12}O_6$, to 500 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm (2.4.7); absorbance at about 284 nm, not more than 0.25.

Bacterial endotoxins (2.2.3). Not more than 10 Endotoxin Units per g of dextrose.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. *For sodium chloride* — Titrate an accurately measured volume containing 0.1 g of Sodium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

For dextrose — To an accurately measured volume containing 2 to 5 g of anhydrous dextrose, $C_6H_{12}O_6$, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume of the injection taken for assay.

Storage. Store in single dose containers. On keeping, small solid particles may separate from a glass container.

Labelling. The label states (1) the strength as the percentages w/v of Sodium Chloride and Dextrose; (2) that a solution containing visible particles must not be used.

When the preparation is intended for intravenous infusion, the label states the approximate concentrations, in millimoles per litre, of the sodium and chloride ions and the number of grams per litre of dextrose, $C_6H_{12}O_6$.

Sodium Chloride and Fructose Injection

Sodium Chloride and Fructose Intravenous Infusion; Sodium Chloride and Fructose Infusion; Fructose and Sodium Chloride Injection.

Sodium Chloride and Fructose Injection is a sterile solution of Sodium Chloride and Fructose in Water for Injections.

Sodium Chloride and Fructose Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of sodium chloride, NaCl, and fructose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Usual strengths. Injections containing the following amounts of Sodium Chloride, NaCl, and Fructose, $C_6H_{12}O_6$.

Per cent w/v of Sodium Chloride (NaCl)	Per cent w/v of Fructose ($C_6H_{12}O_6$)	Per cent w/v of Sodium Chloride (NaCl)	Per cent w/v of Fructose ($C_6H_{12}O_6$)
0.11	5	0.45	2.5
0.18	5	0.45	10
0.225	5	0.9	2.5
0.33	5	0.9	5
0.45	5	0.9	10

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. The solution prepared in the Assay is laevo-rotatory.

C. Gives reaction B of sodium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 3.0 to 6.0.

5-Hydroxymethylfurfural and related substances. Dilute a volume containing 1.0 g of Fructose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm (2.4.7); absorbance at about 284 nm, not more than 0.50.

Heavy metals (2.3.13). Evaporate a volume containing 4.0 g of Fructose to 10 ml and add 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Assay. *For sodium chloride* — Titrate an accurately measured volume containing about 0.1 g of Sodium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

For fructose — To an accurately measured volume containing about 5.0 g of Fructose, add 0.2 ml of *6 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.5427 represents the weight, in g, of fructose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers. On keeping, small solid particles may separate from glass containers.

Labelling. The label states (1) the strength as the percentages w/v of Sodium Chloride and Fructose; (2) when the preparation is intended for intravenous infusion, the approximate concentrations, in millimoles per litre, of the sodium and chloride ions and the number of grams per litre of fructose; (3) that a solution containing visible particles should not be used.

Compound Sodium Chloride and Dextrose Injection

Compound Sodium Chloride and Dextrose Intravenous Infusion

Compound Sodium Chloride and Dextrose Injection is a sterile solution containing 0.86 per cent w/v of Sodium Chloride, 0.03 per cent w/v of Potassium Chloride, 0.033 per cent w/v of Calcium Chloride and 5 per cent w/v of Dextrose in Water for Injections.

Compound Sodium Chloride and Dextrose Injection contains not less than 0.82 per cent and not more than 0.90 per cent w/v of sodium chloride, NaCl, not less than 0.0285 per cent and not more than 0.0315 per cent w/v of potassium chloride, KCl, not less than 0.030 per cent and not more than 0.036 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than 0.523 per cent and not more than 0.580 per cent w/v of chloride, Cl, and not less than 4.75 per cent and not more than 5.25 per cent w/v of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Category. Fluid and electrolyte replenisher.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. Gives reaction B of sodium salts and reaction A of chlorides (2.3.1).

C. After evaporation to one half of its original volume, the solution gives reaction A of potassium salts and reaction B of calcium salts (2.3.1).

Tests

pH (2.4.24). 3.5 to 6.5.

5-Hydroxymethylfurfural and related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium chloride* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

1 g of Na is equivalent to 2.542 g of NaCl.

For potassium chloride — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

1 g of K is equivalent to 1.907 g of KCl.

For calcium chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *eriochrome black T mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For total chloride — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total Chloride, calculated as Cl.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient

water to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers of glass or plastic at a temperature not exceeding 30°. On keeping, small solid particles may separate from the solution in glass containers.

Labelling. The label states (1) the strength as the percentages w/v of Sodium Chloride, Potassium Chloride, Calcium Chloride and Dextrose; (2) that the injection contains, in millimoles per litre, the following approximate amounts of the ions. sodium, 147.5, potassium, 4, calcium, 4.5, and Chloride, 156; (3) the total osmolar concentration in mOsmol per litre; (4) that the injection should not be used if it contains visible particles.

Sodium Chloride Hypertonic Injection

Hypertonic Saline

Sodium Chloride Hypertonic Injection is a sterile 1.6 per cent w/v solution of Sodium Chloride in Water for Injections. It contains no antimicrobial agent.

Sodium Chloride Hypertonic Injection contains not less than 1.52 per cent w/v and not more than 1.68 per cent w/v of sodium chloride, NaCl.

Description. A clear, colourless solution.

Identification

Gives the reactions of sodium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5.

Heavy metals (2.3.13). Evaporate 40 ml to about 20 ml, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (0.5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. To an accurately measured volume containing about 0.16 g of Sodium Chloride in a glass-stoppered flask add 50 ml of *water* and 50.0 ml of *0.1 M silver nitrate*, 5 ml of *2 M nitric acid* and 2 ml of *dibutyl phthalate*. Shake well and titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution* as indicator, until the colour becomes reddish yellow.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from a glass container.

Labelling. The label states (1) the strength as the percentage w/v of Sodium Chloride; (2) that a solution containing visible solid particles must not be used.

When the preparation is intended for intravenous infusion, the label states that the injection contains approximately 270 millimoles each of sodium and chloride ions per litre.

Sodium Chloride Injection

Sodium Chloride Intravenous Infusion

Sodium Chloride Injection is a sterile 0.9 per cent w/v solution of Sodium Chloride in Water for Injections. It contains no antimicrobial agent.

Sodium Chloride Injection contains not less than 0.85 per cent w/v and not more than 0.95 per cent w/v of sodium Chloride, NaCl.

Description. A clear, colourless solution.

Identification

Gives the reactions of sodium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 7.0.

Heavy metals (2.3.13). Evaporate 67 ml to about 20 ml, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (0.3 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. To an accurately measured volume containing about 0.16 g of Sodium Chloride in a glass-stoppered flask add 50 ml of *water* and 50.0 ml of *0.1 M silver nitrate*, 5 ml of *2 M nitric acid* and 2 ml of *dibutyl phthalate*. Shake well and titrate with *0.1 M ammonium thiocyanate* using 2 ml of *ferric ammonium sulphate solution* as indicator, until the colour becomes reddish yellow.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from a glass container.

Labelling. The label states (1) the strength as the percentage w/v of Sodium Chloride; (2) that a solution containing visible solid particles must not be used.

When the preparation is intended for intravenous infusion, the label states that the injection contains approximately 150 millimoles each of sodium and chloride ions per litre.

Compound Sodium Chloride Injection

Ringer's Injection

Compound Sodium Chloride Injection is a sterile solution containing 0.86 per cent w/v of Sodium Chloride, 0.03 per cent w/v of Potassium Chloride and 0.033 per cent w/v of Calcium Chloride in Water for Injections. It contains no antimicrobial agent.

Compound Sodium Chloride Injection contains not less than 0.82 per cent w/v and not more than 0.90 per cent w/v of sodium chloride, NaCl, not less than 0.0285 per cent w/v and not more than 0.0315 per cent w/v of potassium chloride, KCl, and not less than 0.030 per cent w/v and not more than 0.036 per cent w/v of calcium chloride, $CaCl_2, 2H_2O$.

Description. A clear, colourless solution.

Identification

Gives reaction B of sodium salts and reaction A of chlorides (2.3.1). When concentrated to one half of its original volume, it gives reaction A of potassium salts and reaction B of calcium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5.

Heavy metals (2.3.13). Evaporate 67 ml to about 20 ml, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (0.3 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. *For sodium chloride* — Dilute appropriately with water and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using sodium solution FP, suitably diluted with water for the standard solutions.

1 g of Na is equivalent to 2.54 g of NaCl.

For potassium Chloride — Dilute appropriately with *water* and determine by Method A for flame photometry (2.4.4), measuring at 767 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *potassium solution FP*, suitably diluted with water for the standard solutions.

1 g of K is equivalent to 1.007 g of KCl.

For Calcim Chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with 0.01M disodium edetate using *mordant black II mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of 0.01M magnesium sulphate added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from a glass container.

Labelling. The label states (1) the strength as the percentages w/v of Sodium Chloride, Potassium Chloride and Calcium Chloride; (2) that a solution containing visible solid particles must not be used.

When the preparation is intended for intravenous infusion, the label states that the Injection contains in millimoles per litre, the following approximate amounts of ions; sodium, 147.5; potassium, 4; calcium, 2.25 and chloride, 156.

Compound Sodium Chloride Solution

Ringer's Solution

Compound Sodium Chloride Solution is a solution containing 0.86 per cent w/v of Sodium Chloride, 0.03 per cent w/v of Potassium Chloride and 0.033 per cent w/v of Calcium Chloride in Purified Water. The solution may be clarified by filtration.

Compound Sodium Chloride Solution contains not less than 0.82 per cent w/v and not more than 0.90 per cent of sodium chloride, NaCl, not less than 0.025 per cent and not more than 0.035 per cent w/v of potassium chloride, KCl, and not less than 0.030 per cent and not more than 0.036 per cent w/v of calcium chloride, $CaCl_2, 2H_2O$.

Category. Irrigation solution (for external use).

Description. A clear, colourless solution.

Identification

Gives reaction B of sodium salts and reaction A of chlorides (2.3.1). When concentrated to one half of its original volume,

it gives reaction A of potassium salts and reaction B of calcium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.5.

Heavy metals (2.3.13). Evaporate 67 ml to about 20 ml, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (0.3 ppm).

Assay. *For sodium chloride* — Dilute appropriately with water and determine by Method A for flame photometry (2.4.4), measuring at 589 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *sodium solution FP,* suitably diluted with water for the standard solutions.

1 g of Na is equivalent to 2.54 g of NaCl.

For potassium chloride — Dilute appropriately with water and determine by Method A for flame photometry (2.4.4), measuring at 767 nm or by Method A for atomic absorption spectrophotometry (2.4.2), using *potassium solution FP,* suitably diluted with water for the standard solutions.

1 g of K is equivalent to 1.007 g of KCl.

For calcium Chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *mordant black II mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of 0.01M magnesium sulphate added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light.

Labelling. The label states the strength as the percentages w/v of Sodium Chloride, Potassium Chloride and Calcium Chloride. If the contents of the container are sterile, the label states (1) Sterile Compound Sodium Chloride Solution; (2) that the solution is not intended for injection.

Sodium Chloride Irrigation Solution

Sodium Chloride Irrigation Solution is a sterile solution containing 0.9 per cent w/v of Sodium Chloride in Water for Injections.

Sodium Chloride Irrigation Solution contains not less than 0.85 per cent w/v and not more than 0.95 per cent w/v of sodium Chloride, NaCl. It contains no antimicrobial agent.

Description. A clear, colourless solution.

Identification

Gives the reactions of sodium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.5 to 7.0.

Heavy metals (2.3.13). Evaporate 67 ml to about 20 ml, add 2 ml of *dilute acetic acid* and dilute to 25 ml with *water*. The solution complies with limit test for heavy metals, Method A (0.3 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Titrate an accurately measured volume containing about 0.135 g of Sodium Chloride with *0.1 M silver nitrate* using *potassium chromate solution* as indicator.

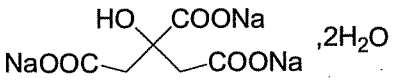
1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Storage. Store in single dose containers. The container may be designed to empty rapidly and may contain a volume of more than 1 litre.

Labelling. The label states (1) the strength as the percentage w/v of Sodium Chloride; (2) that the solution should not be used if it contains visible particles; (3) 'For Irrigation only' and 'Not for Injection'; (4) that once the container is opened, the unused portion should be discarded.

Sodium Citrate

Trisodium Citrate



$C_6H_5Na_3O_7,2H_2O$ Mol. Wt. 294.1

Sodium Citrate is trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate.

Sodium Citrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_5Na_3O_7$, calculated on the anhydrous basis.

Category. Systemic alkalinising agent.

Dose. 1 to 10 g.

Description. White, granular crystals or a white, crystalline powder; odourless; slightly deliquescent in moist air.

Identification

A. 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) gives the reactions of sodium salts and reaction A of citrates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Titrate 20 ml of solution A with *0.05 M sulphuric acid* or *0.1 M sodium hydroxide* using *thymol blue solution* as indicator; not more than 0.5 ml of *0.05 M sulphuric acid* or *0.1 M sodium hydroxide* is required.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 15 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of *water*, 5 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). 25 ml of solution A complies with the limit test for chlorides (100 ppm).

Oxalate. Dissolve 0.5 g in 4 ml of *water*, add 3 ml of *hydrochloric acid* and 1 g of *zinc*, in granules, and heat on a water-bath for 1 minute. Allow to stand for 2 minutes, decant the liquid into a test-tube containing 0.25 ml of a 1 per cent w/v solution of *phenylhydrazine hydrochloride* and heat to boiling. Cool rapidly, transfer to a graduated cylinder and add an equal volume of *hydrochloric acid* and 0.25 ml of *potassium ferricyanide solution*. Shake and allow to stand for 30 minutes. Any pink colour produced is not more intense than that obtained by treating at the same time and in the same manner 4 ml of a 0.005 per cent w/v solution of *oxalic acid* (300 ppm, calculated as anhydrous oxalic acid).

Sulphates (2.3.17). To 10 ml of solution A add 2 ml of *7 M hydrochloric acid* and dilute to 15 ml with *distilled water*; the resulting solution complies with the limit test for sulphates (150 ppm).

Tartrates. To a solution of 1 g in 2 ml of *water* in a test-tube, add 1 ml of a 10 per cent w/v solution of *potassium acetate* and 1 ml of *6 M acetic acid*. Scratch the walls of the test-tube with a glass rod; no crystalline precipitate is formed.

Readily carbonisable substances. Heat 0.2 g, in powder, with 10 ml of *sulphuric acid (96 per cent w/w)* in a water-bath at 90° ± 1° for 1 hour and cool rapidly. The solution is not more intensely coloured than reference solution YS2 or GYS2 (2.4.1).

Water (2.3.43). 11.0 to 13.0 per cent, determined on 0.3 g and after stirring for 15 minutes before titrating.

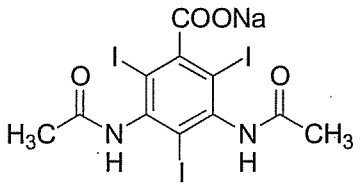
Assay. Weigh accurately about 0.15 g and dissolve in 20 ml of *anhydrous glacial acetic acid*, warming to about 50°. Allow to cool. Titrate with *0.1 M perchloric acid*, using 0.25 ml of *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.008602 g of $C_6H_5Na_3O_7$.

Storage. Store protected from light.

Sodium Diatrizoate

Diatrizoate Sodium



$C_{11}H_8I_3N_2NaO_4$ Mol. Wt. 635.9

Sodium Diatrizoate is sodium 3,5-diacetamido-2,4,6-triiodobenzoate.

Sodium Diatrizoate contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{11}H_8I_3N_2NaO_4$, calculated on the anhydrous basis.

Category. Urographic radio-opaque substance.

Dose. To be decided by the physician in accordance with the needs of the patient.

Description. A white powder; odourless or almost odourless.

Identification

Tests A and D may be omitted if tests B, C, E and F are carried out. Tests B, C and F may be omitted if tests A, D and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sodium diatrizoate RS* or with the reference spectrum of sodium diatrizoate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 20 volumes of *chloroform*, 10 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of 0.08 per cent w/v solution of *sodium hydroxide* in *methanol*.

Reference solution. A 0.1 per cent w/v of *sodium diatrizoate RS* in 0.08 per cent w/v solution of *sodium hydroxide* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Heat 0.5 g in a crucible; violet vapours of iodine are evolved.

D. To 20 mg add 5 ml of *1 M sodium hydroxide* and boil gently under a reflux condenser for 10 minutes. Cool, add 5 ml of *2 M hydrochloric acid* and cool in ice for 5 minutes. Add 4 ml of a 1 per cent w/v solution of *sodium nitrite*, cool in ice for 5 minutes, add 0.3 g of *sulphamic acid*, shake gently until effervescence ceases and add 2 ml of a 0.4 per cent w/v solution of *N-(1-naphthyl) ethylenediamine dihydrochloride*; an orange-red colour is produced.

E. Heat 0.5 g with 1 ml of *sulphuric acid* on a water-bath until a pale violet solution is produced, add 2 ml of *ethanol (95 per cent)* and heat again; odour of ethyl acetate is produced.

F. Gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 7.5 to 9.5, determined in a 50 per cent w/v solution.

Free amine. Place 1.0 g in a 50-ml glass-stoppered volumetric flask, add 5 ml of *water*, 10 ml of *0.1 M sodium hydroxide* and 25 ml of *dimethyl sulphoxide*. Stopper the flask, mix the contents gently and cool in ice, protected from light. After 5 minutes, slowly add 2 ml of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 2 per cent w/v solution of *sodium nitrite*, mix and allow to stand for 5 minutes. Add 1 ml of an 8 per cent w/v solution of *sulphamic acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in a 70 per cent w/v solution of *1,2-propanediol* and mix. Remove the flask from the ice and allow to stand in water at 25° ± 2° for 10 minutes, with occasional shaking. Add sufficient *dimethyl sulphoxide* to produce 50 ml and mix. Within 5 minutes, measure the absorbance of the resulting solution at the maximum at about 470 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* in the same manner; absorbance, not more than 0.40, calculated on the anhydrous basis.

Free iodine and iodide. Dissolve 2.0 g in 24 ml of *water* taken in a 50-ml glass-stoppered centrifuge tube. Add 5 ml of *toluene* and 5 ml of *1 M sulphuric acid*, shake well and centrifuge; no red colour appears in the toluene layer. To the mixture add 1 ml of a 2 per cent w/v solution of *sodium nitrite*, shake and centrifuge; any red colour in the toluene layer is not more intense than that produced in a solution prepared in the same manner using 2.0 ml of a 0.025 per cent w/v solution of *potassium iodide* and 22 ml of *water* (220 ppm).

Heavy metals. Dissolve 1.0 g in 20.0 ml of *water* and 5 ml of *1 M sodium hydroxide*, transfer the solution to a 50-ml Nessler cylinder, dilute with *water* to 40 ml and mix. Add 10 ml of *sodium sulphide solution*, shake and allow to stand for 5 minutes (20 ppm), the colour of the solution when viewed downward over a white surface is not more intense than that produced by treating 2.0 ml of *lead standard solution (10 ppm Pb)* in the same manner in place of the substance under examination.

Water (2.3.43). 4.0 to 7.0 per cent, determined on 0.4 g.

Assay. Weigh accurately about 0.4 g in a glass-stoppered conical flask, add 12 ml of *5 M sodium hydroxide* and 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through cotton and wash the flask and filter with two quantities, each of 20 ml, of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the dark brown colour becomes pale brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless.

1 ml of *0.05 M potassium iodate* is equivalent to 0.02120 g of $C_{11}H_8I_3N_2NaO_4$.

Storage. Store protected from light.

Sodium Diatrizoate Injection

Diatrizoate Sodium Injection

Sodium Diatrizoate Injection is a sterile solution of Sodium Diatrizoate in Water for Injections. It may contain small amounts of suitable buffers, stabilisers and antimicrobial agents but the preparation intended for intravenous administration contains no antimicrobial preservative.

Sodium Diatrizoate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sodium diatrizoate, $C_{11}H_8I_3N_2NaO_4$.

Usual strengths. 25 per cent w/v; 40 per cent w/v.

Identification

Evaporate a volume of the injection containing 1 g of Sodium Diatrizoate to dryness. The residue complies with following tests.

A. Heat 0.5 g of residue in a crucible; violet vapours of iodine are evolved.

B. To 20 mg of the residue add 5 ml of *1 M sodium hydroxide* and boil gently under a reflux condenser for 10 minutes. Cool,

add 5 ml of *2 M hydrochloric acid* and cool in ice for 5 minutes. Add 4 ml of a 1 per cent w/v solution of *sodium nitrite*, cool in ice for 5 minutes, add 0.3 g of *sulphamic acid*, shake gently until effervescence ceases and add 2 ml of a 0.4 per cent w/v solution of *N-(1-naphthyl) ethylenediamine dihydrochloride*; an orange-red colour is produced.

C. Heat 0.5 g of residue with 1 ml of *sulphuric acid* on a water-bath until a pale violet solution is produced, add 2 ml of *ethanol (95 per cent)* and heat again; odour of ethyl acetate is produced.

Tests

pH (2.4.24). 6.6 to 7.6.

Free amine. To a volume containing 1.0 g of Sodium Diatrizoate in a 50-ml glass-stoppered volumetric flask, add 5 ml of *water*, 10 ml of *0.1 M sodium hydroxide* and 25 ml of *dimethyl sulphoxide*. Stopper the flask, mix the contents gently and cool in ice, protected from light. After 5 minutes, slowly add 2 ml of *hydrochloric acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 2 per cent w/v solution of *sodium nitrite*, mix and allow to stand for 5 minutes. Add 1 ml of an 8 per cent w/v solution of *sulphamic acid*, mix and allow to stand for 5 minutes. Add 2 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in a 70 per cent w/v solution of *1,2-propanediol* and mix. Remove the flask from the ice and allow to stand in water at 25° ± 2° for 10 minutes, with occasional shaking. Add sufficient *dimethyl sulphoxide* to produce 50 ml and mix. Within 5 minutes, measure the absorbance of the resulting solution at the maximum at about 470 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *water* in the same manner; absorbance, not more than 0.30, calculated on the anhydrous basis.

Free iodine and iodide. To a volume containing 2.0 g of Sodium Diatrizoate in 24 ml of *water* taken in a 50-ml glass-stoppered centrifuge tube add 5 ml of *toluene* and 5 ml of *1 M sulphuric acid*, shake well and centrifuge; no red colour appears in the toluene layer. To the mixture add 1 ml of a 2 per cent w/v solution of *sodium nitrite*, shake and centrifuge; any red colour in the toluene layer is not more intense than that produced in a solution prepared in the same manner using 2.0 ml of a 0.025 per cent w/v solution of *potassium iodide* and 22 ml of *water* (220 ppm).

Pyrogens (2.2.8). Complies with test for pyrogens, using per kg of the rabbit's weight a volume containing 2.5 g of Sodium Diatrizoate.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Weigh accurately about 0.5 g in a glass-stoppered conical flask, add 12 ml of *5 M sodium hydroxide* and 1 g of *zinc powder* and boil under a reflux condenser for 30 minutes. Cool, rinse the condenser with 30 ml of *water*, filter through cotton and wash the flask and filter with two quantities, each of 20 ml, of *water*. To the combined filtrate and washings add 80 ml of *hydrochloric acid*, cool and titrate with *0.05 M potassium iodate* until the dark brown colour becomes pale brown. Add 5 ml of *chloroform* and continue the titration, shaking well after each addition, until the chloroform becomes colourless.

1 ml of *0.05 M potassium iodate* is equivalent to 0.02120 g of $C_{11}H_8I_3N_2NaO_4$.

Storage. Store protected from light.

Labelling. The label states (1) the concentration of the active ingredient; (2) whether the contents are intended for intravenous injection and, if so, that the unused portion remaining in the container after use must be discarded.

Sodium Dihydrogen Phosphate Dihydrate

Sodium Acid Phosphate

$NaH_2PO_4,2H_2O$ Mol. Wt. 156.0

Sodium Dihydrogen Phosphate Dihydrate contains not less than 98.0 per cent and not more than 100.5 per cent of NaH_2PO_4, calculated on the dried basis.

Category. Urinary acidifier.

Dose. 2 to 4 g.

Description. Colourless crystals or a white powder; odourless.

Identification

A. Dissolve 10.0 g in sufficient *carbon dioxide-free water* to produce 100 ml (solution A). Solution A is faintly acid.

B. Solution A neutralised with a 10 per cent w/v solution of *potassium hydroxide* gives reaction A of sodium salts (2.3.1).

C. Solution A gives the reactions of phosphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.2 to 4.5, determined in a mixture of 5 ml of solution A and 5 ml of *carbon dioxide-free water*.

Arsenic (2.3.10). Dissolve 0.5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 12 ml of solution A complies with the limit test for heavy metals, Method D (10 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Chlorides (2.3.12). 10 ml of solution A diluted to 20 ml with *water* complies with the limit test for chlorides (250 ppm)

Sulphates (2.3.17). To 5 ml of solution A add 0.5 ml of *hydrochloric acid* and dilute to 15 ml with distilled water; the solution complies with the limit test for sulphates (300 ppm).

Reducing substances. To 5 ml of solution A add 0.25 ml of *0.02 M potassium permanganate* and 5 ml of *1 M sulphuric acid* and heat in a water-bath for 5 minutes; the pink colour is not completely discharged.

Disodium phosphate. Dilute 10 ml of solution A to 50 ml with *water* and titrate with *0.05 M sulphuric acid* using *bromocresol green solution* as indicator; not more than 1 ml of *0.05 M sulphuric acid* is required.

Loss on drying (2.4.19). 21.5 to 24.0 per cent, determined on 0.25 g by drying in an oven at 130°.

Assay. Weigh accurately about 2.5 g, dissolve in 40 ml of *water* and titrate with carbonate-free *1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *1 M sodium hydroxide* is equivalent to 0.120 g of NaH_2PO_4.

Storage. Store protected from moisture.

Sodium Fluoride

NaF Mol. Wt. 42.0

Sodium Fluoride contains not less than 98.5 per cent and not more than 100.5 per cent of NaF, calculated on the dried basis.

Category. Preventive for dental caries.

Description. A white powder or colourless crystals.

Identification

A. Dissolve 2.5 g in sufficient *carbon dioxide-free water* without heating to produce 100 ml (solution A). To 2 ml of solution A add 0.5 ml of *calcium chloride solution*; a gelatinous white precipitate is produced which dissolves on adding 5 ml of *ferric chloride solution*.

B. Add about 4 mg to a mixture of 0.1 ml of *alizarin red S solution* and 0.1 ml of *zirconyl nitrate solution* and mix; the colour changes to yellow.

C. Gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 2.5 g of *potassium nitrate* in 40 ml of solution A, dilute to 50 ml with *carbon dioxide-free water*, cool to 0° and add 0.2 ml of *dilute phenolphthalein solution*. If the solution is colourless, not more than 1.0 ml of *0.1 M sodium hydroxide* is required to produce a red colour that persists for not less than 15 seconds. If the solution is red, not more than 0.25 ml of *0.1 M hydrochloric acid* is required to change the colour of the solution. Reserve the neutralised solution for the test for Fluorosilicate.

Chlorides (2.3.12). 40 ml of solution A complies with the limit test for chlorides (250 ppm).

Fluorosilicate. Heat to boiling the solution reserved in the test for Acidity or alkalinity and titrate while hot with *0.1 M sodium hydroxide* until a red colour is produced. Not more than 1.5 ml of *0.1 M sodium hydroxide* is required.

Sulphates (2.3.17). Dissolve 0.25 g in 10 ml of a saturated solution of *boric acid* in *distilled water* and add 5 ml of *distilled water* and 0.6 ml of *7 M hydrochloric acid*. The solution complies with the limit test for sulphates (200 ppm). Prepare the standard by mixing together 0.6 ml of *7 M hydrochloric acid*, 5 ml of *sulphate standard solution (10 ppm SO_4)* and 10 ml of a saturated solution of *boric acid* in *distilled water*.

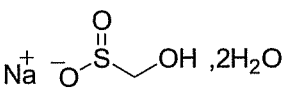
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 130° for 3 hours.

Assay. Weigh accurately about 80 mg, add a mixture of 5 ml of *acetic anhydride* and 20 ml of *anhydrous glacial acetic acid* and heat to dissolve. Cool, add 20 ml of *dioxan*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator, until a green colour is produced. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.004199 g of NaF.

Storage. Store protected from moisture.

Sodium Formaldehyde Sulphoxylate



$CH_3NaO_3S,2H_2O$ Mol Wt. 154.1

Sodium Formaldehyde Sulphoxylate is monosodium hydroxymethane sulphinate dihydrate. It may contain a suitable stabilising agent such as sodium carbonate.

Sodium Formaldehyde Sulphoxylate contains an amount of CH_3NaO_3S equivalent to not less than 45.0 per cent and

not more than 55.0 per cent of SO_2, calculated on the dried basis.

Category. Pharmaceutical aid (antioxidant).

Description. White crystals or hard white masses; odour, characteristic and garlic-like.

Identification

A. Dissolve about 4 g in 10 ml of *water* in a test-tube and add 1 ml of *ammoniacal silver nitrate solution*; metallic silver is produced either as a finely divided grey precipitate or as a bright metallic mirror on the inner surface of the tube.

B. Add about 50 mg to a solution of 40 mg of *salicylic acid* in 5 ml of *sulphuric acid* and warm very gently; a permanent deep red colour develops.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

Alkalinity. Dissolve 1.0 g in 50 ml of *water* and add 0.15 ml of *dilute phenolphthalein solution*; not more than 3.5 ml of *0.05 M sulphuric acid* is required to change the colour of the solution.

pH (2.4.24). 9.5 to 10.5, determined in a 2.0 per cent w/v solution in *carbon dioxide-free water*.

Iron (2.3.14). Ignite 1.0 g, initially at a low temperature until thoroughly charred and finally at about 600°, preferably in a muffle furnace, until all the carbon has been burnt off. Cool, dissolve the residue in 2 ml of *hydrochloric acid* and dilute to 50 ml with *water*. Add about 50 mg of *ammonium persulphate* and 5 ml of *ammonium thiocyanate solution*, mix and transfer to a Nessler cylinder. The red colour of the solution is not more intense than that of 1.0 ml of *iron standard solution (10 ppm)* treated in the same manner.

Sulphides. Dissolve 6 g in 14 ml of *water* in a test-tube and wet a strip of *lead acetate paper* in the clear solution; no discolouration is evident within 5 minutes.

Sodium sulphite. Not more than 5.0 per cent, calculated as Na_2SO_3, determined by the following method. Transfer 4.0 ml of the solution obtained in the Assay to a flask, add 2 ml of *formaldehyde solution* and titrate with *0.1 M iodine* that is used for the Assay, adding *starch solution* towards the end of the titration as indicator.

Calculate the percentage of Na_2SO_3 from the expression $78.775(V_2-V_1)/W$,

where V_1 and V_2 are the volumes, in ml, of *0.1 M iodine* consumed in this test and in the Assay respectively and W is the weight, in g, of the substance under examination taken for the Assay.

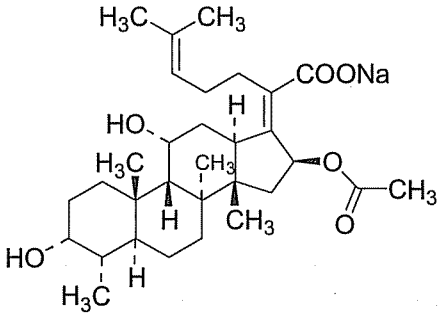
Loss on drying (2.4.19). Not more than 27.0 per cent, determined on 0.5 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 1.0 g, dissolve in 25 ml of *water*, add sufficient *water* to produce 50.0 ml and mix. To 4.0 ml of this solution add 100 ml of *water* and titrate with *0.1 M iodine* using 3 ml of *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.1 M iodine* is equivalent to 0.001602 g of SO_2.

Storage. Store protected from light and moisture.

Sodium Fusidate



$C_{31}H_{47}NaO_6$ Mol. Wt. 538.7

Sodium Fusidate is sodium (17*Z*)-16β-acetoxy-3α,11α-dihydroxyfusida-17(20),24-diene-21-oate, produced by the growth of certain strains of *Fusidium coccineum* or by any other means.

Sodium Fusidate contains not less than 97.5 per cent and not more than 101.0 per cent of $C_{31}H_{47}NaO_6$, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. 1.5 g daily, in divided doses.

Description. A white or almost white, crystalline powder; slightly hygroscopic.

Identification

A. Dissolve 0.1 g in 5 ml of *water*, add 5 ml of *chloroform* and 0.1 ml of a 10 per cent w/w solution of *phosphoric acid*, shake vigorously for 1 minute, allow to separate and filter the lower layer through absorbent cotton covered with *anhydrous sodium sulphate*. Repeat the extraction with two quantities, each of 5 ml, of *chloroform*, evaporate the combined extracts at a pressure of 2 kPa, dry the residue over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 2 hours and dissolve in 1 ml of *chloroform IR*.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that

obtained with *fusidic acid RS* or with the reference spectrum of fusidic acid.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Ignite 1 g. The residue gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 15.0 per cent w/v solution in *carbon dioxide-free water* is not more intensely coloured than reference solution BS6 (2.4.1).

pH (2.4.24). 7.5 to 9.0, determined in a 1.25 per cent w/v solution.

Specific optical rotation (2.4.22). +5.0° to +8.0°, determined at 20° by dissolving 1.5 g in 25 ml of *water*, adding 0.1 ml of *5 M ammonia* and diluting to 50 ml with *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg of *3-ketofusidic acid RS* in 5 ml of the mobile phase. To 1.0 ml of this solution add 0.2 ml of the test solution and dilute to 20.0 ml with the mobile phase.

Reference solution (b). Dilute 20 µl of the test solution to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 10 volumes of *methanol*, 20 volumes of 1.0 per cent w/v solution of *orthophosphoric acid*, 20 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to 3-ketofusidic acid and sodium fusidate is not less than 2.5.

Inject the test solution, reference solution (a) and (b). The signal-to-noise ratio of the principal peak is not less than 3. Run the chromatogram 3.5 times the retention time of principal peak. The sum of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (4.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.2 g and dissolve in a mixture of 15 ml of *water* and 20 ml of *ethanol (95 per cent)*. Titrate with *0.1 M hydrochloric acid* to pH 4.1, stirring continuously, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.05387 g of $C_{31}H_{47}NaO_6$.

Storage. Store protected from light and moisture.

Sodium Hydroxide

Caustic Soda

NaOH Mol. Wt. 40.0

Sodium Hydroxide contains not less than 97.0 per cent and not more than 100.5 per cent of total alkali, calculated as NaOH.

Category. Pharmaceutical aid (alkalising agent).

Description. White, crystalline masses supplied as sticks, pellets or slabs; deliquescent. Readily absorbs carbon dioxide.

CAUTION — Great care should be exercised in handling Sodium Hydroxide as it rapidly destroys tissues.

Identification

A. Carefully dissolve 5.0 g in 12 ml of *distilled water*, add 17 ml of *7 M hydrochloric acid*, adjust the pH to 7.0 with *1 M hydrochloric acid* and add sufficient *distilled water* to produce 50 ml (solution A). 2 ml of solution A gives reaction A of sodium salts (2.3.1).

B. pH of a 0.01 per cent w/v solution, not less than 11.0 (2.4.24).

Tests

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 2.5 g in 50 ml of *water*, add 16 ml of *brominated hydrochloric acid AsT*, and remove the excess of bromine with a few drops of *stannous* chloride *solution AsT*. The resulting solution complies with the limit test for arsenic (4 ppm).

Heavy metals (2.3.13). Dissolve 1.0 g in 5 ml of *water* and 10 ml of *3 M hydrochloric acid*, heat to boiling, cool and dilute to 25 ml with *water*. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Carbonates. Not more than 2.0 per cent, calculated as Na_2CO_3, determined in the Assay.

Chlorides (2.3.12). Dissolve 2.0 g in 5 ml of *water*, acidify with about 8 ml of *nitric acid* and dilute to 20 ml with *water*. The solution, without the addition of *dilute nitric acid*, complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). Dissolve 2.0 g in 6 ml of *distilled water*, adjust the pH to 7 with *hydrochloric acid* and dilute to 20 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (75 ppm).

Potassium. Acidify 2.5 ml of solution A with *acetic acid* and add 0.15 ml of *sodium cobaltinitrite solution*; no precipitate is formed.

Assay. Weigh accurately about 2.0 g, dissolve in about 80 ml of *carbon dioxide-free water*, add 0.3 ml of *phenolphthalein solution* and titrate with *1 M hydrochloric acid*. Add 0.3 ml of *methyl orange solution* and continue the titration with *1 M hydrochloric acid*.

1 ml of *1 M hydrochloric acid* used in the second part of the titration is equivalent to 0.1060 g of Na_2CO_3.

1 ml of *1 M hydrochloric acid* used in the combined titrations is equivalent to 0.0400 g of total alkali, calculated as NaOH.

Storage. Store protected from moisture, in non-metallic containers.

Sodium Lactate Injection

Sodium Lactate Intravenous Infusion

Sodium Lactate Injection is a sterile solution containing 1.85 per cent w/v of sodium lactate in Water for Injections. It is prepared from Lactic Acid with the aid of Sodium Hydroxide and sufficient Dilute Hydrochloric Acid to adjust the pH of the solution.

Sodium Lactate Injection contains not less than 1.75 per cent and not more than 1.95 per cent w/v of sodium lactate, $C_3H_5NaO_3$.

Category. Fluid and electrolyte replenisher.

Dose. By intravenous infusion at the rate of 5 ml or less per minute.

Description. A clear, colourless solution.

Identification

A. When warmed with *potassium permanganate*, gives acetaldehyde, recognisable by its odour.

B. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame, imparts a yellow colour to the flame.

C. Carry out reaction C of calcium salts (2.3.1); no white precipitate is produced.

Tests

pH (2.4.24). 5.0 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per millimole.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. Measure accurately 10 ml, evaporate to dryness in a platinum dish and ignite very gently until completely carbonised. Boil the residue with 25.0 ml of *0.05 M sulphuric acid*, filter and wash thoroughly with hot *water*. Titrate the excess of acid in the combined filtrate and washings with *0.1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.01121 g of $C_3H_5NaO_3$.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from the solution in glass containers.

Labelling. The label states (1) that the Injection is one-sixth molar and contains, in one litre, approximately 167 millimoles each of sodium ions and of bicarbonate ions (as lactate); (2) that the injection should not be used if the solution contains visible solid particles.

Compound Sodium Lactate and Dextrose Injection

Compound Sodium Lactate with Dextrose Intravenous Infusion; Ringer-Lactate Solution with Dextrose for Injection; Hartmann's Solution with Dextrose for Injection.

Compound Sodium Lactate and Dextrose Injection is a sterile solution containing 0.24 per cent v/v of Lactic Acid (equivalent to 0.32 per cent w/v of sodium lactate) with 0.6 per cent w/v of Sodium Chloride, 0.04 per cent w/v of Potassium Chloride, 0.027 per cent w/v of Calcium Chloride and 5 per cent w/v of Dextrose in Water for Injections.

Compound Sodium Lactate and Dextrose Injection contains not less than 0.27 per cent and not more than 0.32 per cent w/v of sodium, Na, not less than 0.019 per cent and not more than 0.022 per cent w/v of potassium, K, not less than 0.37 per cent and not more than 0.42 per cent w/v of total chloride, Cl, not less than 0.025 per cent and not more than 0.029 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than

0.23 per cent and not more than 0.28 per cent w/v of lactate, calculated as $C_3H_6O_3$, and not less than 4.50 per cent and not more than 5.25 per cent w/v of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Category. Fluid and electrolyte replenisher.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. When warmed with *potassium permanganate* gives acetaldehyde, recognisable by its odour.

C. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged reddish purple.

D. Gives reaction C of calcium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Heavy metals (2.3.13). Evaporate a volume containing 4 g of dextrose to 10 ml and add 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml. The solution complies with the limit test for heavy metals, Method A (5 ppm).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with water for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total chlorides — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For calcium chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *eriochrome black T mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For lactate — Determine by liquid chromatography (2.4.14).

Test solution. The preparation under examination.

Reference solution. A 0.28 per cent w/v solution of *lithium lactate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *water* and 10 volumes of a 2 per cent v/v solution of *octylamine* in *acetonitrile*, the pH of which is adjusted to 7.0 with a 10 per cent v/v solution of *phosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_3H_6O_3$, in the injection.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers of glass or plastic at a temperature not exceeding 30°. On keeping, small particles may separate from the solution in glass containers.

Labelling. The label states (1) that the injection contains, in millimoles per litre, the following approximate amounts of the

ions. sodium, 131, potassium, 5, calcium, 2; bicarbonate (as lactate), 29 and Chloride, 111; (2) the total osmolar concentration in mOsmol per litre; (3) that the injection should not be used if it contains visible particles.

Half Strength Compound Sodium Lactate and Dextrose Injection

Half Strength Compound Sodium Lactate with Dextrose Intravenous Infusion; Half Strength Ringer-Lactate Solution with Dextrose Injection

Half Strength Compound Sodium Lactate and Dextrose Injection is a sterile solution containing 0.12 per cent v/v of Lactic Acid (equivalent to 0.16 per cent w/v of sodium lactate) with 0.3 per cent w/v of Sodium Chloride, 0.02 per cent w/v of Potassium Chloride, 0.0135 per cent w/v of Calcium Chloride and 5 per cent w/v of Dextrose in Water for Injections.

Compound Sodium Lactate and Dextrose Injection contains not less than 0.135 per cent and not more than 0.16 per cent w/v of sodium, Na, not less than 0.0095 per cent and not more than 0.011 per cent w/v of potassium, K, not less than 0.185 per cent and not more than 0.210 per cent w/v of total chloride, Cl, not less than 0.0125 per cent and not more than 0.0145 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than 0.115 per cent and not more than 0.140 per cent w/v of lactate, calculated as $C_3H_6O_3$, and not less than 4.5 per cent and not more than 5.25 per cent w/v of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Category. Fluid and electrolyte replenisher.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. When warmed with *potassium permanganate* gives acetaldehyde, recognisable by its odour.

C. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged reddish purple.

D. Gives reaction C of calcium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm, not more than 0.25 (2.4.7).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total chlorides — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For calcium chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *eriochrome black T mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For lactate — Determine by liquid chromatography (2.4.14).

Test solution. The preparation under examination.

Reference solution. A 0.28 per cent w/v solution of *lithium lactate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *water* and 10 volumes of a 2 per cent v/v solution of *octylamine* in

acetonitrile, the pH of which is adjusted to 7.0 with a 10 per cent v/v solution of *phosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_3H_6O_3$ in the injection.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and measure the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers of glass or plastic at a temperature not exceeding 30°. On keeping, small particles may separate from the solution in glass containers.

Labelling. The label states (1) that the injection contains, in millimoles per litre, the following approximate amounts of the ions. sodium, 65.5, potassium, 2.5, calcium, 1, bicarbonate (as lactate), 14.5, and chloride, 55.5; (2) the total osmolar concentration in mOsmol per litre; (3) that the injection should not be used if it contains visible particles.

Modified Compound Sodium Lactate and Dextrose Injection

Modified Compound Sodium Lactate with Dextrose Intravenous Infusion; Modified Lactated Ringer's and Dextrose Injection.

Modified Compound Sodium Lactate and Dextrose Injection is a sterile solution containing 0.048 per cent v/v of Lactic Acid (equivalent to 0.064 per cent w/v of sodium lactate) with 0.12 per cent w/v of Sodium Chloride, 0.008 per cent w/v of Potassium Chloride, 0.0054 per cent w/v of Calcium Chloride and 5 per cent w/v of Dextrose in Water for Injections.

Modified Compound Sodium Lactate and Dextrose Injection contains not less than 0.054 per cent and not more than 0.064 per cent w/v of sodium, Na, not less than 0.0038 per cent and not more than 0.0044 per cent w/v of potassium, K, not less than 0.074 per cent and not more than 0.084 per cent w/v of total chloride, Cl, not less than 0.005 per cent and not more than 0.0058 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than 0.046 per cent and not more than 0.056 per cent w/v of lactate, calculated as $C_3H_6O_3$, and not less than 4.5 per cent and not more than 5.25 per cent w/v of dextrose, $C_6H_{12}O_6$. It contains no antimicrobial agent.

Category. Fluid and electrolyte replenisher.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. To 1 ml add 0.05 ml of *potassium cupri-tartrate solution*; the solution remains blue and clear. Heat to boiling, a copious red precipitate is formed.

B. When warmed with *potassium permanganate* gives acetaldehyde, recognisable by its odour.

C. The residue on evaporation, when moistened with hydrochloric acid and introduced on a platinum wire into a flame imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged reddish purple.

D. Gives reaction C of calcium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 6.5.

5-Hydroxymethylfurfural and Related substances. Dilute a volume containing 1.0 g of Dextrose to 500.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 284 nm; absorbance at about 284 nm (2.4.7), not more than 0.25.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For total chlorides — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as

indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For calcium chloride — Evaporate 100.0 ml on a water-bath to 50 ml, add to this solution 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *eriochrome black T mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For lactate — Determine by liquid chromatography (2.4.14).

Test solution. The preparation under examination.

Reference solution. A 0.28 per cent w/v solution of *lithium lactate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *water* and 10 volumes of a 2 per cent v/v solution of *octylamine* in *acetonitrile*, the pH of which is adjusted to 7.0 with a 10 per cent v/v solution of *phosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_3H_6O_3$ in the injection.

For dextrose — To an accurately measured volume containing 2 g to 5 g of Dextrose, add 0.2 ml of *5 M ammonia* and sufficient *water* to produce 100.0 ml. Mix well, allow to stand for 30 minutes and determine the optical rotation in a 2-dm tube (2.4.22). The observed rotation in degrees multiplied by 0.9477 represents the weight, in g, of dextrose, $C_6H_{12}O_6$, in the volume taken for assay.

Storage. Store in single dose containers of glass or plastic at a temperature not exceeding 30°. On keeping, small particles may separate from the solution in glass containers.

Labelling. The label states (1) that the injection contains, in millimoles per litre, the following approximate amounts of the ions. sodium, 26.2, potassium, 1, calcium, 0.4, bicarbonate (as lactate), 5.8, and chloride, 22.2; (2) the total osmolar concentration in mOsmol per litre; (3) that the injection should not be used if it contains visible particles.

Compound Sodium Lactate Injection

Compound Sodium Lactate Intravenous Infusion; Ringer-Lactate Solution for Injection; Hartmann's Solution for Injection

Compound Sodium Lactate Injection is a sterile solution containing 0.24 per cent v/v of Lactic Acid (equivalent to 0.32 per cent w/v of sodium lactate) with 0.6 per cent w/v of Sodium Chloride, 0.04 per cent w/v of Potassium Chloride and 0.027 per cent w/v of Calcium Chloride in Water for Injections.

Compound Sodium Lactate Injection contains not less than 0.27 per cent and not more than 0.32 per cent w/v of sodium, Na, not less than 0.019 per cent and not more than 0.022 per cent w/v of potassium, K, not less than 0.37 per cent and not more than 0.42 per cent w/v of total chloride, Cl, not less than 0.025 per cent and not more than 0.029 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than 0.23 per cent and not more than 0.28 per cent w/v of lactate, calculated as $C_3H_6O_3$.

Category. Fluid and electrolyte replenisher.

Description. A clear, colourless solution.

Identification

A. When warmed with potassium permanganate gives acetaldehyde, recognisable by its odour.

B. The residue on evaporation, when moistened with hydrochloric acid and introduced on a platinum wire into a flame, imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged with reddish purple.

C. Gives reaction C of calcium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Intravenous Infusions).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS*, suitably diluted for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS,* suitably diluted for the standard solutions.

For total chlorides — To 20 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of nitric acid, filter, wash the precipitate with water slightly acidified with nitric acid and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using ferric ammonium sulphate solution as indicator. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For calcium chloride — To 50 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *mordant black 11 mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For lactate, calculated as $C_3H_6O_3$ — Evaporate 50 ml to dryness in a platinum dish and ignite very gently until completely carbonised. Boil the residue with 25.0 ml of *0.05 M sulphuric acid*, filter, and wash thoroughly with hot *water*. Titrate the excess of acid in the combined filtrate and washings with *0.1 M sodium hydroxide* using *methyl orange solution* as indicator.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.009008 g of $C_3H_6O_3$.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from the solution in glass containers.

Labelling. The label states (1) that the injection contains, in millimoles per litre, the following approximate amounts of the ions. sodium, 131, potassium, 5, calcium, 2, bicarbonate (as lactate), 29 and chloride, 111; (2) that the injection should not be used if the solution contains visible solid particles.

Compound Sodium Lactate Solution for Irrigation

Ringer-Lactate Solution for Irrigation; Hartmann's Solution for Irrigation

Compound Sodium Lactate Solution for Irrigation is a sterile solution containing 0.24 per cent v/v of Lactic Acid (equivalent to 0.32 per cent w/v of sodium lactate) with 0.6 per cent w/v of Sodium Chloride, 0.04 per cent w/v of Potassium Chloride and 0.027 per cent w/v of Calcium Chloride in Water for Injections.

Compound Sodium Lactate Solution for Irrigation contains not less than 0.27 per cent and not more than 0.32 per cent w/v of sodium, Na, not less than 0.019 per cent and not more than 0.022 per cent w/v of potassium, K, not less than 0.37 per cent and not more than 0.42 per cent w/v of total chloride, Cl, not less than 0.025 per cent and not more than 0.029 per cent w/v of calcium chloride, $CaCl_2,2H_2O$, and not less than 0.23 per cent and not more than 0.28 per cent w/v of lactate, calculated as $C_3H_6O_3$. It contains no antimicrobial agent.

Category. Irrigation fluid.

Description. A clear, colourless solution.

Identification

A. When warmed with *potassium permanganate* gives acetaldehyde, recognisable by its odour.

B. The residue on evaporation, when moistened with *hydrochloric acid* and introduced on a platinum wire into a flame imparts a yellow colour to the flame. When viewed through a suitable blue glass, the flame is tinged reddish purple.

C. Gives reaction C of calcium salts and reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 7.0.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sodium* — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution FP* or *sodium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For potassium — Dilute suitably with *water* and determine by Method A for flame photometry (2.4.4), or by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 767 nm and using *potassium solution FP* or *potassium solution AAS* respectively, suitably diluted with *water* for the standard solutions.

For calcium chloride — To 50.0 ml add 5.0 ml of *0.01 M magnesium sulphate* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *eriochrome black T mixture* as indicator. From the volume of *0.01 M disodium edetate* required subtract the volume of *0.01 M magnesium sulphate* added.

1 ml of the remainder of *0.01 M disodium edetate* is equivalent to 0.00147 g of $CaCl_2, 2H_2O$.

For total chlorides — To 20.0 ml add 30 ml of *water*, 50.0 ml of *0.1 M silver nitrate* and 2 ml of *nitric acid*. Filter, wash the

precipitate with *water* slightly acidified with *nitric acid* and titrate the excess of silver nitrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator until a reddish yellow colour is produced. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of total chloride, calculated as Cl.

For lactate — Determine by liquid chromatography (2.4.14).

Test solution. The preparation under examination.

Reference solution. A 0.28 per cent w/v solution of *lithium lactate RS* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *water* and 10 volumes of a 2 per cent v/v solution of *octylamine* in *acetonitrile*, the pH of which is adjusted to 7.0 with a 10 per cent v/v solution of *phosphoric acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_3H_6O_3$, in the injection.

Storage. Store in single dose containers of glass or plastic. On keeping, small solid particles may separate from the solution in glass containers. The container may be designed to empty rapidly and may contain a volume of more than one litre.

Labelling. The label states (1) that the irrigation solution contains, in millimoles per litre, the following approximate amounts of the ions. sodium,131, potassium, 5, calcium, 2, bicarbonate (as lactate), 29, and chloride, 111; (2) that the solution should not be used if it contains visible particles; (3) 'For irrigation only' and 'Not for injection'; (4) that once the container is opened, the unused portion should be discarded.

Sodium Lauryl Sulphate

Sodium Lauryl Sulphate is a mixture of sodium alkyl sulphates consisting mainly of sodium dodecyl sulphate, $CH_3[CH_2]_{10}CH_2OSO_3Na$.

Sodium Lauryl Sulphate contains not less than 85.0 per cent of sodium alkyl sulphates, calculated as $C_{12}H_{25}NaO_4S$.

Category. Pharmaceutical aid (anionic emulsifying agent).

Description. A white or pale yellow powder or crystals.

Identification

A. A 1 per cent w/v solution, when shaken, produces plenty of foam.

B. Mix 0.1 ml of a 1 per cent w/v solution with 0.1 ml of a 0.1 per cent w/v solution of *methylene blue* and 2 ml of *1 M sulphuric acid*, add 2 ml of *dichloromethane* and shake; the dichloromethane layer is intensely blue.

C. Mix about 10 mg with 10 ml of *ethanol* and heat to boiling on a water-bath, shaking frequently. Filter immediately and evaporate the *ethanol*. Dissolve the residue in 8 ml of *water*, add 3 ml of *2 M hydrochloric acid*, evaporate the solution to half its volume and cool. Filter and to the filtrate add 1 ml of *barium chloride solution*; a white, crystalline precipitate is produced.

D. Gives reaction B of sodium salts (2.3.1).

Tests

Alkalinity. Dissolve 1.0 g in 100 ml of *carbon dioxide-free water* and add 0.1 ml of *phenol red solution*. Not more than 0.5 ml of *0.1 M hydrochloric acid* is required to change the colour of the solution.

Non-esterified alcohols. Not more than 4 per cent, determined by the following method. Dissolve 10.0 g in 100 ml of *water*, add 100 ml of *ethanol (95 per cent)* and extract the solution with three quantities, each of 50 ml, of *n-pentane*, adding *sodium chloride*, if necessary, to promote separation of the two layers. Wash the combined organic layers with three quantities, each of 50 ml, of *water*. Dry the organic solution over *anhydrous sodium sulphate*, filter and evaporate on a water-bath until the odour of pentane is no longer detectable. Heat the residue at 105° for 15 minutes, cool and weigh.

Sodium Chloride and Sodium Sulphate. Not more than a total of 8.0 per cent, determined by the following methods.

For sodium chloride — Dissolve 5.0 g in 50 ml of *water,* add *2 M nitric acid* dropwise until the solution is neutral to *litmus paper*, add 2 ml of *potassium chromate solution* and titrate with *0.1 M silver nitrate.*

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

For sodium sulphate — Dissolve 0.5 g in 20 ml of *water,* warming gently if necessary, and add 1 ml of a 0.05 per cent w/v solution of *dithizone* in *acetone*. If the solution is red, add *1 M nitric acid,* dropwise, until it becomes bluish green. Add 2 ml of *dichloroacetic acid solution* and 80 ml of *acetone* and titrate with *0.01 M lead nitrate* until a permanent orange-red colour is obtained.

1 ml of *0.01 M lead nitrate* is equivalent to 0.001420 g of Na_2SO_4.

Assay. Weigh accurately about 1.15 g, dissolve in sufficient *water* to produce 1000.0 ml, warming if necessary. To 20.0 ml add 15 ml of *chloroform*, 10 ml of *dilute sulphuric acid* and 1 ml of *dimethyl yellow-oracet blue B solution* and titrate with *0.004 M benzethonium chloride*, shaking vigorously and allowing the layers to separate after each addition, until the chloroform layer acquires a permanent clear green colour.

1 ml of *0.004 M benzethonium chloride* is equivalent to 0.001154 g of sodium alkyl sulphates, calculated as $C_{12}H_{25}NaO_4S$.

Storage. Store protected from moisture.

Sodium Metabisulphite

Sodium Pyrosulphite; Sodium Disulphite

$Na_2S_2O_5$ Mol. Wt. 190.1

Sodium Metabisulphite may be prepared by saturating a solution of Sodium Hydroxide with sulphur dioxide and allowing crystallisation to occur.

Sodium Metabisulphite contains not less than 95.0 per cent and not more than 100.5 per cent of $Na_2S_2O_5$.

Category. Pharmaceutical aid (antioxidant).

Description. Colourless, prismatic crystals or a white or creamy white powder; odour, sulphurous.

Identification

A. Gives the reactions of sodium salts (2.3.1).

B. A solution decolorises *iodinated potassium iodide* solution and the resulting solution gives the reactions of sulphates (2.3.1).

Tests

Acidity. A solution is acidic to *phenol red solution*.

Arsenic (2.3.10). Mix 2.5 g in a porcelain dish with 10 ml of *water*, 1.25 g of *potassium chlorate* and 16 ml of *hydrochloric acid* and heat to expel chlorine, remove the last traces of chlorine with a few drops of *stannous chloride solution AsT* and add 35 ml of *water*. The resulting solution complies with the limit test for arsenic (4 ppm).

Heavy metals (2.3.13). Dissolve 1.0 g in 10 ml of *water*, add 5 ml of *hydrochloric acid* and evaporate to dryness on a water-bath. Dissolve the residue in 25 ml of *water* containing 2 ml of *dilute acetic acid*. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Iron. To 0.5 g add 2 ml of *hydrochloric acid* and evaporate on a water-bath to dryness. Dissolve the residue in 2 ml of *hydrochloric acid* and 20 ml of *water* and add a few drops of *bromine solution*, cool, dilute with *water* to 25 ml, then add 50 mg of *ammonium persulphate* and 5 ml of *ammonium thiocyanate solution*. Any colour produced is not more intense than that obtained by adding 5 ml of *ammonium thiocyanate solution* to a mixture of *iron standard solution (20 ppm Fe)*, 2 ml of *hydrochloric acid*, 50 mg of *ammonium persulphate* and sufficient *water* to produce 25 ml (40 ppm).

Thiosulphate. Dissolve 1.0 g in 10 ml of *2 M hydrochloric acid* and heat on a water-bath for 10 minutes; not more than a faint opalescence is produced.

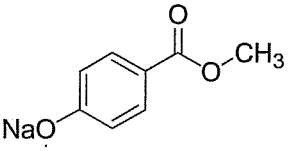
Assay. Weigh accurately about 0.1 g and dissolve in 50.0 ml *0.05 M iodine*, add 1 ml of *hydrochloric acid* and titrate the excess of iodine with *0.1 M sodium thiosulphate* using *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.004753 g of $Na_2S_2O_5$.

Storage. Store protected from light and moisture. On exposure to air and moisture it is slowly oxidised to sulphate with disintegration of the crystals.

Sodium Methylparaben

Sodium Methyl Hydroxybenzoate



$C_8H_7NaO_3$ Mol. Wt. 174.1

Sodium Methylparaben is the sodium salt of methyl 4-hydroxybenzoate.

Sodium Methylparaben contains not less than 99.0 per cent and not more than 102.0 per cent of $C_8H_7NaO_3$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white, crystalline powder; odourless or almost odourless; hygroscopic.

Identification

A. Dissolve 0.5 g in 5 ml of *water* and acidify to *litmus paper* with *hydrochloric acid*; a white precipitate is produced. Wash the precipitate with *water* and dry.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *methylparaben RS*.

B. The residue on ignition gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1).

pH (2.4.24). 9.5 to 10.5, determined in a 0.1 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 1 volume of *glacial acetic acid,* 30 volumes of *water* and 70 volumes of *methanol.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *water.* Immediately add 2 ml of *hydrochloric acid* and shake with 50 ml of *ether.* Evaporate the upper layer to dryness and take up the residue with 10 ml of *acetone.*

Reference solution (a). Dissolve 34.3 mg of *4-hydroxybenzoic acid* (sodium propylparaben impurity A) in 100.0 of *acetone.*

Reference solution (b). Dilute 0.5 ml of test solution (a) to 100.0 ml with *acetone.*

Reference solution (c). Dissolve 10 mg of *ethyl parahydroxybenzoate RS* in 1 ml of test solution (a) and dilute to 10.0 ml with *acetone.*

Apply 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot due to 4- hydroxybenzoic acid is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (4.0 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (2.3.12). Dissolve 1.0 g in 20 ml of *water,* add 0.2 ml of *nitric acid* and filter. 15 ml of the filtrate complies with the limit test for chlorides (330 ppm).

Sulphates (2.3.17). Dissolve 0.5 g in 40 ml of *water,* add 3.5 ml of *2 M hydrochloric acid,* dilute to 60 ml with water and filter. 15 ml of the filtrate complies with the limit test for sulphates (0.12 per cent).

Water (2.3.43). Not more than 5.0 per cent, determined on 1.0 g.

Assay. Dissolve 0.15 g in 50 ml of *anhydrous acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01741 g of $C_8H_7NaO_3$.

Storage. Store protected from moisture.

Sodium Phosphate

Disodium Hydrogen Phosphate; Disodium Hydrogen Phosphate Dodecahydrate

$Na_2HPO_4,12H_2O$ Mol. Wt. 358.1

Sodium Phosphate contains not less than 98.0 per cent and not more than 101.0 per cent of Na_2HPO_4, calculated on the anhydrous basis.

Category. Cathartic; pharmaceutical aid (buffering agent).

Description. Colourless, transparent crystals; very efflorescent.

Identification

A 10.0 per cent w/v solution in *distilled water* (solution A) gives the reactions of sodium salts and of phosphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT.* The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 10 ml of *water,* adding 4 ml of *1 M acetic acid* and diluting to 25 ml with *water.* The solution complies with the limit test for heavy metals, Method A (10 ppm).

Iron (2.3.14). 20 ml of solution A complies with the limit test for iron (20 ppm).

Chlorides (2.3.12). To 10 ml of solution A add 10 ml of *2 M nitric acid* and dilute to 20 ml with *water.* The resulting solution complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). To 2.5 ml of solution A add 2 ml of *2 M hydrochloric acid* and dilute to 15 ml with *distilled water.* The resulting solution complies with the limit test for sulphates (600 ppm).

Reducing substances. To 5 ml of solution A add 5 ml of *1 M sulphuric acid* and 0.25 ml of *0.02 M potassium permanganate* and heat on a water-bath for 5 minutes; the red colour is not completely discharged.

Sodium dihydrogen phosphate. The value of the expression $(n_2 - 25)/(25 - n_1)$,

where n_1 and n_2 are the titres of *1 M sodium hydroxide* obtained in the Assay, does not exceed 0.025.

Water (2.3.43). 57.0 to 61.0 per cent, determined on 0.1 g dissolved in a mixture of 10 volumes of *methanol* and 40 volumes of *dimethylformamide.*

Assay. Weigh accurately about 4.0 g *(w)*, dissolve in 25 ml of *water*, add 25.0 ml of *1 M hydrochloric acid* and titrate potentiometrically with *1 M sodium hydroxide* to the first inflection of the pH curve (n_1 *ml*). Continue the titration until the second inflection of the curve is reached; the total volume of sodium hydroxide required is n_2 ml.

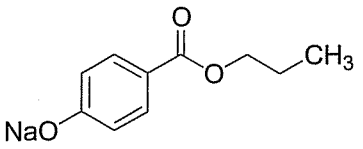
Calculate the content of Na_2HPO_4 from the expression $1420(25 - n_1)/w\,(100 - d)$,

where d is the percentage water content.

Storage. Store protected from moisture.

Sodium Propylparaben

Sodium Propyl Hydroxybenzoate



$C_{10}H_{11}NaO_3$ Mol. Wt. 202.2

Sodium Propylparaben is the sodium salt of propyl 4- hydroxybenzoate.

Sodium Propylparaben contains not less than 99.0 per cent and not more than 102.0 per cent of $C_{10}H_{11}NaO_3$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white, crystalline powder; odourless or almost odourless; hygroscopic.

Identification

A. Dissolve 0.5 g in 5 ml of *water* and acidify to *litmus paper* with *hydrochloric acid*; a white precipitate is produced. Wash the precipitate with *water* and dry.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *propylparaben RS*.

B. The residue on ignition gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1).

pH (2.4.24). 9.5 to 10.5, determined in a 0.1 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *water*. Immediately add 2 ml of *hydrochloric acid* and shake with 50 ml of *ether*. Evaporate the upper layer to dryness and take up the residue with 10 ml of *acetone*.

Reference solution (a). Dissolve 34.3 mg of *4-hydroxybenzoic acid* (sodium propylparaben impurity A) in 100.0 of *acetone*.

Reference solution (b). Dilute 0.5 ml of test solution (a) to 100 ml with *acetone*.

Reference solution (c). Dissolve 10 mg of *ethyl parahydroxybenzoate RS* in 1 ml of test solution (a) and dilute to 10.0 ml with *acetone*.

Apply 5 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot due to 4- hydroxybenzoic acid is not more intense than the corresponding spot in the chromatogram obtained with reference solution (a) (4.0 per cent) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Chlorides (2.3.12). Dissolve 1.0 g in 20 ml of *water*, add 0.2 ml of *nitric acid* and filter. 15 ml of the filtrate complies with the limit test for chlorides (330 ppm).

Sulphates (2.3.17). Dissolve 0.5 g in 40 ml of *water*, add 3.5 ml of *2 M hydrochloric acid*, dilute to 60 ml with *water* and filter. 15 ml of the filtrate complies with the limit test for sulphates (0.12 per cent).

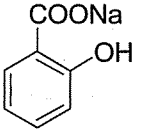
Water (2.3.43). Not more than 5.0 per cen, determined on 1.0 g.

Assay. Dissolve 0.15 g in 50 ml of *anhydrous acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out the blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02022 g of $C_{10}H_{11}NaO_3$.

Storage. Store protected from moisture.

Sodium Salicylate



$C_7H_5NaO_3$ Mol. Wt. 160.1

Sodium Salicylate is sodium 2-hydroxybenzoate.

Sodium Salicylate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_7H_5NaO_3$, calculated on the dried basis.

Category. Anti-inflammatory; analgesic.

Dose. 500 mg to 2 g, with food; in the treatment of acute rheumatism, 5 to 10 g daily, in divided doses.

Description. Colourless, small crystals or shiny flakes or a white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sodium salicylate RS* or with the reference spectrum of sodium salicylate.

B. A 10.0 per cent w/v solution in *carbon dioxide-free water* prepared from *distilled water* (solution A) gives the reactions of salicylates (2.3.1).

C. Gives reaction B of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity. To 20 ml of solution A add 0.1 ml of *phenol red solution*; the solution is yellow. Titrate with *0.01 M sodium hydroxide* to a reddish violet colour; not more than 2.0 ml of *0.01 M sodium hydroxide* is required.

Arsenic (2.3.10). Mix 5.0 g with 10 ml of *bromine solution* and evaporate to dryness on a water-bath. Ignite gently, cool, dissolve the residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT* and remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 2.0 g in 46 ml of *water*, add with constant stirring 4 ml of *dilute hydrochloric acid*, filtering and using 25 ml of the filtrate. The solution complies with the limit test for heavy metals, Method A (20ppm).

Chlorides (2.3.12). To 25 ml of solution A add 15 ml of *water* and 10 ml of *2 M nitric acid* and filter. 25 ml of the filtrate complies with the limit test for chlorides (200 ppm).

Sulphates (2.3.17). 2.5 ml of solution A diluted to 15 ml with *distilled water* complies with the limit test for sulphates (600 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01601 g of $C_7H_5NaO_3$.

Storage. Store protected from light and moisture.

Sodium Starch Glycollate

Sodium Carboxymethyl Starch

Sodium Starch Glycollate is the sodium salt of a poly-α-glucopyranose in which some of the hydroxyl groups are in the form of carboxymethyl ether.

Sodium Starch Glycollate contains not less than 2.8 per cent and not more than 4.5 per cent of sodium, Na, calculated on the material washed with Ethanol (95 per cent) and dried as described under Assay.

Category. Pharmaceutical aid (tablet disintegrant).

Description. A very fine, white or off-white, free-flowing powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sodium starch glycollate RS* or with the reference spectrum of sodium starch glycollate.

B. To 5 ml of a 2 per cent w/v dispersion in *water* add 0.05 ml of *0.005 M iodine*; a dark blue colour is produced.

C. The solution obtained in the test for Heavy metals gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 2.0 per cent w/v dispersion in *carbon dioxide-free water*.

Heavy metals (2.3.13). To 4.0 g in a silica or platinum dish add 2 ml of a 50 per cent w/v solution of *sulphuric acid*, heat in a water-bath and then cautiously over a flame at about 600°. Continue heating until all black particles have disappeared, allow to cool, add 0.1 ml of *1 M sulphuric acid*, heat to ignition once again and allow to cool. Add 0.1 ml of *2 M ammonium carbonate*, evaporate to dryness and cautiously ignite. To the residue add 5 ml of *hydrochloric acid*, evaporate to dryness on a water-bath and dissolve the residue in 100 ml of *water*. 25 ml of a solution complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). 50 ml of the solution obtained in the test for Heavy metals complies with the limit test for iron (20 ppm).

Sodium Chloride. Not more than 10.0 per cent, determined by the following method. To 1.0 g add 20.0 ml of *0.1 M silver nitrate* and 30 ml of *nitric acid* and boil carefully for 30 minutes. Cool and add a sufficient volume of a saturated solution of *potassium permanganate* to change the colour of the solution to red. Discharge the colour by the dropwise addition of *hydrogen peroxide solution (10 vol)*, add 3 ml of *dibutyl phthalate* and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator, shaking vigorously after each addition of titrant. Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.005844 g of NaCl.

Sodium glycollate. To 0.2 g add 5 ml of *glacial acetic acid*, mix well and add 5 ml of *water*, stirring occasionally until solution is complete. Slowly add 50 ml of *acetone* with stirring and then add 1 g of *sodium chloride*. Filter, wash the residue with *acetone* and dilute the filtrate to 100 ml with *acetone*. Transfer 2 ml of this solution to an open flask, heat on a water-bath for exactly 20 minutes, cool, add 5 ml of *naphthalenediol reagent* and mix thoroughly. Add a further 15 ml of the same reagent, mix, cover the flask with aluminium foil and heat on a water-bath for 20 minutes. Cool and dilute to 25 ml with *sulphuric acid*. The absorbance (2.4.7) of the resulting solution at the maximum at about 540 nm using *water* as the blank, is not more than that of a solution prepared in the following manner. To 5 ml of a 0.062 per cent w/v solution of *glycollic acid*, previously dried at a pressure not exceeding 2 kPa for 16 hours, add 5 ml of *glacial acetic acid*, dilute to 100 ml with *acetone* and complete the procedure described above beginning at the words "Transfer 2 ml...." (2.0 per cent).

Microbial Contamination (2.2.9). 1.0 g is free from *Escherichia coli* and *Salmonellae*.

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 0.5 g by drying in an oven at 105°.

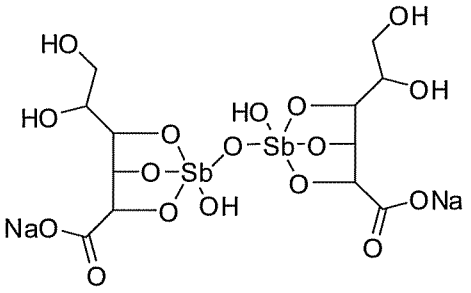
Assay. Weigh accurately about 4.0 g, add 350 ml of a mixture of 4 volumes of *ethanol (95 per cent)* and 1 volume of *water*, add 0.25 ml of *phenolphthalein solution* and mix. Add *1 M sodium hydroxide* dropwise until the colour of the suspension becomes faintly pink, shake for 30 minutes and decant through a sintered glass crucible. Repeat the extraction three times, or until a test for chloride ions is negative. Transfer the bulk of the residue to the crucible, wash the residue with *ethanol (95 per cent)* and dry at 110° to constant weight. Weigh accurately 0.5 g of the residue, add 80 ml of *anhydrous glacial acetic acid*, heat under a reflux condenser for 2 hours, cool. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M perchloric acid* is equivalent to 0.0023 g of Na.

Storage. Store protected from light and moisture.

Sodium Stibogluconate

Sodium Antimony Gluconate



Sodium Stibogluconate is mainly the disodium salt of μ-oxy-bis[gluconato(3)-O^2,O^3,O^4-hydroxoantimony].

The method of manufacture is such as to ensure consistently controlled reaction stoichiometry in order to yield sodium stibogluconate that is satisfactory with regard to intrinsic toxicity.

Sodium Stibogluconate contains not less than 30.0 per cent and not more than 34.0 per cent of pentavalent antimony, calculated on the dried and methanol-free basis.

Category. Antiprotozoal.

Dose. By intramuscular or intravenous injection, 600 mg to 2 g daily, for 10 to 30 days.

Description. A colourless, mostly amorphous powder; odourless or almost odourless.

Identification

A. An aqueous solution is *dextro-rotatory*.

B. Pass *hydrogen sulphide* into a 5 per cent w/v solution for several minutes; an orange precipitate is produced.

C. When heated, it chars without melting, emitting an odour of burnt sugar and leaving a residue which gives the reactions of antimony compounds and the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 5.6, determined in the solution obtained in the test for Stability of solution.

Stability of solution. Heat a solution containing 10.0 per cent w/v of pentavalent antimony in an autoclave at 115.5° and at a pressure of 70 kPa for 30 minutes. The resulting solution is colourless or almost colourless.

Trivalent antimony. Dissolve 2.0 g in 30 ml of *water*, add 15 ml of *hydrochloric acid* and titrate with *0.00833 M potassium bromate* using *methyl orange solution* as indicator. Not more than 1.3 ml of *0.00833 M potassium bromate* is required.

Chlorides. Dissolve 2.5 g in 50 ml of *water* and add 2 ml of *2 M nitric acid* and 75 ml of *acetate buffer pH 5.0*. Titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Not more than 3.0 ml of *0.1 M silver nitrate* is required.

Methanol. Not more than 2.0 per cent w/w, determined by gas chromatography (2.4.13).

Test solution. Add 5 ml of *water* to 0.5 g of the substance under examination and mix with the aid of ultrasound until the solution is complete.

Reference solution (a). Add 5 ml of a 0.2 per cent v/v solution of *ethanol* (internal standard) to 0.5 g of the substance under examination and mix with the aid of ultrasound until the solution is complete.

Reference solution (b). Add 1 ml of a 1.0 per cent v/v solution of *methanol* to 5 ml of a 0.2 per cent v/v solution of the internal standard.

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with porous polymer beads (80 to 100 mesh),
- temperature: column. 130°,

Calculate the percentage w/w of methanol taking 0.792 g as its weight per ml at 20°.

Undue toxicity. Dissolve a suitable quantity of the substance under examination in *water for injections* to give a solution containing 28 mg of pentavalent antimony per ml. Inject intravenously 0.3 ml of the solution into each of 10 mice that have been deprived of food for not less than 17 hours. After injections allow the mice access to food and water. None of the mice dies within 24 hours. If one of the mice dies within 24 hours, repeat the test. None of the second group of mice dies within 24 hours.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.25 g by drying in an oven at 130° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.16 g, dissolve in 30 ml of *hydrochloric acid*, add 70 ml of *phosphoric acid* and stir carefully until completely mixed. Titrate with *0.05 M ferrous ammonium sulphate* prepared using *sulphuric acid (1 per cent)*, determining the end-point potentiometrically (2.4.25), using a platinum electrode and a silver-silver chloride reference electrode.

1 ml of *0.05 M ferric ammonium sulphate* is equivalent to 0.003044 g of pentavalent antimony.

Storage. Store protected from moisture.

Sodium Stibogluconate Injection

Sodium Antimony Gluconate Injection

Sodium Stibogluconate Injection is a sterile solution of Sodium Stibogluconate in Water for Injections. Injection in multiple dose containers must not contain phenol as preservative.

Sodium Stibogluconate Injection contains not less than 9.5 per cent w/v and not more than 10.5 per cent w/v of pentavalent antimony, Sb.

Usual strength. The equivalent of 100 mg of pentavalent antimony per ml.

Description. A clear, colourless or faintly straw-coloured solution.

Identification

A. It is *dextro*-rotatory.

B. Dilute 2 ml to 10 ml with *water* and pass hydrogen sulphide through the solution; no immediate precipitate is produced. Continue to pass hydrogen sulphide through the solution for several minutes; an orange precipitate is produced.

C. The residue obtained by evaporation to dryness, after incineration, gives the reactions of antimony compounds and the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 5.0 to 5.6.

Undue toxicity. Dilute a suitable volume of the injection under examination with sufficient *water for injections* to give a solution containing the equivalent of 28 mg of pentavalent antimony per ml. Inject intravenously 0.3 ml of the solution into each of 10 mice that have been deprived of food for not less than 17 hours. After injections allow the mice access to food and water. None of the mice dies within 24 hours. If one of the mice dies within 24 hours, repeat the test. None of the second group of mice dies within 24 hours.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To 1.0 ml, accurately measured, add 30 ml of *hydrochloric acid* and 70 ml of *phosphoric acid* and mix. Titrate with *0.05 M ferric ammonium sulphate* prepared using *sulphuric acid (1 per cent)*, determining the end-point potentiometrically (2.4.25), using a platinum electrode and a silver-silver chloride reference electrode.

1 ml of *0.05 M ferric ammonium sulphate* is equivalent to 0.003044 g of pentavalent antimony.

Labelling. The label states the strength in terms of the equivalent amount of pentavalent antimony per ml.

Sodium Thiosulphate

Sodium Hyposulphite

$Na_2S_2O_3,5H_2O$ Mol. Wt. 248.2

Sodium Thiosulphate contains not less than 99.0 per cent and not more than 101.0 per cent of $Na_2S_2O_3,5H_2O$.

Category. Antidote to cyanide poisoning

Dose. By intravenous or intramuscular injection, 300 mg to 1 g.

Description. Colourless large crystals or a coarse, crystalline powder; odourless; deliquescent in moist air and effloresces in dry air at temperature above 33°. It dissolves in its water of crystallisation at about 49°.

Identification

A. To 0.5 ml of a 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) add 0.5 ml of *water* and 2 ml of *0.1 M silver nitrate*; a white precipitate is produced which quickly becomes yellowish and finally black.

B. To a portion of solution A add a few drops of *iodine solution*; the colour is discharged.

C. Dilute 2.5 ml of solution A to 5 ml with *water* and add 1 ml of *hydrochloric acid*; a gas is evolved which turns starch-iodate paper blue and a precipitate of sulphur is produced.

D. 1 ml of solution A gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 6.0 to 8.4, determined in solution A.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Dissolve 1.0 g in 10 ml of *water*. Slowly add 5 ml of *dilute hydrochloric acid* and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of *water* for 2 minutes and filter. Heat the filtrate to boiling, add sufficient *bromine solution* to the hot filtrate to produce a clear solution and add a slight excess of *bromine solution*. Boil the solution to expel the bromine completely, cool to room temperature and add a drop of *phenolphthalein solution* and *sodium hydroxide solution* until a slight pink colour is produced. Add 2 ml of *dilute acetic acid* and dilute with *water* to 25 ml. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). To 12.5 ml of solution A add 15 ml of *2 M nitric acid*, boil gently for 3 to 4 minutes, cool and filter. The filtrate complies with the limit test for chlorides (200 ppm).

Sulphides. To 10 ml of solution A add 0.05 ml of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside*; the solution does not become violet.

Sulphates and sulphites (2.3.17). Dilute 2.5 ml of solution A to 10 ml with *distilled water*. To 3 ml of this solution add 2 ml of *iodine solution* and gradually add more *iodine solution* and dilute to 15 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (0.2 per cent).

Assay. Weigh accurately about 0.5 g, dissolve in 20 ml of *water* and titrate with *0.05 M iodine* using *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.02482 g of $Na_2S_2O_3,5H_2O$.

Storage. Store protected from moisture.

Sodium Thiosulphate Injection

Sodium Hyposulphite Injection

Sodium Thiosulphate Injection is a sterile solution of Sodium Thiosulphate in Water for Injections.

Sodium Thiosulphate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sodium thiosulphate, $Na_2S_2O_3,5H_2O$.

Usual strength. 100 mg per ml.

Description. A clear, colourless solution.

Identification

A. To 0.5 ml of a 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) add 0.5 ml of *water* and 2 ml of *0.1 M silver nitrate*; a white precipitate is produced which quickly becomes yellowish and finally black.

B. To a portion of solution A add a few drops of *iodine solution*; the colour is discharged.

C. Dilute 2.5 ml of solution A to 5 ml with *water* and add 1 ml of *hydrochloric acid*; a gas is evolved which turns starch-iodate paper blue and a precipitate of sulphur is produced.

D. 1 ml of solution A gives reaction A of sodium salts (2.3.1).

Tests

pH (2.4.24). 7.0 to 9.0.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

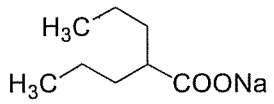
Assay. To an accurately measured volume containing about 0.5 g of Sodium Thiosulphate add about 20 ml of *water* and titrate with *0.05 M iodine*, using 3 ml of *starch solution*, added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.02482 g of $Na_2S_2O_3,5H_2O$.

Storage. Store in single dose containers.

Sodium Valproate

Divalproex Sodium



$C_8H_{15}NaO_2$ Mol. Wt. 166.2

Sodium Valproate is sodium 2-propylpentanoate.

Sodium Valproate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{15}NaO_2$, calculated on the dried basis.

Category. Anticonvulsant.

Dose. 600 mg to 1.6 g daily, in divided doses.

Description. A white or almost white, crystalline powder; hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sodium valproate RS* or with the reference spectrum of sodium valproate.

B. In the test for Related substances, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

C. Dissolve 1.25 g in 20 ml of *distilled water* in a separating funnel, add 5 ml of *2 M nitric acid*, shake and allow the mixture to stand for 12 hours; the lower layer (solution A) gives reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 20.0 per cent w/v solution is not more opalescent than opalescence standard OS2 (2.4.1), and is not more intensely coloured than reference solution YS6 (2.4.1).

Acidity or alkalinity. To 10 ml of a 10.0 per cent w/v solution in *carbon dioxide-free water* add 0.1 ml of *dilute phenolphthalein solution*; not more than 0.75 ml of either *0.1 M sodium hydroxide* or *0.1 M hydrochloric acid* is required to change the colour of the solution.

Related substances. Determine by gas chromatography (2.3.13).

Test solution (a). Add 5 ml of *1 M sulphuric acid* to 10 ml of a 0.04 per cent w/v solution of the substance under examination and shake with three quantities, each of 20 ml, of *ether*. Add 10 ml of a 0.02 per cent w/v solution of *butyric acid* (internal standard) in *ether* to the combined ether extracts, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Test solution (b). Add 0.5 ml of *1 M sulphuric acid* to 10 ml of a 0.04 per cent w/v solution of the substance under examination and shake with three quantities, each of 5 ml, of *ether*. Shake the combined ether extracts with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Test solution (c). Dissolve 0.5 g of the substance under examination in 10 ml of *water*, add 5 ml of *1 M sulphuric acid* and treat as described for test solution (a) beginning at the words "shake with three...".

Reference solution. Prepare in the same manner as test solution (b) but using *sodium valproate RS* in place of the substance under examination.

Chromatographic system

- a glass column 2.6 m × 2 mm, packed with *silanised diatomaceous support* (125 to 180 mesh) impregnated with 5 per cent w/w of *polyethylene glycol 20,000 2-nitroterephthalate* and 1 per cent w/w of *phosphoric acid*,
- temperature. column.150° to 170° to obtain a retention time of about 12 minutes for valproic acid [the principal peak in test solution (b)],
  inlet port at 200° and detector at 300°,
- flow rate. 20 ml per minute of the carrier gas.

Allow the chromatography to proceed for 2.5 times the retention time of valproic acid. Adjust the sensitivity so that the height of the peak due to the internal standard in the chromatogram obtained with test solution (a) is not less than 70 per cent of the full-scale deflection on the recorder. In the chromatogram obtained with test solution (c), the sum of the areas of any secondary peaks is not greater than the area of the peak due to the internal standard. Ignore any peaks the area of which is less than 1 per cent of the area of the peak due to the internal standard. The test is not valid unless the resolution between the peak due to the internal standard and the principal peak in the chromatogram obtained with test solution (a) is at least 12.

Chlorides (2.3.12). Dissolve 1.25 g in 10 ml of *water*. The resulting solution complies with the limit test for chlorides (200 ppm).

Sulphates (2.3.17). Solution A complies with the limit test for sulphates (200 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B. Use 2 ml of *lead standard solution (10 ppm)* to prepare the standard (20 ppm).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g and dissolve in 25 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01662 g of $C_8H_{15}NaO_2$.

Storage. Store protected from moisture.

Sodium Valproate Oral Solution

Sodium Valproate Elixir

Sodium Valproate Oral Solution is a solution of Sodium Valproate in a suitable flavoured vehicle.

Sodium Valproate Oral Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sodium valproate, $C_8H_{15}NaO_2$.

Usual strength. 200 mg in 5 ml.

Identification

A. Shake a quantity containing about 0.25 g of Sodium Valproate with two quantities, each of 25 ml, of *chloroform* and discard the chloroform extracts. Add 10 ml of a saturated solution of *sodium chloride* and 10 ml of *2 M hydrochloric acid*, mix and shake with 25 ml of *chloroform*. Wash the chloroform layer with 5 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate to dryness.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *valproic acid RS* or with the reference spectrum of valproic acid.

B. Shake a quantity containing about 0.25 g of Sodium Valproate with a mixture of 10 ml of a saturated solution of *sodium chloride*, 10 ml of *2 M hydrochloric acid* and 25 ml of *chloroform*. Evaporate the chloroform layer to dryness, dissolve the residue in 2 ml of *water*, make just alkaline with *2 M sodium hydroxide* and add 0.5 ml of a 10 per cent w/v solution of *cobalt nitrate*; a purple precipitate is produced which is soluble in *dichloromethane*.

Tests

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Mix a quantity of the oral solution containing 0.50 g of Sodium Valproate with 10 ml of *water*, acidify with *2 M sulphuric acid* and shake with three quantities, each of 20 ml, of *dichloromethane*. Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Test solution (b). Mix a quantity of the oral solution containing 0.5 g of Sodium Valproate with 10 ml of a 0.02 per cent w/v solution of *octanoic acid* (internal standard) in *0.1 M sodium hydroxide* and continue as described for test solution (a) beginning at the words "acidify with *2 M sulphuric acid...*".

Reference solution. A 0.02 per cent w/v solution of the internal standard in *dichloromethane*.

Chromatographic system

- a glass column 1.5 m x 4 mm. packed with *acid-washed, silanised diatomaceous support* (80 to 180 mesh) coated with 15 per cent w/w of free fatty acid phase (such as Supelco FFAP 2-1063) and *1 per cent w/w* of *phosphoric acid,*
- temperature. column. 170°.

In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks is not greater than the area of the peak due to the internal standard.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Weigh accurately a quantity containing about 0.15 g of Sodium Valproate, add 50 ml of *water*, mix thoroughly and add 10 ml of saturated solution of *sodium chloride*, 10 ml of *2 M hydrochloric acid* and 40 ml of a mixture of 2 volumes of *ether* and 1 volume of *light petroleum (40° to 60°),* shake, allow to separate and reserve the ether layer. Shake the aqueous layer with a further 40 ml of the *ether-light petroleum mixture* and discard the aqueous layer. Shake each of the ether extracts with the same three quantities, each of 10 ml, of a saturated solution of *sodium chloride*, combine the extracts and evaporate to a volume of about 1 ml at a temperature not exceeding 30°. Add 50 ml of *ethanol (95 per cent),* previously neutralised with *0.01 M sodium hydroxide* using *dilute phenolphthalein solution* as indicator, and titrate with *0.1 M sodium hydroxide.*

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01662 g of $C_8H_{15}NaO_2$.

Determine the weight per ml of the preparation (2.4.29) and calculate the content of $C_8H_{15}NaO_2$ weight in volume.

Sodium Valproate Tablets

Sodium Valproate Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sodium valproate, $C_8H_{15}NaO_2$.

Usual strengths. 100 mg; 200 mg.

Identification

A. Shake a quantity of the powdered tablets containing about 0.5 g of Sodium Valproate with 10 ml of *water* and centrifuge. Acidify 5 ml of the supernatant liquid with *2 M sulphuric acid*, shake with 25 ml of *chloroform* and wash the chloroform layer with 5 ml of *water*. Dry by shaking with *anhydrous sodium sulphate*, filter and evaporate the chloroform.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *valproic acid RS* or with the reference spectrum of valproic acid.

B. Shake a quantity of the powdered tablets containing about 0.25 g of Sodium Valproate with 3 ml of *water* and centrifuge. To 2 ml of the supernatant liquid add 0.5 ml of a 10 per cent w/v solution of *cobalt nitrate*; a purple precipitate is produced which is soluble in *dichloromethane*.

Tests

Related substances. Determine by gas chromatography (2.4.13).

Test solution (a). Shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of *water*, acidify with *2 M sulphuric acid* and shake with three quantities, each of 20 ml, of *dichloromethane*. Wash the combined dichloromethane extracts with 10 ml of *water*, shake with *anhydrous sodium sulphate*, filter and evaporate the filtrate to a volume of about 10 ml at a temperature not exceeding 30° using a rotary evaporator.

Test solution (b). Shake a quantity of the powdered tablets containing 0.50 g of Sodium Valproate with 10 ml of a 0.020 per cent w/v solution of *octanoic acid* (internal standard) in *0.1 M sodium hydroxide* and continue as described for test solution (a) beginning at the words "acidify with *2 M sulphuric acid...*".

Reference solution. A 0.02 per cent w/v of the internal standard in *dichloromethane*.

Chromatographic system

- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 180 mesh) coated with 15 per cent w/w of free fatty acid phase (such as Supelco FFAP 2-1063) and *1 per cent w/w* of *phosphoric acid,*
- temperature. column. 170°,

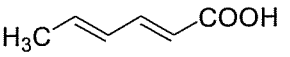
In the chromatogram obtained with test solution (b) the sum of the areas of any secondary peaks is not greater than the area of the peak due to the internal standard.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of Sodium Valproate, add 70 ml of *water*, shake for 10 minutes, dilute to 100.0 ml with *water* and filter. To 50.0 ml of the filtrate add 10 ml of a 30 per cent w/v solution of *sodium chloride* and 10 ml of *2 M hydrochloric acid.* Extract with 40 ml of a mixture of 2 volumes of *ether* and 1 volume of *light petroleum (boiling range 40° to 60°)* and allow to separate. Shake the aqueous layer with a further 40 ml of the ether-light petroleum mixture. Shake each of the ether extracts with the same three quantities, each of 10 ml, of a saturated solution of *sodium chloride*, combine the ether extracts and evaporate to a volume of about 1 ml at a temperature not exceeding 30°. Add 50 ml of *ethanol (95 per cent),* previously neutralised with *0.01 M sodium hydroxide* using dilute *phenolphthalein solution* as indicator, and titrate with *0.1 M sodium hydroxide.*

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01662 g of $C_8H_{15}NaO_2$.

Sorbic Acid



$C_6H_8O_2$ Mol. Wt. 112.1

Sorbic Acid is (2*E*,4*E*)-hexa-2,4-dienoic acid.

Sorbic Acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_6H_8O_2$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (antimicrobial preservative).

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sorbic acid RS* or with the reference spectrum of sorbic acid.

B. Dissolve 50 mg in sufficient *water* to produce 250 ml and dilute 2 ml of this solution to 200 ml with *0.1 M hydrochloric acid.*

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 264 nm; absorbance at about 264 nm, 0.43 to 0.51.

C. Dissolve 0.2 g in 2 ml of *ethanol (95 per cent)* and add 0.2 ml of *bromine water*; the solution is decolorised.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1), and colourless (2.4.1).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Aldehydes. Not more than 0.15 per cent, determined by the following method. Dissolve 1.0 g in a mixture of 50 ml of *2-propanol* and 30 ml of *water*, adjust the pH of the solution to 4.0 with *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* and dilute to 100 ml with *water*. To 10 ml of the resulting solution add 1 ml of *decolorised fuchsin solution* and allow to stand for 30 minutes. Any colour produced is not more intense than that obtained in a solution prepared simultaneously by adding 1 ml of *decolorised fuchsin* solution to a mixture of 1.5 ml of *acetaldehyde standard solution (100 ppm C_2H_4O)*, 4 ml of *2-propanol* and 4.5 ml of *water*.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

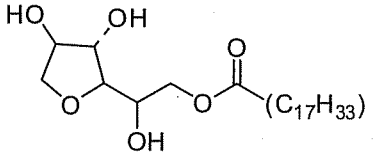
Water (2.3.43). Not more than 1.0 per cent, determined on 2.0 g.

Assay. Weigh accurately about 0.2 g, dissolve in 20 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide*, using 0.2 ml of *phenolphthalein solution* as indicator, until a pink colour is produced.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01121 g of $C_6H_8O_2$.

Storage. Store protected from light and moisture.

Sorbitan Oleate



$C_{24}H_{44}O_6$ Mol. Wt. 428.6

Sorbitan Oleate is mixture usually obtained by esterification of 1 mole of sorbitol and its mono-and di- anhydrides per mole of oleic (*cis*-9-octadecenoic) acid. A suitable antioxidant may be added.

NOTE—When sorbitan mono-oleate is demanded, Sorbitan Oleate shall be supplied.

Description. A brownish-yellow, viscous liquid.

Identification

A. Complies with the test for hydroxyl value (2.3.27).

B. Complies with the test for iodine value (2.3.28).

C. Complies with the test for composition of fatty acids.

D. *Margaric acid.* Not more than 0.2 per cent for oleic acid of vegetable origin and not more than 4.0 per cent for oleic acid of animal origin.

Tests

Relative density (2.4.29). About 0.99.

Acid value (2.3.23). Not more than 8.0, determined on 5.0 g.

Hydroxyl value (2.3.27). 190 to 210.

Saponification value (2.3.37). 145 to 160. Carry out the saponification for 1 hour.

Iodine value (2.3.28). 62 to 76.

Peroxide value (2.3.35). Not more than 10.0.

Composition of fatty acids. Determine by gas chromatography (2.4.13).

Composition of the fatty acid fraction of the substance:

— *myristic acid:* Not more than 5.0 per cent;

— *palmitic acid:* Not more than 16.0 per cent;

— *palmitoleic acid:* Not more than 8.0 per cent;

— *stearic acid:* Not more than 6.0 per cent;

— *oleic acid:* 65.0 per cent to 88.0 per cent;

— *linoleic acid:* Not more than 18.0 per cent;

— *linolenic acid:* Not more than 4.0 per cent;

— *fatty acids with chain length greater than C_{18}:* Not more than 4.0 per cent.

Heavy metals (2.3.13). In a silica crucible, mix thoroughly 2.0 g of the substance under examination with 0.5 g of *magnesium oxide*. Ignite to dull redness until a homogeneous white or greyish-white mass is obtained.

If after 30 min of ignition the mixture remains coloured, allow to cool, mix using a fine glass rod and repeat the ignition. If necessary repeat the operation. Heat at 800° for about 1 hour. Take up the residue in 2 quantities, each of 5 ml, of a mixture of equal volumes of *hydrochloric acid* and *water*. Add 0.1 ml of *phenolphthalein solution* and then *concentrated ammonia* until a pink colour is obtained. Cool, add *glacial acetic acid* until the solution is decolourised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with *water*. 12 ml of resultant solution complies with the limit test for heavy metals, Method D (10 ppm).

Total ash (2.3.19). Not more than 0.5 per cent, determined on 1.5 g.

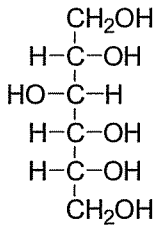
Water (2.3.43). Not more than 1.5 per cent, determined on 1.0 g.

Storage. Store protected from light.

Labelling. The label states the origin of the oleic acid used (animal or vegetable).

Sorbitol

D-Glucitol



$C_6H_{14}O_6$ Mol. Wt. 182.2

Sorbitol is D-glucitol, a hexahydric alcohol related to glucose.

Sorbitol contains not less than 98.0 per cent and not more than 101.0 per cent of $C_6H_{14}O_6$, calculated on the anhydrous basis.

Category. Nutrient (for parenteral administration); pharmaceutical aid (sweetening agent; excipient).

Description. A white, crystalline powder; odourless.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (a).

B. Dissolve 50 mg in 3 ml of *water*, add 3 ml of *catechol solution* and pour the mixture into 6 ml of *sulphuric acid*; a pink colour is produced.

C. Dissolve 5 g in 3 ml of *water* with the aid of gentle heat, cool, add 7 ml of *methanol*, 1 ml of *benzaldehyde* and 1 ml of *hydrochloric acid*, mix and shake continuously for 2 hours. Filter, dissolve the crystals in 20 ml of boiling *sodium bicarbonate solution* and allow to crystallise. The residue, after washing rapidly with 5 ml of a mixture of equal volumes of *methanol* and *water* and drying in a current of air, melts at about 175° (2.4.21).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Dissolve 5.0 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 50 ml (solution A). To 10 ml of solution A add 10 ml of *carbon dioxide-free water*. To 10 ml of the resulting solution add 0.05 ml of *phenolphthalein solution*; not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink. To a further 10 ml of the solution add 0.05 ml of *methyl red solution*; Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Specific optical rotation (2.4.22). +4.0° to +7.0°, determined in a solution prepared in the following manner. Dissolve a mixture of 5.0 g of the substance under examination and 6.4 g of *borax* in 40 ml of *water*, allow to stand for 1 hour, shaking occasionally, dilute to 50 ml with *water* and filter, if necessary.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 5.0 g of the substance under examination in 100.0 ml of *water*.

Reference solution (a). Dissolve 0.5 g of *sorbitol RS* in 10.0 ml of *water*.

Reference solution (b). Dilute 2.0 ml of the test solution to 100.0 ml with *water*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 100.0 ml with *water*.

Reference solution (d). Dissolve 0.5 g each of *sorbitol* and *mannitol (sorbitol impurity A)* in 10.0 ml of *water*.

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature. 85°,
- mobile phase: *water*,
- flow rate. 0.5 ml per minute,
- refractometer at a constant temperature,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to sorbitol and sorbitol impurity A is not less than 2.0. The relative retention time with reference to sorbitol for maltitol (sorbitol impurity C) is about 0.6, for mannitol (sorbitol impurity A) is about 0.8 and for iditol (sorbitol impurity B) is about 1.1.

Inject the test solution, reference solution (b), (c) and (d). Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.1 per cent).

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). 5.0 g complies with the limit test for chlorides (50 ppm).

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol (95 per cent)*. Mix, dilute to 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

Sulphates (2.3.17). 12 ml of solution A complies with the limit test for sulphates (125 ppm).

Reducing sugars. Dissolve 5.0 g in 3 ml of *water* with the aid of gentle heat, cool, add 20 ml of *cupri-citric solution* and a few glass beads, heat in such a manner that the solution boils in 4 minutes and continue boiling for a further 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* followed by 20.0 ml of *0.025 M iodine*. Add, shaking continuously, 25 ml of a 6 per cent v/v solution of *hydrochloric acid* and, when any precipitate has redissolved, titrate the excess iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.5 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14) as described in the test for Related substances with the following modification.

Inject the test solution and reference solution (a).

Calculate the content of $C_6H_{14}O_6$.

Sorbitol intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins *complies with the following additional requirement.*

Bacterial endotoxins (2.2.3). Not more than 4.0 Endotoxin Units per g for parenteral preparations having a concentration of less than 10 per cent w/v of sorbitol and not more than 2.5 Endotoxin Units per g for parenteral preparations containing 10 per cent w/v or more of sorbitol.

Storage. Store protected from moisture.

Labelling. The label states whether or not the contents are intended for use in the manufacture of parenteral preparations.

Sorbitol Solution (70 Per cent) (Crystallising)

Sorbitol (70 Per cent) (Crystallising)

Sorbitol Solution (70 per cent) (Crystallising) is an aqueous solution of hexitols.

Sorbitol Solution (70 per cent) (Crystallising) contains not less than 68.0 per cent w/w and not more than 72.0 per cent w/w of hexitols, calculated as D-glucitol ($C_6H_{14}O_6$).

Category. Pharmaceutical aid (sweetening vehicle); humectant.

Description. A clear, colourless, syrupy liquid.

Identification

A. Dilute 7.0 g with 40 ml of *water*, add 6.4 g of *borax*, allow to stand for 1 hour, shaking occasionally, and dilute to 50.0 ml with *water*. Filter if necessary. The optical rotation of the resulting solution is 0° to +1.5° (2.4.22).

B. Dry 1 g over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa. Heat 0.5 g of the residue with a mixture of 5 ml of *acetic anhydride* and 0.5 ml *pyridine* with the aid of heat and allow to stand for 10 minutes. Pour the mixture into 25 ml of *water*, allow to stand in ice for 2 hours and filter. The precipitate, after recrystallisation from a small volume of *ethanol (95 per cent)* and drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, melts at about 100° (2.4.21).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with a uniform 0.75-mm thick layer of the following mixture. Mix 0.1 g of *carbomer* with 110 ml of *water* and allow to stand, with gentle stirring, for 1 hour. Adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A mixture of 85 volumes of *2-propanol* and 15 volumes of a 0.2 per cent w/v solution of *boric acid*.

Test solution. Dissolve 0.35 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution. A 0.25 per cent w/v solution of *sorbitol RS* in *ethanol (95 per cent)*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool, spray with a 0.5 per cent w/v solution of *potassium permanganate* in *1 M sodium hydroxide* and heat at 100° for 2 minutes. The principal spot in the chromatogram obtained with the test

solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 14.0 per cent w/v solution in *carbon dioxide–free water* (solution A) is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.05 ml of *phenolphthalein solution*; not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink. To a further 10 ml of the solution add 0.05 ml of *methyl red solution*. Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Refractive index (2.4.27). 1.457 to 1.462.

Relative density (2.4.29). Not less than 1.290.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). 5.0 g complies with the limit test for chlorides (50 ppm).

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol (95 per cent)*. Mix, dilute to 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

Sulphates (2.3.17). 12 ml of solution A complies with the limit test for sulphates (125 ppm).

Reducing sugars. Dissolve 5.0 g in 3 ml of *water* with the aid of gentle heat, cool, add 20 ml of *cupri-citric solution* and a few glass beads, heat in such a manner that the solution boils in 4 minutes and continue boiling for a further 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* followed by 20.0 ml of *0.025 M iodine*. Add, shaking continuously, 25 ml of a 6 per cent v/v solution of *hydrochloric acid* and, when any precipitate has redissolved, titrate the excess iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1.0 g of the substance under examination in 50.0 ml of *water*.

Reference solution (a). Dissolve 65 mg of *sorbitol RS* in 5.0 ml of *water*.

Reference solution (b). Dissolve 65 mg each of *mannitol* and *sorbitol* in 5.0 ml of *water*.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature. 85°,
- mobile phase: *water*,
- flow rate. 0.5 ml per minute,
- refractometer at a constant temperature,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to sorbitol and mannitol (sorbitol impurity A) is not less than 2.0. The relative retention time with reference to sorbitol for mannitol (sorbitol impurity C) is about 0.8.

Inject the test solution and reference solution (a).

Calculate the content of D-sorbitol, $C_6H_{14}O_6$.

Storage. Store protected from moisture.

Sorbitol Solution (70 Per cent) (Non-Crystallising)

Sorbitol (70 per cent) (Non-crystallising)

Sorbitol Solution (70 per cent) (Non-crystallising) is an aqueous solution of hydrogenated, partly hydrolysed starch.

Sorbitol Solution (70 per cent) (Non-crystallising) contains not less than 68.0 per cent w/w and not more than 72.0 per cent w/w of solid matter and not less than 62.0 per cent w/w of polyols expressed as D-glucitol ($C_6H_{14}O_6$).

Category. Pharmaceutical aid (sweetening vehicle); humectant.

Description. A clear, colourless or faintly yellow, syrupy liquid; odourless.

Identification

A. Dilute 7.0 g with sufficient *carbon dioxide-free water* to produce 50 ml (solution A). To 3 ml of a freshly prepared 10 per cent w/v solution of *catechol* add 6 ml of *sulphuric acid* while cooling in ice. To 3 ml of the mixture add 0.3 ml of solution A and heat gently over a naked flame for 30 seconds; a pink colour is produced which becomes deep brownish red.

B. Dry 1 g over *phosphorus pentoxide* at 80° at a pressure of 1.5 to 2.5 kPa. Dissolve 0.5 g of the residue in a mixture of 5 ml

of *acetic anhydride* and 0.5 ml of *pyridine* with the aid of heat and allow to stand for 10 minutes. Pour the mixture into 25 ml of *water*, allow to stand in ice for 2 hours and filter. The residue, after recrystallisation from a small volume of *ethanol (95 per cent)* and drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, melts at about 100° (2.4.21).

C. Determine by thin-layer chromatography (2.4.17), coating the plate with a uniform 0.75-mm thick layer of the following mixture. Mix 0.1 g of *carbomer* with 110 ml of *water* and allow to stand, with gentle stirring, for 1 hour. Adjust to pH 7.0 by the gradual addition, with continuous shaking, of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A mixture of 85 volumes of *2-propanol* and 15 volumes of a 0.2 per cent w/v solution of *boric acid.*

Test solution. Dissolve 0.35 g of the substance under examination in 100 ml of *ethanol (95 per cent).*

Reference solution. A 0.25 per cent w/v solution of *sorbitol RS* in *ethanol (95 per cent).*

Apply to the plate 2 µl of each solution. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool, spray with a 0.5 per cent w/v solution of *potassium permanganate* in *1 M sodium hydroxide* and heat at 100° for 2 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 10 ml of *carbon dioxide-free water*. To 10 ml of the resulting solution add 0.05 ml of *phenolphthalein solution*; not more than 0.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink. To a further 10 ml of the solution add 0.05 ml of *methyl red solution*; Not more than 0.3 ml of *0.01 M hydrochloric acid* is required to change the colour of the solution to red.

Optical rotation (2.4.22). +1.5° to + 3.5°, determined in a solution prepared in the following manner. Dilute 7.0 g with 40 ml of *water*, add 6.4 g of *borax*, allow to stand for 1 hour, shaking occasionally, dilute to 50 ml with *water* and filter, if necessary.

Refractive index (2.4.27). 1.455 to 1.465.

Relative density (2.4.29). Not less than 1.285.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). 5.0 g complies with the limit test for chlorides (50 ppm).

Nickel. Dissolve 10.0 g in sufficient *water* to produce 20 ml, add 3 ml of *bromine water* and 2 ml of a 20 per cent w/v solution of *citric acid*, mix and add 10 ml of *6 M ammonia* and 1 ml of a 1 per cent w/v solution of *dimethylglyoxime* in *ethanol (95 per cent)*. Mix, dilute to 50 ml with *water* and allow to stand for 5 minutes; any colour produced is not more intense than that produced by treating in the same manner and at the same time 1.0 ml of *nickel standard solution (10 ppm Ni)* diluted to 20 ml with *water* (1 ppm).

Sulphates (2.3.17). 12 ml of solution A complies with the limit test for sulphates (125 ppm).

Reducing sugars. Dissolve 5.0 g in 3 ml of *water* with the aid of gentle heat, cool, add 20 ml of *cupri-citric solution* and a few glass beads, heat in such a manner that the solution boils in 4 minutes and continue boiling for a further 3 minutes. Cool rapidly and add 100 ml of a 2.4 per cent v/v solution of *glacial acetic acid* followed by 20.0 ml of *0.025 M iodine*. Add, shaking continuously, 25 ml of a 6 per cent v/v solution of *hydrochloric acid* and, when any precipitate has redissolved, titrate the excess iodine with *0.05 M sodium thiosulphate* using 1 ml of *starch solution*, added towards the end of the titration, as indicator. Not less than 12.8 ml of *0.05 M sodium thiosulphate* is required.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1.0 g of the substance under examination in 50.0 ml of *water.*

Reference solution (a). Dissolve 55 mg of *sorbitol RS* in 5.0 ml of *water.*

Reference solution (b). Dissolve 65 mg of *mannitol* and 55 mg of *sorbitol* in 5.0 ml of *water.*

Chromatographic system

- a stainless steel column 30 cm x 7.8 mm, packed with strong cation exchange resin (calcium form) (9 µm),
- column temperature. 85°,
- mobile phase: *water*,
- flow rate. 0.5 ml per minute,
- refractometer at a constant temperature,
- injection volume. 20 µl.

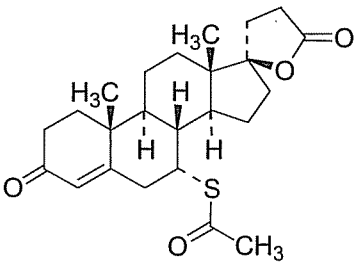
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to sorbitol and mannitol (sorbitol impurity A) is not less than 2.0. The relative retention time with reference to sorbitol for mannitol (sorbitol impurity C) is about 0.8.

Inject the test solution and reference solution (a).

Calculate the content of D-sorbitol, $C_6H_{14}O_6$.

Storage. Store protected from moisture.

Spironolactone



$C_{24}H_{32}O_4S$ Mol. Wt. 416.6

Spironolactone is 7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone.

Spironolactone contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{24}H_{32}O_4S$, calculated on the dried basis.

Category. Diuretic.

Dose. 100 to 200 mg daily, in divided doses.

Description. A yellowish white to buff coloured powder; odourless or with a slight odour of thioacetic acid. It exhibits polymorphism.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *spironolactone RS* or with the reference spectrum of spironolactone. Examine the substances as 5 per cent w/v solutions in *chloroform*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

Mobile phase. A mixture of 40 volumes of *cyclohexane* and 10 volumes of *toluene*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *spironolactone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Shake about 10 mg with 2 ml of *sulphuric acid (50 per cent)*; an orange solution with an intense yellowish fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulphide is evolved which turns *lead acetate paper* black. Add the solution to 10 ml of *water*; a greenish yellow solution is produced which shows opalescence or a precipitate.

Tests

Specific optical rotation (2.4.22). –33.0° to –37.0°, determined in a 1.0 per cent w/v solution in *chloroform*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 62.5 mg of the substance under examination in 2.5 ml of *tetrahydrofuran* and dilute to 25.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 25 mg of *canrenone RS* in 1.0 ml of *tetrahydrofuran* and dilute to 10.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d). Dilute 1.0 ml of the test solution and 1 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (e). Dilute 0.5 ml of reference solution (a) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 8 volumes of *acetonitrile*, 18 volumes of *tetrahydrofuran* and 74 volumes of *water*,
- flow rate. 1.8 ml per minute,

- spectrophotometer set at 254 nm and 283 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to canrenone and spironolactone is not less than 1.4 and the signal-to-noise ratio of the principal peak is not less than 6.

Inject the test solution, reference solution (a), (c), (d) and (e). At 254 nm, run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks other than canrenone, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (e). At 283 nm, the area of the peak due to canrenone is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Calculate the content of canrenone at 283 nm and the content of the other impurities at 254 nm. The sum of areas of all the peaks is not more than 1.0 per cent.

Chromium. Mix 0.2 g with 1 g of *potassium carbonate* and 0.3 g of *potassium nitrate* in a platinum crucible, heat gently until fused and ignite at 600° to 650° until the carbon is removed. Cool, dissolve the residue as completely as possible in 10 ml of *water* with the aid of gentle heat, filter and dilute to 20 ml with *water*. To 10 ml of the solution add 0.5 g of *urea* and just acidify with *sulphuric acid (14 per cent)*. When effervescence ceases add a further 1 ml of *sulphuric acid (14 per cent)*, dilute to 20 ml with *water* and add 0.5 ml of *diphenylcarbazide solution*. Any colour produced is not more intense than that obtained by adding 1 ml of *sulphuric acid (14 per cent)* to 0.5 ml of a freshly prepared 0.00283 per cent w/v solution of *potassium dichromate*, diluting to 20 ml with *water* and adding 0.5 ml of *diphenylcarbazide solution* (50 ppm).

Free mercapto compounds. Shake 2.0 g with 20 ml of *water* for 1 minute and filter. To 10 ml of the filtrate add 0.05 ml of *0.01 M iodine* and 0.1 ml of *starch solution* and mix; a blue colour develops.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours,

Assay. Weigh accurately about 50 mg, dissolve in sufficient *methanol* to produce 250.0 ml, dilute 5.0 ml to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7).

Calculate the content of $C_{24}H_{32}O_4S$ taking 470 as the specific absorbance at 238 nm.

Storage. Store protected from light and moisture.

Spironolactone Tablets

Spironolactone Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of spironolactone, $C_{24}H_{32}O_4S$. The tablets may contain added flavouring agents.

Usual strengths. 25 mg; 50 mg; 100 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.125 g of Spironolactone with two quantities, each of 10 ml, of *chloroform*, filter, evaporate the combined filtrates to dryness and dissolve the residue in 2.5 ml of chloroform.

On the resulting solution determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *spironolactone RS* or with the reference spectrum of spironolactone.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

Mobile phase. A mixture of 40 volumes of *cyclohexane* and 10 volumes of *toluene*.

Test solution. Extract a quantity of the powdered tablets containing 50 mg of Spironolactone with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness. Dissolve 25 mg the residue in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *spironolactone RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Shake about 10 mg of the residue obtained in test B with 2 ml of *sulphuric acid (50 per cent);* an orange solution with an intense yellowish fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulphide is evolved which turns *lead acetate paper* black. Add the solution to 10 ml of *water*; a greenish yellow solution is produced which shows opalescence or a precipitate.

Tests

Dissolution (2.5.2).

Apparatus. No. 1,

Medium. 1000 ml of *0.1 M hydrochloric acid* containing 0.1 per cent w/v of *sodium dodecyl sulphate*,

Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium, filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm. Measure the absorbance of the filtrate, suitably diluted if necessary, at the maximum at about 242 nm (2.4.7). Calculate the content of $C_{24}H_{32}O_4S$ in the medium taking 445 as the specific absorbance at 242 nm.

D. Not less than 70.0 per cent of the stated amount of $C_{24}H_{32}O_4S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing about 62.5 mg of Spironolactone with 25 ml of *chloroform*, sonicate for 15 minutes, centrifuge and filter the supernatant. Repeat the procedure on the residue with a further 25 ml of *chloroform*. Combine the chloroform extracts and evaporate to dryness using a rotary evaporator. Add 2.5 ml of *tetrahydrofuran* and 22.5 ml of the mobile phase to the residue.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 25 mg of *canrenone RS* in 1.0 ml of *tetrahydrofuran* and dilute to 10.0 ml with the mobile phase.

Reference solution (c). Dilute 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d). Dilute 1.0 ml of the test solution and 1.0 ml of reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (e). Dilute 0.5 ml of reference solution (a) to 10.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octylsilane bonded to porous silica (5 µm) (such as Spherisorb C8),
- mobile phase: a mixture of 8 volumes of *acetonitrile*, 18 volumes of *tetrahydrofuran* and 74 volumes of *water*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 254 nm and 283 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to canrenone and spironolactone is not less than 1.4 and the signal-to-noise ratio of the principal peak is not less than 6.

Inject the test solution, reference solution (a), (c), (d) and (e). At 254 nm, run the chromatogram twice the retention time of principal peak. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks other than the canrenone, is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with reference solution (e). At 283 nm, the area of the peak due to canrenone is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Calculate the content of canrenone at 283 nm and the content of the other impurities at 254 nm. The sum of areas of all the peaks is not more than 1.0 per cent.

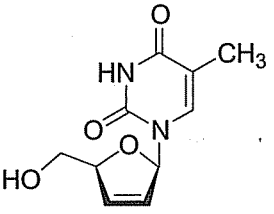
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of Spironolactone, add 100 ml of *methanol* and heat just to boiling, with swirling. Cool, add sufficient *methanol* to produce 250.0 ml, dilute 10.0 ml to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7).

Calculate the content of $C_{24}H_{32}O_4S$ taking 470 as the specific absorbance at 238 nm.

Storage. Store protected from light and moisture.

Stavudine



$C_{10}H_{12}N_2O_4$ Mol. Wt. 224.2

Stavudine is 2',3'-didehydro-3'-deoxythymidine.

Stavudine contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{12}N_2O_4$, calculated on the dried basis.

Category. Antiretroviral.

Dose. 30 to 40 mg twice daily depending on body weight of the patient.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *stavudine RS* or with the reference spectrum of stavudine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to stavudine in the chromatogram obtained with reference solution (c).

C. Melts at about 164° (2.4.21).

Tests

Specific optical rotation (2.4.22). –39.0° to –46.0°, determined in a 0.7 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14), as described in the Assay.

Separately inject the test solution, reference solutions (a) and (b) and record the chromatograms for at least three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to thymine is not greater than the area of the peak in the chromatogram obtained with reference solution (b) (1 per cent); the area of any peak corresponding to β-thymidine is not greater than the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1 per cent); the area of any individual impurity peak other than thymine and β-thymidine is not more than 0.5 per cent and the sum of the areas of all the impurity peaks other than thymine and β-thymidine is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50.0 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A solution containing 0.05 per cent w/v of *stavudine RS*, 0.01 per cent w/v of *thymine* and 0.0005 per cent w/v of *â-thymidine* in the mobile phase.

Reference solution (b). A 0.0005 per cent w/v solution of *thymine* in the mobile phase.

Reference solution (c). A 0.05 per cent w/v solution of *stavudine RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: a mixture of 20 volumes of *methanol* and 80 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject separately reference solutions (a) and (c). The order of elution in the chromatogram obtained with reference solution (a) is thymine, α-thymidine and stavudine with relative retention times of about 0.5, 0.7 and 1.0 respectively. The test is not valid unless the column efficiency determined from the stavudine peak in the chromatogram obtained with reference solution (a) is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections of reference solution (c) is not more than 2.0 per cent.

Inject separately the test solution and reference solution (c) and measure the responses of the principal peak.

Calculate the content of $C_{10}H_{12}N_2O_4$.

Storage. Store protected from light and moisture.

Stavudine Capsules

Stavudine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of stavudine, $C_{10}H_{12}N_2O_4$.

Usual strengths. 30 mg; 40 mg.

Identification

A. Shake a quantity of the mixed contents of the capsules containing about 50 mg of Stavudine with 80 ml of *water* for 10 minutes. Add sufficient *water* to produce 100 ml, mix and filter. Dilute 10 ml of the filtrate to 100 ml with *water*.

When examined in the range 200 nm to 300 nm (2.4.7), the resulting solution shows an absorption maximum at about 265 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to stavudine in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix well the contents of 20 capsules and transfer an accurately weighed quantity containing about 50 mg of Stavudine to a 100-ml volumetric flask. Add about 60 ml of *water*, mix with the aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter.

Reference solution. Weigh 5 mg each of *stavudine RS* and *thymine RS* and transfer to a 25-ml volumetric flask. Add 5 ml of *methanol*, mix with the aid of ultrasound to dissolve and dilute to volume with *water*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: gradient mixtures of *acetonitrile* and *0.1 M ammonium acetate*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min.)	*0.1 M Ammonium acetate* (per cent v/v)	*Acetonitrile* (per cent v/v)
0	95	5
5	95	5
25	20	80
30	20	80
31	95	5
35	95	5

Inject the reference solution. The test is not valid unless the column efficiency determined from the peaks of stavudine and thymine is not less than 7000 theoretical plates and the tailing factor is not more than 2.0.

Inject separately *water* and the test solution. Examine the water chromatogram for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution corresponding to thymine is not more than 3.0 per cent. Any other secondary peak observed in the chromatogram obtained with the test solution is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 4.0 per cent when calculated by percentage area normalisation.

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *0.01 M hydrochloric acid,*

Speed and time. 75 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. In the case of capsules containing 30 mg of Stavudine, weigh accurately about 30 mg of *stavudine RS* and transfer to a 100-ml volumetric flask. Dissolve and dilute to volume with *0.01 M hydrochloric acid.* Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid*; in the case of capsules containing 40 mg of stavudine, weigh accurately about 40 mg of *stavudine RS* and transfer to a 100-ml volumetric flask. Dissolve and dilute to volume with *0.01 M hydrochloric acid.* Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 85 volumes of *water* and 15 volumes of *methanol,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 20 µl.

Inject the reference solution and record the chromatograms for twice the retention time of stavudine. The test is not valid unless the column efficiency determined from the stavudine peak is not less than 3000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.5 per cent

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_{10}H_{12}N_2O_4$ in the medium.

D. Not less than 70 per cent of the stated amount of $C_{10}H_{12}N_2O_4$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Stavudine and transfer to a 100-ml volumetric flask. Add about 60 ml of *water*, mix with the aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter. Further dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

Reference solution. Weigh accurately about 50 mg of *stavudine RS* and transfer to a 100-ml volumetric flask. Add about 60 ml of *water*, mix with the aid of ultrasound to dissolve and dilute to volume with *water*. Dilute 10.0 ml of this solution to 25.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 5 volumes of *acetonitrile* and 95 volumes of *0.1 M ammonium acetate,*

- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl

Inject the reference solution. The test is not valid unless the column efficiency determined from the stavudine peak is not less than 3000 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peak.

Calculate the content of $C_{10}H_{12}N_2O_4$ in the capsules.

Storage. Store protected from moisture.

Stavudine Oral Solution

Stavudine Oral Solution is a mixture consisting of Stavudine with buffering agents and other excipients. It contains a suitable flavouring agent. It is filled in a sealed container.

The oral solution is constituted by dispersing the contents of the sealed container in the specified volume of water just before issue.

Stavudine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of stavudine $C_{10}H_{12}N_2O_4$.

Storage. Store the constituted solution in a refrigerator (2° to 8°). Discard any unused portion after 30 days of reconstitution.

The contents of the sealed container comply with the requirements stated under Oral Liquids and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

pH (2.4.24). 5.0 to 7.0, determined in the reconstituted solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the reconstituted solution containing 10 mg of stavudine and dissolve in 20 ml of *water*.

Reference solution (a). A 0.05 per cent w/v solution of *stavudine RS* in *water*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with *water*.

Reference solution (c). A solution containing 0.0125 per cent w/v each of thymine and thymidine in *water*. Dilute 2 ml of the solution to 100 ml of *water*.

Use the chromatographic system described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject reference solution (c). The test is not valid unless the resolution between thymine and thymidine peak is not less than 8.4.

Inject the test solution and reference solution (b). Run the chromatogram for 4 times the retention time (about 9 minutes) of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 1.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (1.5 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

Other tests. Complies with the tests stated under Oral Liquids.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh accurately a quantity of the reconstituted solution containing 10 mg of Stavudine, disperse in sufficient water and diluted to 100.0 ml with water.

Reference solution (a). A 0.01 per cent w/v solution of *stavudine RS* in *water*.

Reference solution (b). A solution containing 0.0125 per cent w/v each of thymine and thymidine in *water*. Dilute 2 ml of the solution to 100 ml with *water*.

Chromatographic system

- a stainless steel column 3.3 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 95 volumes of 25 mM *ammonium acetate* and 5 volumes of *methanol*,
  B. 50 volumes of 25 mM *ammonium acetate* and 50 volumes of *methanol*.
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 268 nm,

– injection volume. 20 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	100	0
60	100	0
120	0	100
155	100	0

Inject reference solution (b). The test is not valid unless the resolution between thymine and thymidine is not less than 8.4.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of $C_{10}H_{12}N_2O_4$ in the oral solution.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Stavudine and Lamivudine Tablets

Stavudine and Lamivudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of stavudine, $C_{10}H_{12}N_2O_4$ and lamivudine, $C_8H_{11}N_3O_3S$.

Usual strengths. Stavudine, 30 mg and Lamivudine, 150 mg; Stavudine, 40 mg and Lamivudine, 150 mg.

Identification

In the Assay, the two principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to stavudine and lamivudine in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 100 mg of lamivudine and transfer to a 200-ml volumetric flask. Add about 100 ml of *water*, mix with the aid of ultrasound for 10 minutes with occasional shaking to obtain a uniform dispersion, cool to room temperature, dilute to volume with *water* and mix. Filter through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate.

Reference solution (a). Weigh accurately about 65 mg of *stavudine RS* and 6.5 mg of *thymine RS*, and transfer to a 25-ml volumetric flask. Add 5 ml of *methanol*, sonicate to dissolve, dilute to volume with *water* and mix.

Reference solution (b). Weigh accurately about 100 mg of *lamivudine RS*, transfer to a 200 ml volumetric flask, add about 100 ml of *water* and mix with the aid of ultrasound to dissolve. Add 10 ml of reference solution (a) to this solution and dilute to volume with *water* and mix.

Chromatographic system

– a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
– mobile phase: gradient mixtures of *acetonitrile* and *0.1 M ammonium acetate*,
– flow rate. 1 ml per minute,
– a linear gradient programme using the conditions given below,
– spectrophotometer set at 270 nm,
– injection volume. 20 µl.

Time (in min.)	*0.1 M Ammonium acetate* (per cent v/v)	*Acetonitrile* (per cent v/v)
0	95	5
5	95	5
25	20	80
30	20	80
31	95	5
35	95	5

Inject separately reference solutions (b) and (c). The test is not valid unless the column efficiency determined from the thymine, stavudine and lamivudine peaks is not less than 3000 theoretical plates and the tailing factor for the same peaks is not more than 2.0.

Inject separately *water* and the test solution. Examine the chromatogram obtained with water for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution corresponding to thymine is not more than 3.0 per cent. Any other secondary peak observed in the chromatogram obtained with the test solution is not more than 0.5 per cent and the sum of the areas of all the secondary peaks is not more than 4.0 per cent when calculated by percentage area normalisation.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. In the case of tablets containing 30 mg of stavudine, weigh accurately about 33 mg of *stavudine RS* and 167 mg of *lamivudine RS* and transfer to a 100 ml volumetric flask. Add about 20 ml of *methanol*, sonicate to dissolve and dilute to volume with a solvent mixture of equal volumes of *methanol* and *water* (diluent). Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid*; in the case of tablets containing 40 mg of stavudine, weigh accurately about 44 mg of *stavudine RS* and 167 mg of *lamivudine RS* and transfer to a 100-ml volumetric flask. Add about 20 ml of *methanol*, mix with the aid of ultrasound to dissolve and dilute to volume with the diluent. Dilute 5.0 ml of this solution to 50.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of a buffer prepared by dissolving 0.68 g of *potassium dihydrogen phosphate* and 1.0 g of *sodium octanesulphonate* in 1000.0 ml of *water* to which 1 ml of *triethylamine* is added and the pH of which is adjusted to 2.5 with *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual stavudine and lamivudine peaks is not more than 1.5 and the relative standard deviation for replicate injections for each of the peaks corresponding to stavudine and lamivudine is not more than 1.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peaks due to stavudine and lamivudine.

Calculate the contents of $C_{10}H_{12}N_2O_4$ and $C_8H_{11}N_3O_3S$ in the medium.

D. Not less than 70 per cent of the stated amounts of $C_{10}H_{12}N_2O_4$ and $C_8H_{11}N_3O_3S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder containing about 150 mg of Lamivudine to a 100-ml volumetric flask, add about 20 ml of *methanol* and 50 ml of a mixture of equal volumes of *water* and *methanol*, mix with the aid of ultrasound for 5 minutes with occasional shaking to obtain a uniform dispersion. Cool to room temperature and dilute to volume with the same solvent mixture. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Dilute suitably with the same solvent mixture to obtain a solution with a concentration of 0.15 mg per ml of lamivudine.

Reference solution. A similarly prepared solution containing 0.015 per cent w/v of *lamivudine RS* and a concentration of *stavudine RS* similar to that of the concentration of stavudine in test solution.

Use the chromatographic system described under Dissolution.

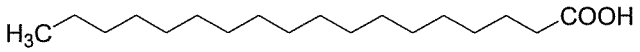
Inject the reference solution. The test is not valid unless the column efficiency determined from the lamivudine peak is not less than 2000 theoretical plates, the tailing factor for the individual peaks due to lamivudine and stavudine is not more than 1.5 and the relative standard deviation for replicate injections for each of the peaks corresponding to stavudine and lamivudine is not more than 2.0 per cent.

Inject separately the test solution and the reference solution and measure the responses for the major peaks.

Calculate the contents of $C_{10}H_{12}N_2O_4$ and $C_8H_{11}N_3O_3S$ in the tablets.

Storage. Store protected from moisture.

Stearic Acid



Stearic Acid consists of a mixture of fatty acids, chiefly stearic acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$). It may contain a suitable antioxidant.

Stearic Acid contains not less than 40.0 per cent of $C_{18}H_{36}O_2$ and the sum of the contents of $C_{18}H_{36}O_2$ and $C_{16}H_{32}O_2$ is not less than 90.0 per cent.

Category. Pharmaceutical aid (tablet lubricant; emulsion adjunct).

Description. A white powder or white, greasy, flaky crystals or hard masses showing signs of crystallisation.

Identification

In the Assay, the chromatogram obtained with the test solution shows two principal peaks which correspond to the two principal peaks in the chromatogram obtained with reference solution (c).

Tests

Congealing temperature (2.4.10). Not lower than 54°.

Acid value (2.3.23). 200 to 212, determined on 1.0 g.

Iodine value (2.3.28). Not more than 4.0 *(iodine monochloride method)*.

Mineral acid. Shake 5 g of the melted substance with an equal volume of hot *water* for 2 minutes, cool and filter. To the filtrate add 0.05 ml of *methyl orange solution*; no red colour is produced.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent, determined on 2.0 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Add 5 ml of *boron trifluoride solution* to 0.1 g of the substance under examination and heat under a reflux condenser for 15 minutes, cool and transfer to a separating funnel with the aid of 10 ml of *hexane*. Add 10 ml of a saturated solution of *sodium chloride* and 10 ml of *water*, shake, discard the lower aqueous layer and dry the upper layer over *anhydrous sodium sulphate*.

Reference solution (a). Add 5 ml of a 1 per cent w/v solution of *nonadecanoic acid* (internal standard) in *toluene* to a mixture of 50 mg of *stearic acid RS* and 50 mg of *palmitic acid RS* and evaporate to dryness. Add 5 ml of *boron trifluoride solution* and complete the procedure described for the test solution beginning at the words "heat under a reflux condenser...."..

Reference solution (b). Add 5 ml of the internal standard solution to 0.1 g of the substance under examination, evaporate to dryness, add 5 ml of *boron trifluoride solution* and complete the procedure described for the test solution beginning at the words "heat under a reflux condenser.....".

Reference solution (c). Prepare in the same manner as reference solution (a) but omitting the internal standard.

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 15 per cent w/w of *diethylene glycol succinate polyester*,
- temperature: column 170°, inlet port and detector at 240°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{18}H_{36}O_2$, and $C_{16}H_{32}O_2$.

Storage. Store protected from moisture.

Stearyl Alcohol

Stearyl Alcohol is a mixture of solid alcohols consisting chiefly of 1-octadecanol, ($C_{18}H_{38}O$).

Category. Pharmaceutical aid (stiffening agent).

Description. A white, unctuous mass or almost white flakes or granules; odour, faint and characteristic.

Tests

Appearance of solution. Dissolve 0.5 g in 20 ml of *ethanol (95 per cent)* by heating to boiling and allow to cool. The solution is clear (2.4.1), and not more intensely coloured than reference solution BS6 (2.4.1).

Melting range (2.4.21). 55° to 60°.

Acid value (2.3.23). Not more than 2.0.

Hydroxyl value (2.3.27). 195 to 220.

Iodine value (2.3.28). Not more than 2.0 *(iodine bromide method)*, determined on 2.0 g dissolved in 25 ml of *chloroform*, warming if necessary to effect solution.

Saponification value (2.3.37). Not more than 2.0, determined on 10.0 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *ethanol (95 per cent)*.

Reference solution (a). Dissolve 50 mg of *cetyl alcohol* in 10.0 ml of *ethanol (95 per cent)*.

Reference solution (b). Dissolve 50 mg of *stearyl alcohol RS* in 5.0 ml of *ethanol (95 per cent)*.

Reference solution (c). To 1.0 ml of reference solution (a), add 1.0 ml of reference solution (b) and dilute to 10.0 ml with *ethanol (95 per cent)*.

Chromatographic system
- a stainless steel column 30 m x 0.32 mm, packed with poly(dimethyl)siloxane (1 µm),
- temperature:

	Time (min)	Temperature (°)
Column	0–20	150–250
	20–40	250

- injector port and detector at 250°,
- flame ionization detector,
- flow rate. 1 ml per minute using nitrogen as the carrier gas.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to cetyl alcohol and stearyl alcohol is not less than 5.0.

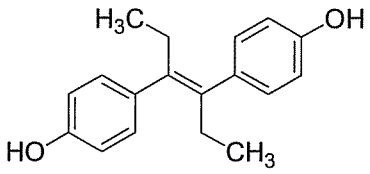
Inject 1 µl of the test solution, reference solution (b) and (c).

Calculate the content of $C_{18}H_{38}O$.

Storage. Store protected from moisture.

Stilboestrol

Diethylstilboestrol



$C_{18}H_{20}O_2$ Mol. Wt. 268.4

Stilboestrol is (*E*)-α,β-diethylstilbene-4,4'-diol.

Stilboestrol contains not less than 97.0 per cent and not more than 101.0 per cent of $C_{18}H_{20}O_2$, calculated on the dried basis.

Category. Oestrogen.

Dose. In the treatment of menopausal symptoms, 100 µg to 1 mg daily; for the suppression of lactation, 5 mg thrice daily for 3 days, followed by 5 mg daily for 6 days; in the treatment of carcinoma of the prostate and mammary carcinoma, 10 to 20 mg daily.

Description. A white or almost white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *stilboestrol RS* or with the reference spectrum of stilboestrol.

B. When examined in the range 230 nm to 450 nm (2.4.7), the irradiated solution prepared as directed in the Assay shows absorption maxima at about 292 nm and 418 nm.

C. In the test for Mono- and di-methyl ethers, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

D. Dissolve 0.5 mg in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat in a water-bath for 3 minutes; a deep yellow colour is produced which almost disappears on dilution with 3 ml of *glacial acetic acid* (distinction from dienoestrol).

Tests

4,4'-Dihydroxystilben and related ethers. Absorbance of a 1.0 per cent w/v solution in *ethanol* at about 325 nm, not more than 0.50 (2.4.7).

Mono- and di-methyl ethers. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *diethylamine*.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution (a). A 0.5 per cent w/v solution of *stilboestrol RS in ethanol (95 per cent)*.

Reference solution (b). A 0.05 per cent w/v solution of *stilboestrol monomethyl ether RS* in *ethanol (95 per cent)*.

Reference solution (c). A 0.05 per cent w/v solution of *stilboestrol dimethyl ether RS* in *ethanol (95 per cent)*.

Reference solution (d). A solution containing 0.25 per cent w/v each of *dienoestrol RS* and *stilboestrol RS*

Apply to the plate 1 µl of each solution. After development, dry the plate in air, spray with *ethanolic sulphuric acid (20 per cent)* and heat at 120° for 10 minutes. Any secondary spots in the chromatogram obtained with the test solution corresponding to the mono-and di-methyl ethers of stilboestrol are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively. Stilboestrol sometimes produces two spots. The test is not valid unless the chromatogram obtained with reference solution (d) shows two clearly separated spots of approximately the same intensity.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 20 mg, dissolve in sufficient *ethanol* to produce 100.0 ml and dilute 10.0 ml of this solution to 100.0 ml with *ethanol*. To 25.0 ml of the resulting solution add 25.0 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen phosphate* in 55 ml of *water*, transfer a portion of the mixture to a 1-cm closed quartz cell, place the cell 10 cm from a 15-watt short-wave, ultraviolet light of mercury lamp and irradiate for 10 minutes. Measure the absorbance of the irradiated solution at the maximum at about 418 nm (2.4.7).

Calculate the content of $C_{18}H_{20}O_2$ from the absorbance obtained by repeating the operation using *stilboestrol RS* in place of the substance under examination.

Storage. Store protected from light and moisture.

Stilboestrol Tablets

Diethylstilboestrol Tablets

Stilboestrol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of stilboestrol, $C_{18}H_{20}O_2$.

Usual strengths. 500 µg; 1 mg; 5 mg; 25 mg.

Identification

A. When examined in the range 230 nm to 450 nm (2.4.7), the irradiated solution prepared as directed in the Assay shows absorption maxima at about 292 nm and 418 nm.

B. Extract a quantity of the powdered tablets containing 3 mg of Stilboestrol with *ether*, filter and evaporate the filtrate to dryness. Dissolve 0.5 mg of the residue in 0.2 ml of *glacial acetic acid*, add 1 ml of *phosphoric acid* and heat in a water-bath for 3 minutes; a deep yellow colour is produced which almost disappears on dilution with 3 ml of *glacial acetic acid* (distinction from dienoestrol).

Tests

Uniformity of content. *For tablets containing 10 mg or less* — Comply with the test stated under Tablets.

Finely crush one tablet, add 10 ml of *ethanol*, shake for 30 minutes, add sufficient *ethanol* to produce 25.0 ml and centrifuge. Pipette an aliquot of the supernatant liquid containing 0.5 mg of Stilboestrol add 25.0 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen phosphate* in 55 ml of *water*, transfer a portion of the mixture to a 1-cm closed quartz cell, place the cell 10 cm from a 15-watt short-wave, ultraviolet light of mercury lamp and irradiate for 10 minutes. Measure the absorbance of the irradiated solution at the maximum at about 418 nm (2.4.7).

Calculate the content of $C_{18}H_{20}O_2$ in the tablet from the absorbance obtained by repeating the operation using *stilboestrol RS* in place of the substance under examination.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Stilboestrol, add 5 ml of *ethanol*, shake for 15 minutes, add sufficient *ethanol* to produce 100.0 ml and centrifuge. Dilute 20.0 ml of the clear, supernatant liquid to 50.0 ml with *ethanol* and mix. To 25.0 ml of the resulting solution, add 25.0 ml of a solution prepared by dissolving 1 g of *dipotassium hydrogen phosphate* in 55 ml of *water*, transfer a portion of the mixture to a 1-cm closed quartz cell, place the cell 10 cm from a 15-watt short-wave, ultraviolet light of mercury lamp and irradiate for 10 minutes. Measure the absorbance of the irradiated solution at 418 nm (2.4.7).

Calculate the content of $C_{18}H_{20}O_2$ in the tablet from the absorbance obtained by repeating the operation using *stilboestrol RS* in place of the substance under examination.

Storage. Store protected from light and moisture.

Streptokinase

Streptokinase is a preparation of a protein obtained from culture filtrates of certain strains of *Streptococcus haemolyticus* group C. It has the property of combining with human plasminogen to form plasminogen activator and is purified to contain not less than 600 Units of Streptokinase activity per µg of nitrogen before addition of any stabiliser or carrier. It usually contains a buffer and may be stabilised by the addition of suitable substances such as Human Albumin.

Category. Fibrinolytic enzyme.

Dose. By intravenous infusion, 250,000 to 600,000 Units over 30 minutes followed by 100,000 Units every hour for upto 1 week.

Description. A white powder or a white, friable solid; hygroscopic.

Identification

A. Place 0.5 ml of citrated human, canine or rabbit plasma in a haemolysis tube maintained in a water-bath at 37°. Add 0.1 ml of a solution of the substance under examination containing 10,000 Units per ml in *citro-phosphate buffer pH 7.2* and 0.1 ml of a solution of *thrombin* containing 20 Units per ml in *citro-phosphate buffer pH 7.2* and shake immediately; a clot forms and lyses within 30 minutes. Repeat the procedure using citrated bovine plasma; lysis does not occur within 1 hour.

B. Dissolve 0.6 g of *agar* in 50.0 ml of *mixed barbitone buffer pH 8.6*, heating until a clear solution is obtained. Place glass plates (50 mm x 50 mm) that are free from traces of grease on a level surface. Apply to each plate 4 ml of the agar solution and allow to cool until set. Bore a hole 6 mm in diameter in the centre of the agar and an appropriate number of holes (not exceeding six) at distances of 11 mm from the central hole removing the residual agar by means of a cannula connected to a vacuum pump. Place a quantity of 80 µl of goat or rabbit antistreptokinase serum containing 10,000 Units of antistreptokinase activity per ml in the central hole and 80 µl of a solution of the substance under examination containing 125,000 Units of streptokinase activity per ml of each of the surrounding holes. Place the plates in a humidified tank for 24 hours.

Only one precipitation arc is produced which is well-defined and localised between the application point of the serum and each hole containing the solution of the substance under examination.

Tests

pH (2.4.24). 6.8 to 7.5, determined on a solution prepared in freshly boiled and cooled *water* containing 5000 Units per ml.

Streptodornase. Introduce 0.5 ml of a 0.1 per cent w/v solution of *sodium deoxyribonucleate* in *imidazole buffer pH 6.5* into each of eight centrifuge tubes. To each of the first two tubes add 0.25 ml of *imidazole buffer pH 6.5* and 0.25 ml of solution of the substance under examination in *imidazole buffer pH 6.5* containing 150,000 Units per ml (solution A) followed immediately by 3.0 ml of *0.25 M perchloric acid.* Mix the contents of each tube, centrifuge for 5 minutes at 3000 rpm and measure the absorbance of each of the supernatant liquids at about 260 nm (2.4.7), using as the blank a mixture of 1.0 ml of *imidazole buffer pH 6.5* and 3.0 ml of *0.25 M perchloric acid.* Calculate the sum of the two absorbances (A_1). To each of the remaining six tubes add, respectively, 0.25, 0.25, 0.125, 0.125, 0 and 0 ml of *imidazole buffer pH 6.5* followed by 0.25 ml of solution A and finally 0, 0, 0.125, 0.125, 0.25 and 0.25 ml respectively of a solution of the Standard Preparation containing 20 Units of streptodornase activity per ml in *imidazole buffer pH 6.5.* [The Standard Preparation is the 1st International Standard Preparation for Streptodornase, established in 1964, consisting of a freeze-dried mixture of streptodornase and streptokinase with lactose (supplied in ampoules containing 2400 Units of streptodornase activity), or another suitable preparation the activity of which has been determined in relation to the International Standard]. Mix the contents of each tube, incubate at 37° for 15 minutes and add to each tube 3.0 ml of *0.25 M perchloric acid.* Mix the contents of each tube, centrifuge and measure the absorbance of each of the supernatant liquids at about 260 nm (2.4.7), using as the blank the mixture specified above. If the sum of the absorbances of the liquids in the third and fourth tubes is A_2, that of the liquids in the fifth and six tubes is A_3, and that of the liquids in the seventh and eighth tubes is (A_4), (A_1–A_2) is less than $0.5(A_3+A_4)–A_2$.

Streptolysin. Dissolve a quantity of the substance under examination containing 500,000 Units in 0.5 ml of a mixture of 90 volumes of *saline solution* and 10 volumes of *citro-phosphate buffer pH 7.2* in a haemolysis tube. Add 0.4 ml of a 2.3 per cent w/v solution of *sodium thioglycollate* and incubate in a water-bath at 37° for 10 minutes. Add 0.1 ml of a solution of a reference preparation of human antistreptolysin O containing 5 Units per ml and incubate at 37° for 5 minutes. Add 1 ml of *rabbit erythrocyte suspension*, continue the incubation for 30 minutes and centrifuge at about 1000 rpm. The absorbance of the supernatant liquid at about 550 nm (2.4.7), is not more than 1.5 times the absorbance obtained by repeating the above procedure using 0.5 ml of the mixture of saline solution and citro-phosphate buffer pH 7.2 in place of the solution containing the substance under examination.

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa for 24 hours.

Assay. The potency of streptokinase is determined by comparing its ability to activate human plasminogen to form plasmin with that of the Standard Preparation. The plasmin generated is determined by measurement of the time taken to lyse a fibrin clot under the conditions of a suitable method of assay.

Standard Preparation

The Standard Preparation is the 2nd International Standard for Streptokinase, established in 1989, consisting of freeze-dried streptokinase (supplied in ampoules containing 700 Units of streptokinase activity), or another suitable preparation the activity of which has been determined in relation to the International reference preparation.

Method

Use *citro-phosphate buffer pH 7.2* containing 3 per cent w/v of *bovine serum albumin* for the preparation of solutions and dilutions.

Prepare a solution of the Standard Preparation to contain 1000 Units of streptokinase activity per ml and prepare a solution of the preparation under examination expected to have the same concentration; keep the solutions in ice and use within 6 hours. Prepare three 1.5-fold serial dilutions of the solution of the Standard Preparation so that the longest clot-lysis time is less than 20 minutes and prepare three similar dilutions of the solution of the preparation under examination. Keep the solutions in ice and use within 1 hour. Using 24 tubes, 8 mm in diameter, label the tubes S_1, S_2, S_3 for the dilutions of the Standard Preparation and T_1, T_2, T_3 for the dilutions of the preparation under examination, allocating four tubes to each dilution. Place the tubes in ice. Into each tube introduce 0.2 ml of the appropriate dilution, 0.2 ml of *citro-phosphate buffer pH 7.2* containing 3 per cent w/v of *bovine serum albumin* and 0.1 ml of a solution containing 20 Units of *thrombin* per ml. Place the tubes in a water-bath at 37° and allow to stand for 2 minutes to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the first tube 0.5 ml of a 1 per cent w/v solution to *human euglobulins* ensuring mixing. At 5-second intervals introduce successively into the remaining tubes 0.5 ml of a 1 per cent w/v solution of human euglobulins. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulin and the lysis of the clot.

Using the logarithms of the lysis times, calculate the result of the assay by standard statistical methods.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The fiducial limits of error are not less than 80 per cent and not more than 125 per cent of the stated potency.

Streptokinase intended for use in the manufacture of parenteral preparations complies with the following additional requirements.

Abnormal toxicity (2.2.1). Determine by Method A, using a solution containing 50,000 Units in 0.5 ml of *water for injections administered* in 15 to 20 seconds.

Bacterial endotoxins (2.2.3). Dissolve the contents of the sealed container in *water BET* to give a solution containing 10,000 Units of Streptokinase per ml. Carry out the test on the resulting solution; the maximum allowable endotoxin concentration of the solution is 23.33 Units of endotoxin per ml. Carry out the test using the maximum valid dilution of the prepared solution calculated from the declared sensitively of the lysate used in the test.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in sealed containers, protected from light. The containers should be sterile, tamper-evident and sealed so as to exclude micro-organisms. Under these conditions the contents may be expected to retain their potency for 2 years.

Labelling. The label states (1) the number of Units of streptokinase activity in the container; (2) the number of Units of streptokinase activity per mg, calculated with reference to the dried preparation; (3) the name and quantity of any added substances; (4) the storage conditions; (5) whether or not it is intended for use in the manufacture of parenteral preparations.

Streptokinase Injection

Streptokinase Injection is a sterile material consisting of Streptokinase with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. 50,000 Units; 100,000 Units; 750,000 Units; 1,500,000 Units.

Identification

A. Place 0.5 ml of citrated human, canine or rabbit plasma in a haemolysis tube maintained in a water-bath at 37°. Add 0.1 ml of a solution of the contents of the container containing 10,000 Units per ml in *citro-phosphate buffer pH 7.2* and 0.1 ml of a solution of *thrombin* containing 20 Units per ml in *citro-phosphate buffer pH 7.2* and shake immediately; a clot forms and lyses within 30 minutes. Repeat the procedure using citrated bovine plasma; lysis does not occur within 1 hour.

B. Dissolve 0.6 g of *agar* in 50.0 ml of *mixed barbitone buffer pH 8.6*, heating until a clear solution is obtained. Place glass plates (50 mm x 50 mm) that are free from traces of grease on a level surface. Apply to each plate 4 ml of the agar solution and allow to cool until set. Bore a hole 6 mm in diameter in the centre of the agar and an appropriate number of holes (not exceeding six) at distances of 11 mm from the central hole removing the residual agar by means of a cannula connected to a vacuum pump. Place a quantity of 80 µl of goat or rabbit antistreptokinase serum containing 10,000 Units of antistreptokinase activity per ml in the central hole and 80 µl of a solution of the contents of the container containing 125,000 Units of streptokinase activity per ml of each of the surrounding holes. Place the plates in a humidified tank for 24 hours.

Only one precipitation arc is produced which is well-defined and localised between the application point of the serum and each hole containing the solution of the substance under examination.

Tests

pH (2.4.24). 6.8 to 7.5, determined on a freshly constituted injection containing 5000 Units per ml.

Streptodornase. Introduce 0.5 ml of a 0.1 per cent w/v solution of *sodium deoxyribonucleate* in *imidazole buffer pH 6.5* into each of eight centrifuge tubes. To each of the first two tubes add 0.25 ml of *imidazole buffer pH 6.5* and 0.25 ml of solution of the contents of the container with the substance under examination in *imidazole buffer pH 6.5* containing 150,000 Units per ml (solution A) followed immediately by 3.0 ml of *0.25 M perchloric acid*. Mix the contents of each tube, centrifuge for 5 minutes at 3000 rpm and measure the absorbance of each of the supernatant liquids at about 260 nm (2.4.7), using as the blank a mixture of 1.0 ml of *imidazole buffer pH 6.5* and 3.0 ml of *0.25 M perchloric acid*. Calculate the sum of the two absorbances (A_1). To each of the remaining six tubes add, respectively, 0.25, 0.25, 0.125, 0.125, 0 and 0 ml of *imidazole buffer pH 6.5* followed by 0.25 ml of solution A and finally 0, 0, 0.125, 0.125, 0.25 and 0.25 ml respectively of a solution of the Standard Preparation containing 20 Units of streptodornase activity per ml in *imidazole buffer pH 6.5*.

[The Standard Preparation is the 1st International Standard Preparation for Streptodornase, established in 1964, consisting of a freeze-dried mixture of streptodornase and streptokinase with lactose (supplied in ampoules containing 2400 Units of streptodornase activity), or another suitable preparation the activity of which has been determined in relation to the International Standard]. Mix the contents of each tube, incubate at 37° for 15 minutes and add to each tube 3.0 ml of *0.25 M perchloric acid*. Mix the contents of each tube, centrifuge and measure the absorbance of each of the supernatant liquids at about 260 nm (2.4.7), using as the blank the mixture specified above. If the sum of the absorbances of the liquids in the third and fourth tubes is A_2, that of the liquids in the fifth and six tubes is A_3, and that of the liquids in the seventh and eighth tubes is (A_4), (A_1–A_2) is less than $0.5(A_3+A_4)–A_2$.

Streptolysin. Dissolve a quantity of the contents of the container containing 500,000 Units in 0.5 ml of a mixture of 90 volumes of *saline solution* and 10 volumes of *citro-phosphate buffer pH 7.2* in a haemolysis tube. Add 0.4 ml of a 2.3 per cent w/v solution of *sodium thioglycollate* and incubate in a water-bath at 37° for 10 minutes. Add 0.1 ml of a solution of a reference preparation of human antistreptolysin O containing 5 Units per ml and incubate at 37° for 5 minutes. Add 1 ml of *rabbit erythrocyte suspension*, continue the incubation for 30 minutes and centrifuge at about 1000 rpm. The absorbance of the supernatant liquid at about 550 nm (2.4.7), is not more than 1.5 times the absorbance obtained by repeating the above procedure using 0.5 ml of the mixture of saline solution and citro-phosphate buffer pH 7.2 in place of the solution containing the substance under examination.

Bacterial endotoxins (2.2.3). Dissolve the contents of the sealed container in *water BET* to give a solution containing 10,000 Units of Streptokinase per ml. Carry out the test on the resulting solution; the maximum allowable endotoxin concentration of the solution is 23.33 Units of endotoxin per ml. Carry out the test using the maximum valid dilution of the prepared solution calculated from the declared sensitively of the lysate used in the test.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine on the mixed contents of ten containers.

The potency of streptokinase is determined by comparing its ability to activate human plasminogen to form plasmin with that of the Standard Preparation. The plasmin generated is determined by measurement of the time taken to lyse a fibrin clot under the conditions of a suitable method of assay.

Standard Preparation

The Standard Preparation is the 2nd International Standard for Streptokinase, established in 1989, consisting of freeze-dried streptokinase (supplied in ampoules containing 700 Units of streptokinase activity), or another suitable preparation the activity of which has been determined in relation to the International reference preparation.

Method

Use *citro-phosphate buffer pH 7.2* containing 3 per cent w/v of *bovine serum albumin* for the preparation of solutions and dilutions.

Prepare a solution of the Standard Preparation to contain 1000 Units of streptokinase activity per ml and prepare a solution of the contents of the container expected to have the same concentration; keep the solutions in ice and use within 6 hours. Prepare three 1.5-fold serial dilutions of the solution of the Standard Preparation so that the longest clot-lysis time is less than 20 minutes and prepare three similar dilutions of the solution of the preparation under examination. Keep the solutions in ice and use within 1 hour. Using 24 tubes, 8 mm in diameter, label the tubes S_1, S_2, S_3 for the dilutions of the Standard Preparation and T_1, T_2, T_3 for the dilutions of the preparation under examination, allocating four tubes to each dilution. Place the tubes in ice. Into each tube introduce 0.2 ml of the appropriate dilution, 0.2 ml of *citro-phosphate buffer pH 7.2* containing 3 per cent w/v of *bovine serum albumin* and 0.1 ml of a solution containing 20 Units of *thrombin* per ml. Place the tubes in a water-bath at 37° and allow to stand for 2 minutes to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the first tube 0.5 ml of a 1 per cent w/v solution to *human euglobulins* ensuring mixing. At 5-second intervals introduce successively into the remaining tubes 0.5 ml of a 1 per cent w/v solution of human euglobulins. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulin and the lysis of the clot.

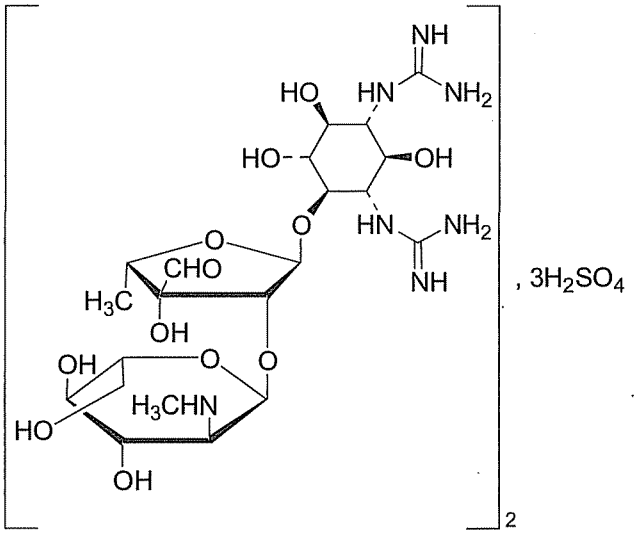
Using the logarithms of the lysis times, calculate the result of the assay by standard statistical methods.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The fiducial limits of error are not less than 80 per cent and not more than 125 per cent of the stated potency.

Storage. Store in sealed containers, protected from light in a refrigerator (2° to 8°). The containers should be sterile and sealed so as to exclude micro-organisms. Under these conditions the contents may be expected to retain their potency for 2 years.

Labelling. The label states the total number of Units of streptokinase activity contained in it.

Streptomycin Sulphate



$(C_{21}H_{39}N_7O_{12})_2,3H_2SO_4$ Mol. Wt. 1457.4

Streptomycin Sulphate is the sulphate of *O*-2-deoxy-2-methylamino-α-L-glucopyranosyl-(1→2)-*O*-5-deoxy-3-*C*-formyl-α-L-lyxofuranosyl-(1→4)-N^1,N^3-diamidino-D-streptamine, a substance produced by the growth of certain strains of *Streptomyces griseus* or obtained by any other means.

Streptomycin Sulphate has a potency equivalent to not less than 700 µg and not more than 850 µg of streptomycin per mg. It contains not less than 90.0 per cent of the stated amount of streptomycin, $C_{21}H_{39}N_7O_{12}$, calculated on the dried basis.

Category. Antitubercular.

Dose. By intramuscular injection, the equivalent of 500 mg to 1 g of streptomycin daily, or at longer intervals.

Description. A white or almost white powder; odourless or with slight odour; hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17). Prepare the plate by mixing 0.3 g of *carbomer* with 240 ml of *water*, allowing to stand with moderate stirring for 1 hour, adjusting the pH to 7.0 by the gradual addition with constant shaking of *2 M sodium hydroxide* and adding 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.1 per cent w/v of *streptomycin sulphate RS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v of *streptomycin sulphate RS*, 0.1 per cent w/v of *neomycin sulphate RS* and 0.1 per cent w/v of *kanamycin monosulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and *sulphuric acid (45 per cent)* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 5 to 10 mg in 4 ml of *water* and add 1 ml of *1 M sodium hydroxide*. Heat in a water-bath for 4 minutes. Add a slight excess of *2 M hydrochloric acid* and 0.1 ml of a 10 per cent w/v solution of *ferric chloride*; a violet colour is produced.

C. Dissolve 0.1 g in 2 ml of *water* and add 1 ml of *dilute 1-naphthol solution* and 2 ml of a mixture of equal volumes of *dilute sodium hypochlorite solution* and *water*; a red colour is produced.

D. Dissolve 10 mg in 5 ml of water and add 1 ml of *1 M hydrochloric acid*. Heat in a water-bath for 2 minutes. Add 2 ml of a 0.5 per cent w/v solution of *1-naphthol* in *1 M sodium hydroxide* and heat in a water-bath for 1 minute; a faint yellow colour is produced.

E. Gives the reactions of sulphates (2.3.1).

Tests

Appearance of solution. A 25.0 per cent w/v solution in *carbon dioxide-free water* is not more intensely coloured than degree 3 of the appropriate range of reference solutions (2.4.1). The solution, after standing protected from light at a temperature of about 20° for 24 hours, is not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 4.5 to 7.0, determined in a 25.0 per cent w/v solution.

Sulphates. 18.0 to 21.5 per cent, calculated on the dried basis, when determined by the following method. Dissolve 0.25 g in 100 ml of *water*, adjust the pH to 11 with *strong ammonia solution* and add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate the excess of barium chloride with *0.1 M disodium edetate*, adding 50 ml of *ethanol (95 per cent)* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of sulphate, SO_4.

Colorimetric test. Dissolve 0.1 g in sufficient *water* to produce 100 ml. To 5 ml, add 5 ml of *0.2 M sodium hydroxide* and heat in a water-bath for exactly 10 minutes. Cool in ice for exactly 5 minutes, add 3 ml of a 1.5 per cent w/v solution of *ferric ammonium sulphate* in *0.25 M sulphuric acid* and sufficient *water* to produce 25 ml and mix. Exactly 20 minutes after the addition of the *ferric ammonium sulphate solution*, measure the absorbance of a 2-cm layer of the solution at the maximum at about 525 nm (2.4.7), using as the blank a solution prepared in the same manner but omitting the substance under examination. The absorbance is not less than 90.0 per cent of that obtained by carrying out the procedure at the same time and in the same manner using *streptomycin sulphate RS* in place of the substance under examination, each absorbance being calculated on the dried basis.

Streptomycin B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *toluene*, 25 volumes of *glacial acetic acid* and 25 volumes of *methanol*.

Test solution. Dissolve 0.2 g of the substance under examination in 5 ml of a freshly prepared mixture of 97 volumes of *methanol* and 3 volumes of *sulphuric acid*, heat under a reflux condenser for 1 hour, cool, wash down the condenser with *methanol* and add sufficient *methanol* to produce 20 ml.

Reference solution. Dissolve 36 mg of *D-mannose* in 5 ml of a freshly prepared mixture of 97 volumes of *methanol* and 3 volumes of *sulphuric acid*, heat under a reflux condenser for 1 hour, cool, wash down the condenser with *methanol* and add sufficient *methanol* to produce 50 ml. Dilute 5 ml of the resulting solution to 50 ml with *methanol*; this solution contains the equivalent of 0.03 per cent w/v of streptomycin B (1 mg of D-mannose is equivalent to 4.13 mg of streptomycin B).

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 13 to 15 cm. Dry the plate in air, and spray with a freshly prepared mixture of equal volumes of a 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and a 20 per cent v/v solution of *sulphuric acid* and heat at 110° for 5 minutes. Any spot in the chromatogram obtained with the test solution is not more intense than the corresponding spot in the chromatogram with the reference solution.

Methanol. Determine by gas chromatography (2.4.13).

Test solution. A 4 per cent w/v solution of the substance under examination in *water*.

Reference solution. A 0.012 per cent w/v solution of *methanol* in *water*.

Chromatographic system
- a glass column 1.5 to 2.0 m × 2 to 4 mm, packed with ethylvinylbenzene-divinylbenzene copolymer (150 to 180 µm) porous polymer beads (such as Porapak Q),
- temperature: column 120° to 140°, inlet port and detector at least 50° higher than that of the column,
- flow rate. 30 to 40 ml per minute of the carrier gas.

The area of any peak corresponding to methanol in the chromatogram obtained with the test solution is not greater than that of the peak in the chromatogram obtained with reference solution (0.3 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 24 hours.

Assay. Determine by the microbiological assay of antibiotics, Method A or B (2.2.10), and express the results in µg of streptomycin per mg.

Streptomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg of streptomycin.

Streptomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the equivalent weight of streptomycin contained in it; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations; (3) the name and quantity of any added stabiliser.

Streptomycin Injection

Streptomycin Sulphate Injection

Streptomycin Injection is a sterile material consisting of Streptomycin Sulphate with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Streptomycin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of streptomycin, $C_{21}H_{39}N_7O_{12}$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. The equivalent of 750 mg and 1 g of streptomycin.

Description. A white or almost white powder which yields a clear, colourless or faintly yellow coloured solution when dissolved in water.

Identification

A. Determine by thin-layer chromatography (2.4.17). Prepare the plate by mixing 0.3 g of *carbomer* with 240 ml of *water*, allowing to stand with moderate stirring for 1 hour, adjusting the pH to 7.0 by the gradual addition with constant shaking of *2 M sodium hydroxide* and adding 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.1 per cent w/v of *streptomycin sulphate RS* in *water*.

Reference solution (b). A solution containing 0.1 per cent w/v of *streptomycin sulphate RS*, 0.1 per cent w/v of *neomycin sulphate RS* and 0.1 per cent w/v of *kanamycin monosulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in warm air, spray with a mixture of equal volumes of a 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and *sulphuric acid (45 per cent)* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 5 to 10 mg in 4 ml of *water* and add 1 ml of *1 M sodium hydroxide*. Heat in a water-bath for 4 minutes. Add a slight excess of *2 M hydrochloric acid* and 0.1 ml of a 10 per cent w/v solution of *ferric chloride*; a violet colour is produced.

C. Dissolve 0.1 g in 2 ml of *water* and add 1 ml of *dilute 1-naphthol solution* and 2 ml of a mixture of equal volumes of *dilute sodium hypochlorite solution* and *water*; a red colour is produced.

D. Dissolve 10 mg in 5 ml of water and add 1 ml of *1 M hydrochloric acid*. Heat in a water-bath for 2 minutes. Add 2 ml of a 0.5 per cent w/v solution of *1-naphthol* in *1 M sodium hydroxide* and heat in a water-bath for 1 minute; a faint yellow colour is produced.

Tests

pH (2.4.24). 4.5 to 7.0, determined in a 25.0 per cent w/v solution.

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 0.1 kPa for 24 hours.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine on the mixed contents of ten containers by the microbiological assay of antibiotics, Method A or B (2.2.10).

Bacterial endotoxins (2.2.3): Not more than 0.25 Endotoxin Unit per mg of streptomycin.

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of streptomycin.

Streptomycin Tablets

Streptomycin Sulphate Tablets

Streptomycin Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of streptomycin, $C_{21}H_{39}N_7O_{12}$.

Usual strength. The equivalent of 500 mg of streptomycin.

Identification

A. Triturate a quantity of the powdered tablets containing 0.2 g of streptomycin in a mixture of 2 ml of *methanol* and 0.1 ml of *sulphuric acid*, filter, if necessary, and allow to stand at about 25°; crystals of streptidine sulphate separate in the course of 2 to 3 days. Dissolve the crystals in a solution of 0.1 g of *picric acid* in 10 ml of hot *water* and cool; the precipitate, after recrystallisation from hot *water*, melts at about 283° (2.4.21).

B. Boil a small quantity of the powdered tablets containing 0.1 g of streptomycin with 5 ml of *1 M sodium hydroxide* for a few minutes, add a slight excess of *2 M hydrochloric acid* and 0.15 ml of a 10 per cent w/v solution of *ferric chloride*; a brilliant violet colour is produced.

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.3 g of streptomycin and, triturate with 20 ml of *buffer solution pH 8.0*. Dilute to 100.0 ml with *water*.

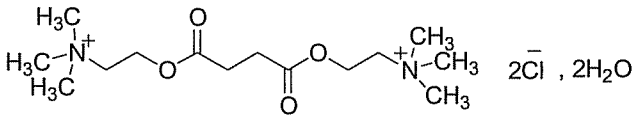
Determine by the microbiological assay of antibiotics, Method A or B (2.2.10).

Storage. Store protected from moisture.

Labelling. The label states the strength in terms of the equivalent amount of streptomycin.

Succinylcholine Chloride

Suxamethonium Chloride



$C_{14}H_{30}Cl_2N_2O_4,2H_2O$ Mol. Wt. 397.3

Succinylcholine Chloride is 2,2'-succinyldioxy-bis(ethyltrimethylammonium) dichloride dihydrate.

Succinylcholine Chloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{14}H_{30}Cl_2N_2O_4$, calculated on the anhydrous basis.

Category. Depolarising skeletal muscle relaxant.

Dose. By intravenous injection, 20 to 100 mg, according to the needs of the patient.

Description. A white or almost white, crystalline powder; almost odourless; hygroscopic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *succinylcholine chloride RS* or with the reference spectrum of succinylcholine chloride.

B. Dissolve about 25 mg in 1 ml of *water*, add 0.1 ml of a 1 per cent w/v solution of *cobalt chloride* and 0.1 ml of *potassium ferrocyanide solution*; a green colour is produced.

C. Dissolve 1 g in sufficient *carbon dioxide-free water* to produce 20 ml. To 1 ml of this solution add 9 ml of *water*, 10 ml of *1 M sulphuric acid* and 30 ml of *ammonium reineckate solution*; a pink precipitate is produced. Allow to stand for 30 minutes, filter and wash with *water*, then with *ethanol (95 per cent)* and finally with *ether*. The residue, after drying at 80° melts at 180° to 185° (2.4.21).

D. Gives the reactions of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1). 4 ml of solution A diluted to 10 ml with *water* is colourless (2.4.1).

pH (2,4,24). 4.0 to 5.0, determined in a 0.5 per cent w/v solution.

Choline chloride. Determine by thin-layer chromatography (2.4.17), coating the plate with *microcrystalline cellulose*.

Mobile phase. A mixture of 50 volumes of *1-butanol*, 40 volumes of *water* and 10 volumes of *anhydrous formic acid*; shake for 10 minutes, allow to separate. Use the upper layer as the mobile phase.

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of *methanol*.

Reference solution. A solution containing 4 per cent w/v of *succinylcholine chloride RS* and 0.02 per cent w/v of *choline chloride* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *potassium iodobismuthate solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot due to choline chloride in the chromatogram obtained with the reference solution. The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 8.0 to 10.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 0.3 g, dissolve in 30 ml of *anhydrous glacial acetic acid*, add 30 ml of *acetic anhydride* and 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator, until a bluish green colour is produced. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01807 g of $C_{14}H_{30}Cl_2N_2O_4$.

Storage. Store protected from light and moisture.

Succinylcholine Injection

Suxamethonium Chloride Injection

Succinylcholine Injection is a sterile solution of Succinylcholine Chloride in Water for Injections.

Succinylcholine Injection contains not less than 90.0 per cent and not more than 107.5 per cent of the stated amount of succinylcholine chloride, $C_{14}H_{30}Cl_2N_2O_4,2H_2O$.

Usual strength. 50 mg per ml.

Identification

Dilute a volume containing 20 mg of Succinylcholine Chloride to 50 ml with *water*. To 0.5 ml add 2 ml of *chloroform*, 2 ml of a solution containing 0.16 per cent w/v of *citric acid* and 6.6 per cent w/v of *disodium hydrogen phosphate* and 0.1 ml of a solution containing 0.15 per cent w/v of each of *bromothymol blue* and *anhydrous sodium carbonate*. Shake for 2 minutes and allow to separate. The chloroform layer is yellow.

Tests

pH (2.4.28). 3.0 to 5.0.

Hydrolysis products. The volume of *0.1 M sodium hydroxide* required for the preliminary neutralisation in the Assay is not more than one tenth of the total volume of *0.1 M sodium hydroxide* required for the preliminary neutralisation and the hydrolysis.

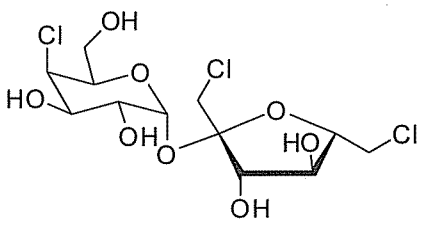
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 0.25 g of Succinylcholine Chloride add 30 ml of *carbon dioxide-free water* and shake with five quantities, each of 25 ml, of *ether*. Wash the combined ether solutions with two quantities, each of 10 ml, of *water* and discard the ether. Shake the combined washings with two quantities, each of 10 ml, of *ether*, add the washings to the original aqueous solution and neutralise with *0.1 M sodium hydroxide* using *bromothymol blue solution* as indicator. Add 25.0 ml of *0.1 M sodium hydroxide*, heat under a reflux condenser for 40 minutes, allow to cool and titrate the excess of alkali with *0.1 M hydrochloric acid* using *bromothymol blue solution* as indicator. Repeat the operation using 40 ml of *carbon dioxide-free water* beginning at the words "Add 25.0 ml of *0.1 M sodium hydroxide*.....". The difference between the titrations represents the amount of sodium hydroxide required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01987 g of $C_{14}H_{30}Cl_2N_2O_4,2H_2O$.

Storage. Store protected from light, at a temperature between 2° to 8°. The injection should not be allowed to freeze.

Sucralose



$C_{12}H_{19}Cl_3O_8$ Mol. Wt. 397.6

Sucralose is 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside.

Sucralose contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{19}Cl_3O_8$, calculated on the anhydrous basis.

Category. Pharmaceutical aid (sweetening vehicle).

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sucralose RS* or with the reference spectrum of sucralose.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +84.0° to +87.5°, determined at 20° in a 1.0 per cent w/v solution in *water*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 70 volumes of 5 per cent w/v solution of *sodium chloride* and 30 volumes of *acetonitrile*.

Test solution. Dissolve about 1.0 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). A 1.0 per cent w/v solution of *sucralose RS* in *methanol*.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray the plate with 15 per cent w/v solution of *sulphuric acid* in *methanol*. Heat the plate at 125° for 10 minutes. The Rf value of the principal spot obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solution (a). Any secondary spot in the chromatogram obtained with the test

solution is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent).

Hydrolysis products. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel.*

NOTE—This test does not require a developing solvent.

Test solution. Dissolve about 2.5 g of the substance under examination in 10 ml of *methanol.*

Reference solution (a). A 10.0 per cent w/v solution of *mannitol* in *water.*

Reference solution (b). A solution containing 0.04 per cent w/v of *fructose* and 10 per cent w/v of *mannitol* in *water.*

Apply to the plate 5 µl of each solution. Spray with a solution containing 1.23 g of *p-anisidine* and 1.66 g of *phthalic acid* in 100 ml of *methanol*, heat the plate at 105° for 15 minutes. If the spot from reference solution (a) has darkened, repeat the test, heating for a shorter period of time. Immediately after heating, view the plate against a dark background: the color of the spot obtained from the test solution is not more intense than that obtained from reference solution (b) (0.1 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.7 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 25 ml of the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *sucralose RS* in the mobile phase.

Chromatographic system
- a stainless steel column 10 cm x 8.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 17 volumes of *water* and 3 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- refractive index detector
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

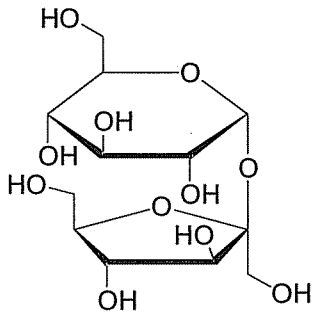
Inject the test solution and the reference solution.

Calculate the content of $C_{12}H_{19}Cl_3O_8$.

Storage. Store protected from moisture, at a temperature not exceeding 21°.

Sucrose

Refined Sugar



$C_{12}H_{22}O_{11}$ Mol. Wt. 342.3

Sucrose is β-D-fructofuranosyl α-D-glucopyranoside.

Category. Pharmaceutical aid (sweetening agent; tablet excipient).

Description. An almost white or colourless crystals, dry crystalline powder; odourless; taste, sweet.

Identification

Dissolve 150.0 g in sufficient *carbon dioxide-free water* prepared from *distilled water* to produce 300 ml (solution A). Dilute 1 ml of solution A to 100 ml with *water.* To 5 ml of the solution add 2 ml of freshly prepared *2 M sodium hydroxide* and 0.15 ml of a freshly prepared *copper sulphate solution*; the solution is clear and blue and remains so on boiling. To the hot solution add 4 ml of *2 M hydrochloric acid*, heat to boiling and add 4 ml of *2 M sodium hydroxide*; an orange precipitate is produced immediately.

Tests

Acidity or alkalinity. To 10 ml of solution A add 0.3 ml of *phenolphthalein solution.* The solution is colourless and not more than 0.6 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to pink.

Specific optical rotation (2.4.22). +65.9° to +67.0°, determined in a 10 per cent w/v solution.

Barium. To 10 ml of solution A add 1 ml of *1 M sulphuric acid.* When examined immediately and after 1 hour any opalescence is not more intense than that of a mixture of 1 ml of *distilled water* and 10 ml of solution A.

Calcium. To 1 ml of solution A add 9 ml of *water* and 1 ml of *ammonium oxalate solution*; the solution remains clear for at least 1 minute.

Heavy metals (2.3.13). Add 0.1 ml of *dilute hydrochloric acid* to 4 ml of solution A and dilute with sufficient *water* to produce

25 ml. The solution complies with the limit test for heavy metals, Method A (10 ppm).

Sulphites. To 4 ml of solution A add sufficient *water* to produce 20 ml, add 0.05 ml of *0.1 M iodine* and 0.05 ml of *starch solution*; a blue colour develops.

Dextrins. To 2 ml of solution A add 8 ml of *water*, 0.05 ml of *2 M hydrochloric acid* and 0.05 ml of *0.05 M iodine*; the solution remains yellow or becomes faint bluish green.

Glucose and invert sugar. Dissolve 20 g in sufficient *water* to make 100 ml and filter if necessary. Place 50 ml of the clear solution in a 250-ml beaker, add 50 ml of *alkaline cupric tartarate solution*, cover the beaker with a watch glass, heat the mixture at such a rate that it comes to a boil in approximately 4 minutes and continue boiling for exactly 2 minutes. Add immediately 100 ml of recently boiled and cooled *water* and collect the precipitated cuprous oxide on a tared sintered-glass crucible. Wash the residue with the hot *water*, then with 10 ml of *ethanol (95 per cent)* and finally with 10 ml of *ether*. Dry at 105° for 1 hour; the weight of the cuprous oxide is not more than 112 mg.

Colouring matter. A. To 100 ml of solution A in a ground-glass-stoppered tube add 1 ml of *dilute hypophosphorous acid* and allow to stand for 1 hour; no unpleasant odour is detectable.

B. Examine solution A in ultraviolet light at 365 nm. Any fluorescence is not more intense than that of a solution containing 0.4 µg per ml of *quinine sulphate* in *0.005 M sulphuric acid.*

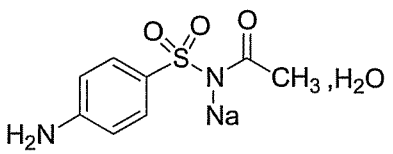
Sulphated ash (2.3.18). Not more than 0.1 per cent determined by dissolving 5.0 g in 5 ml of *water*, adding 2 ml of *sulphuric acid*, evaporating to dryness and igniting to constant weight.

Sucrose intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin unit per mg of Sucrose.

Storage. Store protected from light and moisture.

Sulphacetamide Sodium



$C_8H_9N_2NaO_3S,H_2O$ Mol. Wt. 254.2

Sulphacetamide Sodium is the monohydrate of the sodium salt of N^1-acetylsulphanilamide.

Sulphacetamide Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_9N_2NaO_3S$, calculated on the anhydrous basis.

Category. Antibacterial.

Description. A white or yellowish white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C, D, E and F are carried out. Tests B, C, D and E may be omitted if tests A and F are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphacetamide sodium RS* or with the reference spectrum of sulphacetamide sodium.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. Dissolve 1 g in 10 ml of *water*, add 6 ml of *2 M acetic acid* and filter. Wash the precipitate with a small volume of *water* and dry at 105° for 4 hours. The melting range of the precipitate is 181° to 185° (2.4.21).

D. Dissolve 0.1 g of the precipitate obtained in test C in 5 ml of *ethanol (95 per cent)*, add 0.2 ml of *sulphuric acid* and heat; ethyl acetate, recognizable by its odour, is produced.

E. Dissolve 1 mg of the precipitate obtained in test C in 5 ml of *water* with the aid of heat. The solution gives the reaction of primary aromatic amines (2.3.1), producing an orange-red precipitate.

F. A 5 per cent w/v solution gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution GYS4 (2.4.1).

pH (2.4.28). 8.0 to 9.5, determined in a 5.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Note—Prepare the solutions immediately before use and protected from light.

Test solution. Dissolve 0.2 g of the substance under examination in 10.0 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg each of *sulphacetamide sodium RS* and *sulphanilamide* (sulphacetamide sodium impurity A) in 1.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm)
- mobile phase: a mixture of 1 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 89 volumes of *water*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to sulphacetamide impurity A and sulphacetamide is not less than 5.0. The relative retention time with reference to sulphacetamide for sulphacetamide impurity A is about 0.5.

Inject the test solution and reference solution (b). Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to sulphacetamide impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphates (2.3.17). Dissolve 1.5 g in sufficient *distilled water* to produce 25 ml, add 25 ml of *2 M acetic acid*, shake for 30 minutes and filter. 25 ml of the filtrate complies with the limit test for sulphates (200 ppm).

Water (2.3.43). 6.0 to 8.0 per cent, determined on 0.2 g.

Assay. Weigh accurately about 0.25 g, dissolve in a mixture of 50 ml of *water* and 20 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02362 g of $C_8H_9N_2NaO_3S$.

Storage. Store protected from light and moisture.

Sulphacetamide Eye Drops

Sulphacetamide Sodium Eye Drops

Sulphacetamide Eye Drops are a sterile solution of Sulphacetamide Sodium in Purified Water. It may contain a suitable antimicrobial agent.

Sulphacetamide Eye Drops contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sulphacetamide sodium, $C_8H_9N_2NaO_3S,H_2O$.

Usual strengths. 10 per cent w/v; 20 per cent w/v; 30 per cent w/v.

Identification

To a volume containing 0.5 g of Sulphacetamide Sodium add 6 ml of *5 M acetic acid*, stirring constantly. Filter the precipitate, wash with *water* and dry at 105° for 4 hours. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphacetamide RS* or with the reference spectrum of sulphacetamide.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

C. Dissolve 10 mg in 2 ml of *2 M hydrochloric acid*. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. Dilute the eye drops, if necessary, to contain 10.0 per cent w/v of Sulphacetamide Sodium. The solution is not more intensely coloured than reference solution BYS4 (2.4.1).

pH (2.4.24). 6.6 to 8.6.

Related substances. Determine by liquid chromatography (2.4.14).

Note—Prepare the solutions immediately before use and protected from light.

Test solution. Dissolve a volume of solution containing about 0.2 g of Sulphacetamide Sodium in 10.0 ml of the mobile phase.

Reference solution (a). Dissolve 5 mg each of *sulphacetamide sodium RS* and *sulphanilamide* (sulphacetamide sodium impurity A) in 1.0 ml of the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm)

- mobile phase: a mixture of 1 volumes of *glacial acetic acid*, 10 volumes of *methanol* and 89 volumes of *water*,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to sulphacetamide impurity A and sulphacetamide is not less than 5.0. The relative retention time with reference to sulphacetamide for sulphacetamide impurity A is about 0.5.

Inject the test solution and reference solution (b). Run the chromatogram 7 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to sulphacetamide impurity A is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent), the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Other tests. Comply with the tests stated under Eye Drops.

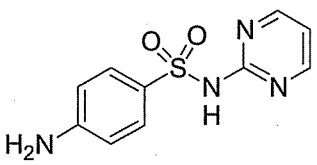
Assay. To an accurately measured volume containing about 0.5 g of Sulphacetamide Sodium add 75 ml of *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02542 g of $C_8H_9N_2NaO_3S,H_2O$.

Storage. Store protected from light and moisture. The Eye Drops should not be allowed to freeze.

Labelling. The label states (1) the name and concentration of any antimicrobial agent used; (2) that it is not meant for injection; (3) that the solution should be used within one month of opening the container; (4) that the solution should not be used if it is dark brown in colour; (5) that it should not be allowed to freeze.

Sulphadiazine



$C_{10}H_{10}N_4O_2S$ Mol. Wt. 250.3

Sulphadiazine is *N*^{1}-(pyrimidin-2-yl)sulphanilamide.

Sulphadiazine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{10}N_4O_2S$, calculated on the dried basis.

Category. Antibacterial.

Dose. Initial dose, 3 g; subsequent doses, upto 4 g daily, in divided doses.

Description. White, yellowish white or pinkish white crystals or crystalline powder; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphadiazine RS* or with the reference spectrum of sulphadiazine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

C. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* and dilute 1 ml of this solution to 10 ml with *water*. The solution, without further acidification, gives the reaction of primary aromatic amines (2.3.1).

D. Heat 3 g in a test-tube inclined at an angle of 45° with the lower part immersed in a silicone oil-bath at about 270°. It decomposes and a white or yellowish white sublimate is produced. The sublimate, after recrystallisation from *toluene* and drying at 100° melts at 123° to 127° (2.4.21).

Tests

Appearance of solution. Dissolve 0.8 g in 10 ml of *1 M sodium hydroxide*. The solution is not more intensely coloured than reference solution YS5, BYS5 or GYS5 (2.4.1).

Acidity. Heat 1.25 g of the finely powdered substance at about 70° with 25 ml of *carbon dioxide-free water* for 5 minutes. Cool for about 15 minutes in ice and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances (2.3.7). Complies with test C.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in a mixture of 20 ml of *2 M hydrochloric acid* and 50 ml of *water*. Add 3 g of

potassium bromide, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02503 g of $C_{10}H_{10}N_4O_2S$.

Storage. Store protected from light and moisture.

Sulphadiazine Tablets

Sulphadiazine Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sulphadiazine, $C_{10}H_{10}N_4O_2S$.

Usual strength. 500 mg.

Identification

A. Triturate a quantity of the powdered tablets containing 0.5 g Sulphadiazine with two successive quantities, each of 5 ml, of *chloroform* and reject the chloroform. Triturate the residue with 10 ml of *dilute ammonia solution* for 5 minutes, add 10 ml of *water* and filter. Warm the filtrate until most of the ammonia has been expelled, cool and acidify with *acetic acid*. Collect the precipitate, wash with *water* and dry at about 100°; the residue melts at about 256°, with decomposition (2.4.21).

B. On the residue obtained in test A determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphadiazine RS* or with the reference spectrum of sulphadiazine.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with the reference solution.

Tests

Related substances (2.3.7). Complies with test C, but using the following solutions.

Test solution (a). Extract a quantity of the powdered tablets containing 0.5 g of Sulphadiazine with 25 ml of a mixture of 90 volumes of *methanol* and 10 volumes of *strong ammonia solution* by shaking for 10 minutes, filter and use the filtrate.

Test solution (b). Dilute 1 volume of test solution (a) to 5 volumes with a mixture of 24 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (c). Dilute 1 volume of test solution (a) to 200 volumes with the same solvent mixture.

Reference solution. A 0.4 per cent w/v solution of *sulphadiazine RS* in the same solvent mixture.

Dissolution (2.5.2).

Apparatus. No. 1,

Medium. 900 ml of *0.1 M hydrochloric acid*,

Speed and time. 100 rpm and 60 minutes.

Withdraw a suitable volume of the sample and filter promptly through a membrane filter disc with an average pore diameter not greater than 1.0 mm. Reject the first few ml of the filtrate and dilute a suitable volume of the filtrate with the same solvent. Dilute suitably with *0.01 M sodium hydroxide*. Measure the absorbances of the resulting solution and of a standard solution of *sulphadiazine RS* of similar concentration in the same medium at the maximum at about 254 nm (2.4.7).

Calculate the content of $C_{10}H_{10}N_4O_2S$ in the medium.

D. Not less than 70.0 per cent of the stated amount of $C_{10}H_{10}N_4O_2S$.

Other tests. Comply with the tests stated under Tablets.

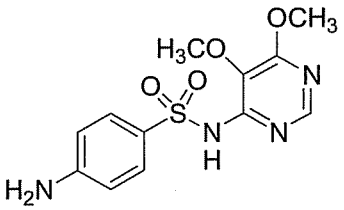
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Sulphadiazine and dissolve as completely as possible in a mixture of 50 ml of *water* and 10 ml of *hydrochloric acid*. Carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02503 g of $C_{10}H_{10}N_4O_2S$.

Storage. Store protected from light and moisture.

Sulphadoxine

Sulphormethoxine; Sulphoethomidine



$C_{12}H_{14}N_4O_4S$ Mol. Wt. 310.3

Sulphadoxine is *N*^{1}-(5,6-dimethoxypyrimidin-4-yl)sulphanilamide.

Sulphadoxine contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{14}N_4O_4S$, calculated on the dried basis.

Category. Antibacterial; antimalarial in combination with pyrimethamine.

Dose. Orally, initially, 2 g; subsequent doses, 1 to 1.5 g weekly. By deep intramuscular or slow intravenous injection, 2.5 g initially, followed by 1.5 g after four days.

Description. White or yellowish white crystals or crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphadoxine RS* or with the reference spectrum of sulphadoxine.

B. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with the reference solution (c).

C. Dissolve 0.5 g in 1 ml of *sulphuric acid (40 per cent)*, heating gently to effect solution, and continue heating until a crystalline precipitate is produced. Allow to cool, add 10 ml of *2 M sodium hydroxide*, cool again, add 25 ml of *ether* and shake for 5 minutes. Dry the upper layer over *anhydrous sodium sulphate*, filter and evaporate the solvent by heating on a water-bath. The residue melts either at 80° to 82° or at 90° to 92° (2.4.21).

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* and dilute 1 ml to 10 ml with *water*. The resulting solution, without further acidification, gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. Dissolve 0.8 g in 10 ml of *1 M sodium hydroxide*. The solution is not more intensely coloured than reference solution YS5, BYS5 or GYS5 (2.4.1).

Acidity. Heat 1.25 g of the finely powdered substance at about 70° with 25 ml of *carbon dioxide-free water* for 5 minutes. Cool for about 15 minutes in ice and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Complies with the test for related substances in sulphonamides (2.3.7), Method C, but using the following solution.

Test solution. A 2 per cent w/v solution of the substance under examination in a mixture of 24 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Heavy metals (2.3.13).1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

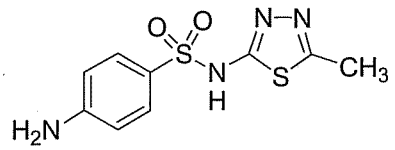
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.03103 g of $C_{12}H_{14}N_4O_4S$.

Storage. Store protected from light and moisture.

Sulphamethizole



$C_9H_{10}N_4O_2S_2$ Mol. Wt. 270.3

Sulphamethizole is N^1-(5-methyl-1,3,4-thiadiazol-2-yl)sulphanilamide.

Sulphamethizole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_9H_{10}N_4O_2S_2$, calculated on the dried basis.

Category. Antibacterial.

Dose. 100 to 200 mg every four to six hours.

Description. White or yellowish white crystals or crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphamethizole RS* or with the reference spectrum of sulphamethizole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Dissolve 50 mg in 4 ml of *methanol* and add 0.2 ml of a 4.0 per cent w/v solution of *cupric acetate*; a flocculent, yellowish green precipitate is produced which becomes dark green.

D. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* and dilute 1 ml of this solution to 10 ml with *water*. The solution, without further acidification, gives the reaction of primary aromatic amines (2.3.1).

Tests

Appearance of solution. Dissolve 0.8 g in 10 ml of *1 M sodium hydroxide*. The solution is not more intensely coloured than reference solution YS5, BYS5 or GYS5 (2.4.1).

Acidity. Heat 1.25 g of the finely powdered substance at about 70° with 25 ml of *carbon dioxide-free water* for 5 minutes. Cool for about 15 minutes in ice and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *chloroform* and 15 volumes of *methanol*.

Test solution (a). Dissolve 0.3 g of the substance under examination in 10 ml of *acetone*.

Test solution (b). Dissolve 0.3 g of the substance under examination in 100 ml of *acetone*.

Reference solution (a). Dissolve 15 mg of the substance under examination in 100 ml of *acetone*.

Reference solution (b). A 0.3 per cent w/v solution of *sulphamethizole RS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate at 105° and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

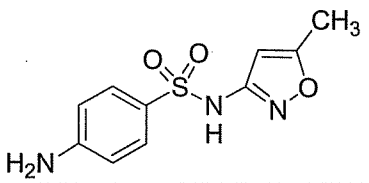
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02703 g of $C_9H_{10}N_4O_2S_2$.

Storage. Store protected from light and moisture.

Sulphamethoxazole



$C_{10}H_{11}N_3O_3S$ Mol. Wt. 253.3

Sulphamethoxazole is *N*[1]-(5-methylisoxazol-3-yl)sulphanilamide.

Sulphamethoxazole contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{11}N_3O_3S$, calculated on the dried basis.

Category. Antibacterial.

Dose. Initial dose, 2 g; subsequent doses, 1 g two or three times daily.

Description. A white or almost white, crystalline powder; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphamethoxazole RS* or with the reference spectrum of sulphamethoxazole.

B. In the test for Related substances, the principal spot in the chromatogram obtained with reference solution (a) corresponds to that in the chromatogram obtained with the reference solution (c).

C. Dissolve about 5 mg in 10 ml of *1 M hydrochloric acid* and dilute 1 ml to 10 ml with *water*. The resulting solution, without further acidification, gives the reaction of primary aromatic amines (2.3.1).

D. Melting range (2.4.21). 169° to 172°.

Tests

Appearance of solution. Dissolve 0.8 g in 10 ml of *1 M sodium hydroxide*. The solution is not more intensely coloured than reference solution YS5, BYS5 or GYS5 (2.4.1).

Acidity. Heat 1.25 g of the finely powdered substance with 25 ml of *carbon dioxide-free water* at 70° for 5 minutes. Cool for about 15 minutes in ice and filter. To 20 ml of the filtrate add 0.1 ml of *bromothymol blue solution*. Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Complies with the test for Related Substances in Sulphonamides (2.3.7), Method C, but using the following solution.

Test solution. Dissolve 0.1 g of the substance under examination in sufficient of a mixture of 1 volume of *strong ammonia solution* and 24 volumes of *methanol* to produce 5 ml.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

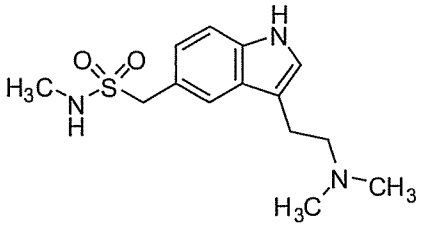
Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.2 g, dissolve in 50 ml of *2 M hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02533 g of $C_{10}H_{11}N_3O_3S$.

Storage. Store protected from light and moisture.

Sumatriptan



$C_{14}H_{21}N_3O_2S$ Mol. Wt. 295.4

Sumatriptan is 3-(2-dimethylaminoethyl)indol-5-yl-*N*-methylmethanesulphonamide.

Sumatriptan contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{14}H_{21}N_3O_2S$, calculated on the anhydrous basis.

Category. Antimigraine.

Description. A white to pale yellow powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sumatriptan RS*.

Tests

Impurities A and H. Determine by liquid chromatography (2.4.14).

Solvent mixture. 1 volume of *acetonitrile* and 3 volumes of *0.025 M sodium dihydrogen orthophosphate*, adjusted to pH 6.5.

Test solution (a). A 0.2 per cent w/v solution of the substance under examination in the solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with the solvent mixture.

Reference solution. Dissolve the contents of a vial of *sumatriptan for system suitability RS* to 1 ml with *1 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with silica gel (5 µm) (such as Spherisorb silica S5W),
- mobile phase: a mixture of 10 volumes of *10 M ammonium acetate* and 90 volumes of *methanol*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume. 20 µl.

Inject the reference solution. Run the chromatogram 5 times the retention time of the principal peak. The test is not valid unless the resolution between [3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*- methylmethanesulphonamide (sumatriptan impurity A) and sumatriptan is not less than 1.5.

Inject test solution (a) and (b). In the chromatogram obtained with test solution (a) the area of any peak corresponding to sumatriptan impurity A is not more than the area of the principal peak in the chromatogram obtained with test solution (b) (0.6 per cent, taking into account the correction factor of 0.6 for impurity A) and the area of any peak due to [3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*- methylmethanesulphonamide (sumatriptan impurity H) is not more than 0.3 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.3 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *acetonitrile* and 75 volumes of *0.025 M sodium dihydrogen orthophosphate*, adjusted to pH 6.5.

Test solution (a). A 0.2 per cent w/v solution of the substance under examination in the solvent mixture.

Test solution (b). Dilute 1 ml of test solution (a) to 100 ml with the solvent mixture and further dilute 1 ml of the resulting solution to 10 ml with the solvent mixture.

Reference solution. Dilute the contents of a vial of *sumatriptan impurity mixture RS* to 1 ml with *0.1 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 75 volumes of a solution containing 0.97 g of *dibutylamine*, 0.735 g of *sodium orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate* in 750 ml *water*, adjusted to pH 6.5 with *10 M sodium hydroxide* and diluted to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 282 nm,

– injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the resolution between the peaks due to [3-[2-(dimethylamino) ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*- methylmethanesulphonamide (sumatriptan impurity C) and sumatriptan is not less than 1.5.

Inject test solution (a) and (b). In the chromatogram obtained with test solution (a) the areas of any peaks corresponding to *N*-methyl[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulphonamide (sumatriptan impurity B), [3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide (sumatriptan impurity C) and *N*,*N*-dimethyl-2-[5-[(methylsulphamoyl)methyl]-1*H*-indol-3-yl]ethanamine *N*-oxide (sumatriptan impurity D) is not more than 5 times the area of the principal peak in the chromatogram obtained with test solution (b) (0.5 per cent); the area of any peak corresponding to [3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide (sumatriptan impurity E) is not more than the principal peak in the chromatogram obtained with test solution (b) (0.1 per cent); the area of any other secondary peak is not more than the principal peak in the chromatogram obtained with test solution (b) (0.1 per cent). The total impurity content in the test for Impurities A and H and in the test for Related substances is not more than 1.5 per cent. Ignore any peak with an area less than 0.5 times the area of the peak in the chromatogram obtained with test solution (b) (0.05 per cent).

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14) as described under Related substances, using the following solutions.

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of the solvent mixture.

Reference solution (a). A 0.014 per cent w/v solution of *sumatriptan succinate RS* in the solvent mixture.

Reference solution (b). Dilute the contents of a vial of *sumatriptan impurity mixture RS* to 1 ml with *0.1 M hydrochloric acid.*

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to sumatriptan and sumatriptan impurity C is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{21}N_3O_2S$.

Storage. Store protected from light.

Sumatriptan Injection

Sumatriptan Injection is a sterile isotonic solution of Sumatriptan Succinate in Water for Injections.

Sumatriptan Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sumatriptan succinate, $C_{14}H_{21}N_3O_2S,C_4H_6O_4$.

Usual strength. 1 mg per ml.

Description. A clear, colourless to pale yellow solution.

Identification

To a volume of the injection containing 40 mg of sumatriptan succinate add 1 ml of *saturated sodium chloride solution* and 1 ml of *saturated sodium carbonate solution.* Shake vigorously for 30 seconds, add two quantities of 2 ml of *propan-2-ol,* shake, allow to separate (this may take up to 24 hours) and discard the aqueous layer. Evaporate under a stream of nitrogen and dry at 100°. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sumatriptan succinate RS.*

Tests

pH (2.4.24). 4.2 to 5.3.

For impurities A and H. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *0.025 M sodium dihydrogen orthophosphate*, adjusted to *pH 6.5* and 25 volumes of *acetonitrile.*

Test solution. Dilute a volume of injection containing 30 mg of Sumatriptan Succinate to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with the solvent mixture.

Reference solution (b). Dilute the contents of a vial of *sumatriptan for system suitability RS* (containing impurities A ([3-[2-(dimethylamino)ethyl]-2-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethane sulphonamide) and H ([3-[2-(dimethylamino)ethyl]-1-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide) to 1 ml with *1 M hydrochloric acid.*

Chromatographic system
– a stainless steel column 25 cm × 4.6 mm, packed with silica (such as Spherisorb silica S5W),
– mobile phase: a mixture of 10 volumes of *10 M ammonium acetate* and 90 volumes of methanol,
– flow rate. 2 ml per minute,
– spectrophotometer set at 282 nm,
– injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to sumatriptan and sumatriptan impurity A is not less than 1.5.

Inject the test solution and reference solution (a). Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any peak corresponding to sumatriptan impurity A is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.5 per cent) and the area of secondary peak corresponding to sumatriptan impurity H is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 75 volumes of *0.025 M sodium dihydrogen orthophosphate*, the pH of which has been adjusted to 6.5 and 25 volumes *acetonitrile*.

Test solution (a). Dilute a volume of injection containing 30 mg of Sumatriptan Succinate in 10 ml of the solvent mixture.

Test solution (b). Dilute 1.0 ml of test solution (a) to 100.0 ml with the solvent mixture.

Reference solution (a). Dilute the contents of a vial of *sumatriptan impurity mixture RS* (contains sumatriptan impurity B (*N*-methyl[3-[2-(methylamino)ethyl]-1*H*-indol-5-yl]methanesulphonamide), C ([3-[2-(dimethylamino)ethyl]-1-(hydroxymethyl)-1*H*- indol-5-yl]-*N*-methylmethanesulphonamide), D (*N,N*-dimethyl-2-[5-[(methylsulphamoyl)methyl]-1*H*-indol-3-yl]ethanamine N- oxide), and E ([3-(2-aminoethyl)-1*H*-indol-5-yl]-*N*-methylmethanesulphonamide) to 1 ml with *0.1 M hydrochloric acid*.

Reference Solution (b). A 0.3 per cent w/v solution of *sumatriptan impurity standard RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Spherisorb ODS 1),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of a solution containing 0.97 g of *dibutylamine*, 0.735 g of *orthophosphoric acid* and 2.93 g of *sodium dihydrogen orthophosphate*, adjust the pH to 7.5 with *10 M sodium hydroxide* and diluted to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 282 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to sumatriptan impurity C and sumatriptan is not less than 1.5.

Inject test solution (a), (b) and reference solution (b). In the chromatogram obtained with test solution (a) the area of any peak corresponding to (3a-hydroxy,1,1-dimethyl-5-[(methylamino)sulfonyl]methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-1-ium trifuoroacetate) (sumatriptan impurity A) is not more than 1.5 times the area of the principal peak in the chromatogram obtained with test solution (b) (1.5 per cent), the area of any peak corresponding to 2 (1-3-[2-(dimethylamino)ethyl]-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-5-yl-*N*-methylmethanesulfonamide) (sumatriptan impurity H) is not more than the area of the principal peak in the chromatogram obtained with test solution (b) (1.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with test solution (b) (1.0 per cent) and sum of areas of the peaks due to sumatriptan impurity A and H is not more than 4 times the area of the principal peak in the chromatogram obtained with test solution (b) (4.0 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 350.0 Endotoxin Units per ml of sumatriptan succinate.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the volume of injection containing 3 mg of Sumatriptan succinate in 100 ml of the solvent mixture.

Reference solution (a). A 0.003 per cent w/v solution of *sumatriptan succinate RS* in the solvent mixture.

Reference solution (b). Dilute the contents of a vial of *sumatriptan impurity mixture RS* (contains impurities B, C, D and E) to 1 ml with *0.1 M hydrochloric acid*.

Use chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless resolution between the peaks due to sumatriptan succinate and sumatriptan succinate impurity C is not less than 1.5.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{14}H_{21}N_3O_2S,C_4H_6O_4$ in the injection.

Storage. Store protected from light.

T

Talc

Purified Talc; Talcum

Talc is a powdered, selected natural hydrated magnesium silicate. It may contain varying amounts of aluminium and iron in forms insoluble in *1 M sulphuric acid.*

Category. Pharmaceutical aid (dusting powder).

Description. A white or almost white powder, free from grittiness; readily adheres to the skin; unctuous to the touch; odourless.

Production

Talc derived from deposits that are known to contain associated asbestos is not suitable for pharmaceutical use. The manufacturer is responsible for demonstrating by the test for amphiboles and serpentines that the product is free from asbestos. The presence of amphiboles and of serpentines is revealed by infrared spectrophotometry. If detected, the specific morphological criteria of asbestos are investigated by a suitable method of optical microscopy to determine whether tremolite asbestos or chrysotile is present, as described below.

Determine by infrared absorption spectrophotometry (2.4.6).

In the range 740 cm^{-1} to 760 cm^{-1} using scale expansion, any absorption band at 758 cm^{-1} may indicate the presence of tremolite or of chlorite. If the absorption band remains after ignition of the substance under examination at 850 ± 50° for at least 30 minutes, it indicates the presence of the tremolite. In the range 600 cm^{-1} to 650 cm^{-1} using scale expansion, any absorption band or shoulder may indicate the presence of serpentines.

Identification

Test A may be omitted if tests B, C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6), shows absorption bands at 3677 cm^{-1}, 1018 cm^{-1} and 669 cm^{-1}.

B. In a platinum crucible, melt a mixture of 0.2 g of *anhydrous sodium carbonate* and 2.0 g of *potassium carbonate.* To the melted mass add 0.1 g of the substance under examination and heat until the mixture is completely melted. Allow to cool and transfer the melted mass into an evaporating dish with 50 ml of hot *water.* Add *hydrochloric acid* until effervescence ceases. Add 10 ml of *hydrochloric acid* and evaporate to dryness on a water-bath. Allow to cool. Add 20 ml of *water,* heat to boiling and filter (the residue is used for identification test C). To 5 ml of the filtrate add 1 ml of *ammonia* and 1 ml of *ammonium chloride solution* and filter. To the filtrate add 1 ml of *disodium hydrogen phosphate solution.* A white, crystalline precipitate is formed.

C. The residue obtained in identification test B gives the reaction of silicates (2.3.1).

Tests

Acidity or alkalinity. Shake 5.0 g with 25 ml of *carbon dioxide-free water* for 1 minute, filter and add to the filtrate 0.5 ml of *bromothymol blue solution*; the solution is not acidic and requires not more than 0.3 ml of *0.1 M hydrochloric acid* to make it acidic.

Iron (2.3.14). Boil 4.0 g with 25 ml of *water* for 30 minutes, replacing the water lost by evaporation, and filter. The filtrate, after the addition of 5 ml of *nitric acid,* complies with the limit test for iron (10 ppm).

Acid-soluble substances. Not more than 2.0 per cent, determined by the following method. Digest 2.0 g with 40 ml of *dilute hydrochloric acid* for 15 minutes, filter, evaporate the filtrate; to the residue add 0.1 ml of *sulphuric acid* and ignite to constant weight.

Water-soluble substances. Shake 5.0 g with 25 ml of *water* for 1 minute, filter, evaporate the filtrate and dry to constant weight; the residue weighs not more than 10 mg.

Carbonates. To 1 g add 20 ml of *dilute hydrochloric acid*; no effervescence is produced.

Chlorides (2.3.12). Suspend 2.0 g in 10 ml of *water,* add 10 ml of *2 M nitric acid,* shake for 15 minutes and filter. 10 ml of the filtrate complies with the limit test for chlorides (250 ppm).

Organic compounds. The residue obtained in the test for Loss on drying is not more than slightly yellow or grey.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 180° for 1 hour.

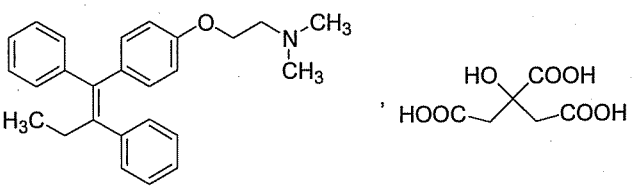
Microbial contamination (2.2.9).

If intended for topical administration, the total viable aerobic count is not more than a total of 10^2 bacteria and fungi per gram.

If intended for oral administration, the total viable aerobic count is not more than 10^3 bacteria and not more than 10^2 fungi per gram.

Storage. Store protected from moisture.

Tamoxifen Citrate



$C_{26}H_{29}NO,C_6H_8O_7$ Mol. Wt. 563.7

Tamoxifen Citrate is (*Z*)-2-[4-(1,2-diphenylbut-1-enyl)-1- phenoxy]ethyldimethylamine citrate.

Tamoxifen Citrate contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{26}H_{29}NO$, $C_6H_8O_7$, calculated on the dried basis.

Category. Antioestrogen.

Dose. The equivalent of 20 to 40 mg of tamoxifen daily as a single dose or in 2 divided doses.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests C and D may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tamoxifen citrate RS* or with the reference spectrum of tamoxifen citrate.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows absorption maxima at about 237 nm and 275 nm.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *triethylamine.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol.*

Reference solution. A 1.0 per cent w/v solution of *tamoxifen citrate RS* in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. To 10 mg add 4 ml of *pyridine* and 2 ml of *acetic anhydride* and shake; a yellow colour is produced. Heat in a water-bath for 2 minutes; a pink to red colour is produced.

Tests

E-Isomer and related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of a mixture of 60 volumes of *acetonitrile*, 25 volumes of *water* and 15 volumes of *tetrahydrofuran.*

Reference solution (a). A 0.0025 per cent w/v solution of the substance under examination in a mixture of 60 volumes of *acetonitrile*, 25 volumes of *water* and 15 volumes of *tetrahydrofuran.*

Reference solution (b). A 0.25 per cent w/v solution of *tamoxifen citrate impurity standard RS* in the same solvent mixture.

Chromatographic system
- a stainless steel column 20 cm × 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile*, 25 volumes of *water*, 15 volumes of *tetrahydrofuran* and 0.4 volume of *strong ammonia solution,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

In the chromatogram obtained with reference solution (b) a peak due to *E*-isomer appears immediately following the peak due to *Z*-tamoxifen. Adjust the sensitivity of the instrument so that the height of the peak due to *E*-isomer is about 15 per cent of the full-scale deflection on the recorder. Measure the height of the peak due to *E*-isomer by dropping a perpendicular from the top of the peak to a line drawn tangentially between the troughs on each side of the *E*-isomer peak or the trough between the E- and *Z*-isomer peaks and the baseline, whichever is appropriate.

The test is not valid unless the height of the trough separating the peaks due to *E*- and *Z*-tamoxifen in the chromatogram obtained with reference solution (b) is less than 7 per cent of full-scale deflection on the recorder and the retention time of the principal peak is less than 30 minutes. (The retention time decreases with increasing concentration of ammonia in the mobile phase).

The content of *E*-isomer in the substance under examination is not more than 1.0 per cent calculated from the declared content of *E*-isomer in *tamoxifen citrate impurity standard RS.* The area of any secondary peak in the chromatogram obtained with the test solution, other than a peak due to *E*-isomer, is not greater than half that of the peak due to tamoxifen citrate in the chromatogram obtained with reference solution (a) and the sum of the areas of all such peaks is not greater than the area of the peak due to tamoxifen in the chromatogram obtained with reference solution (a). Ignore any peak with a retention time less than 2.5 minutes and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 1.0 g, dissolve in 150 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric*

acid, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05636 g of $C_{26}H_{29}NO, C_6H_8O_7$.

Storage. Store protected from light and moisture.

Tamoxifen Tablets

Tamoxifen Citrate Tablets

Tamoxifen Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tamoxifen, $C_{26}H_{29}NO$.

Usual strengths. The equivalent of 10 mg, 20 mg and 40 mg of tamoxifen.

Identification

A. To a quantity of the powdered tablets containing 0.1 g of tamoxifen add 20 ml of *water*, warm, add 2 ml of *5 M sodium hydroxide* and cool. Extract with two quantities, each of 10 ml, of *ether*, filtering after each extraction. Combine the ether extracts and evaporate to dryness in a stream of nitrogen at room temperature. Dry the residue at a pressure not exceeding 0.7 kPa for 30 minutes.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tamoxifen citrate RS* or with the reference spectrum of tamoxifen citrate.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* of the residue obtained in test A shows absorption maxima at about 237 nm and 275 nm.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Shake a quantity of the powdered tablets containing 0.1 g tamoxifen with 10 ml of *methanol*, filter, evaporate the filtrate to dryness on a water-bath, dry the residue at 100° for 30 minutes and dissolve 10 mg of the residue in 10 ml of *methanol*.

Reference solution. A 0.1 per cent w/v solution of *tamoxifen citrate RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

D. Dissolve 10 mg of the residue obtained in the preparation of the test solution in test C in 4 ml of *pyridine*, add 2 ml of *acetic anhydride* and heat on a water-bath for 2 minutes; a pink to red colour is produced.

Tests

E-Isomer and Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Mix a quantity of the powdered tablets containing 60 mg of tamoxifen with 60 ml of a mixture of 60 volumes of *acetonitrile*, 25 volumes of *water* and 15 volumes of *tetrahydrofuran* with the aid of ultrasound for 5 minutes, dilute to 100 ml with the same solvent mixture and filter through Whatman No. 1 filter paper.

Reference solution (a). Dilute 1.0 ml of the test solution to 100 ml with the same solvent mixture.

Reference solution (b). A 0.1 per cent w/v solution of *tamoxifen citrate impurity standard RS* in the same solvent mixture.

Chromatographic system

- a stainless steel column 20 cm x 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile*, 25 volumes of *water*, 15 volumes of *tetrahydrofuran* and 0.4 volume of *strong ammonia solution*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 20 µl.

In the chromatogram obtained with reference solution (b) a peak due to *E*-isomer appears immediately following the peak due to *Z*-tamoxifen. Adjust the sensitivity of the instrument so that the height of the peak due to *E*-isomer is about 15 per cent of full-scale deflection on the recorder. Measure the height of the peak due to *E*-isomer by dropping a perpendicular from the top of the peak to a line drawn tangentially between the troughs on each side of the *E*-isomer peak or the trough between the *E*- and *Z*-isomer peaks and the baseline, whichever is appropriate.

The test is not valid unless the height of the trough separating the peaks due to *E*- and *Z*-tamoxifen in the chromatogram obtained with reference solution (b) is less than 7 per cent of the full-scale deflection on the recorder and the retention time of the principal peak is less than 30 minutes. (The retention time decreases with increasing concentration of ammonia in the mobile phase).

The content of *E*-isomer in the substance under examination is not more than 1 per cent calculated from the declared content

of *E*-isomer in *tamoxifen citrate impurity standard RS*. The area of any secondary peak in the chromatogram obtained with the test solution, other than a peak due to *E*-isomer, is not greater than half that of the peak due to tamoxifen citrate in the chromatogram obtained with reference solution (a) and the sum of the areas of all such peaks is not greater than the area of the peak due to tamoxifen in the chromatogram obtained with reference solution (a). Ignore any peak with a retention time less than 2.5 minutes and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a).

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

Crush one tablet and transfer to a 100-ml volumetric flask with the aid of 75 ml of *methanol*. Shake well for 5 minutes, add sufficient *methanol* to produce 100.0 ml and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7). Calculate the content of $C_{26}H_{29}NO$ in the tablet taking 325 as the specific absorbance at 275 nm.

Other tests. Comply with the tests stated under Tablets.

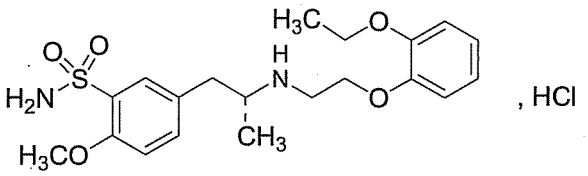
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 25 mg of tamoxifen, shake with 100 ml of *methanol* for 15 minutes, add sufficient *methanol* to produce 250.0 ml and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 275 nm (2.4.7).

Calculate the content of $C_{26}H_{29}NO$ taking 325 as the specific absorbance at 275 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of tamoxifen.

Tamsulosin Hydrochloride



$C_{20}H_{28}N_2O_5S,HCl$ Mol. Wt. 445.0

Tamsulosin Hydrochloride is (*R*)-5-(2{[2-(*o*-ethoxyphenoxy) ethyl]aminopropyl)-2-methoxybenzenesulfonamide hydrochloride.

Tamsulosin Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{20}H_{28}N_2O_5S,HCl$, calculated on the dried basis.

Description. A white to off white powder.

Dose. 400 to 800 µg daily.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tamsulosin hydrochloride RS* or with the reference spectrum of tamsulosin hydrochloride.

B. When examined in the range 200 nm to 350 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows absorption maxima at about 279 and 225 nm.

C. Dissolve with heating 0.75 g in *water* and dilute to 100 ml with *water*. To 5 ml of the solution, cooled in an ice-bath add 3 ml of *dilute nitric acid* and shake. Allow to stand at room temperature for 30 minutes and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Specific optical rotation (2.4.22). –17.0° to –21.0°, determined in a 0.75 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

A. *Test solution*. Dissolve 50.0 mg of the substance under examination in 10 ml of the mobile phase.

Reference solution(a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 4 mg of *2-methoxy-5-[(2R)-2-[(2-(2-methoxyphenoxy)ethyl]amino]propyl]benzenesulphonamide RS* (*tamsulosin impurity A RS*) and 4 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Reference solution (c). Dissolve 4 mg of *(2R)-N- [2-(2-ethoxyphenoxy)ethyl]-1-(4-methoxyphenyl)propan-2-amine RS* (*tamsulosin impurity B RS*) and 4 mg of the substance under examination in the mobile phase and dilute to 20.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 600 ml of *acetonitrile* and 1400 ml of a solution prepared by dissolving 3.0 g of *sodium hydroxide* in a mixture of 8.7 ml of *perchloric*

acid and 1900 ml of *water*, adjusting the pH to 2.0 with *0.5 M sodium hydroxide* and diluting to 2000 ml with *water*,
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 10 µl.

Inject the test solution and reference solutions (a) and (b) and record the chromatograms for 1.5 times the retention time of tamsulosin (retention time = about 6 minutes). In the chromatogram obtained with reference solution (b), the resolution between the peaks due to tamsulosin impurity A and tamsulosin is not less than 6.

In the chromatogram obtained with the test solution, for each unspecified impurity eluting before tamsulosin, the area of the peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

B. Repeat the chromatographic procedure with the following modifications.
- mobile phase: dissolve 3.0 g of *sodium hydroxide* in a mixture of 8.7 ml of *perchloric acid* and 900 ml of *water*, adjust the pH to 2.0 with *0.5 M sodium hydroxide* and dilute to 2000 ml with *water*; add 2000 ml of *acetonitrile*,
- flow rate. 1 ml per minute,

Inject the test solution and reference solutions (a) and (c). Run the chromatograms for 5 times the retention time of tamsulosin (retention time = 2.5 minutes). In the chromatogram obtained with reference solution (c), the resolution between the peaks due to tamsulosin and tamsulosin impurity B is not less than 2.

In the chromatogram obtained with the test solution, for each unspecified impurity eluting after tamsulosin, the area of any peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of the areas of the impurities eluting before tamsulosin in test A and after tamsulosin in test B is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

***S*-isomer**. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.05 g of the substance under examination in *methanol* and add sufficient *methanol* to produce 25.0 ml.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol*. Dilute 1.0 ml of this solution to 10.0 ml with *methanol*.

Reference solution (b). Dissolve 5.0 mg of *tamsulosin racemate RS* in *methanol* and dilute to 25.0 ml with *methanol*. Dilute 2.0 ml of this solution to 10.0 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with silica gel OD for chiral separations (Such as Chiralpak AD-H) (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 1 volume of *diethylamine*, 150 volumes of *methanol*, 200 volumes of *anhydrous ethanol* and 650 volumes of *hexane*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 10 µl.

Inject reference solution (b). Record the chromatogram for about 20 minutes. The resolution between tamsulosin and impurity C (5-[(2*S*)-2-[[2-(2-ethoxyphenoxy) ethyl]amino] propyl]-2-methoxybenzenesulphonamide) is not less than 2. The relative retention with reference to tamsulosin (retention time = about 14 minutes) of tamsulosin impurity C is about 0.8.

Inject the test solution and reference solution (a). In the chromatogram obtained, the area of the peak obtained due to tamsulosin impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

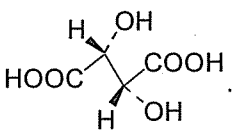
Assay. Weigh accurately about 0.35 g and dissolve in 5.0 ml of *anhydrous formic acid*, add 75 ml of a mixture of 2 volumes of *acetic anhydride* and 3 volumes of *glacial acetic acid*. Titrate immediately with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.0445 g of $C_{20}H_{28}N_2O_5S,HCl$.

Storage. Store protected from light.

Tartaric Acid

L-Tartaric Acid



$C_4H_6O_6$ Mol. Wt. 150.1

Tartaric Acid is (2*R*,3*R*)-2,3-dihydroxybutanedioic acid.

Tartaric Acid contains not less than 99.5 per cent and not more than 101.0 per cent of $C_4H_6O_6$, calculated on the dried basis.

Category. Pharmaceutical aid (buffering agent).

Description. Colourless crystals or a white or almost white crystalline powder.

Identification

A. Ignite a few mg; it gradually decomposes giving off an odour resembling that of burnt sugar (distinction from citric acid).

B. A 10 per cent w/v solution in *distilled water* (solution A) is strongly acidic.

C. Gives reactions A and B of tartrates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

Specific optical rotation (2.4.22). +12.0° to +12.8°, determined in a 20.0 per cent w/v solution.

Arsenic (2.3.10). Dissolve 5.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method A (10 ppm).

Chlorides (2.3.12). 20 ml of solution A complies with the limit test for chlorides (125 ppm).

Sulphates (2.3.17). 10 ml of solution A complies with the limit test for sulphates (150 ppm).

Oxalate. Neutralise 10 ml of solution A with *dilute ammonia solution*, add 0.1 ml of *dilute acetic acid* and 10 ml of *calcium sulphate solution*; no opalescence is produced within 20 minutes.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

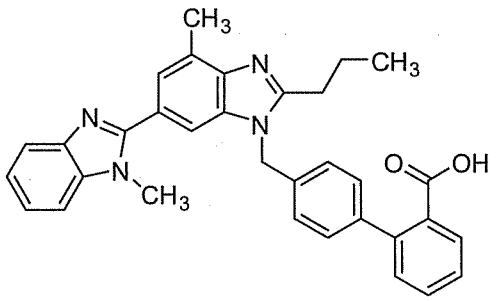
Loss on drying (2.4.19). Not more than 0.2 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 1.0 g, dissolve in 25 ml of *water* and titrate with *1 M sodium hydroxide* using 0.5 ml of *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.07505 g of $C_4H_6O_6$.

Storage. Store protected from moisture.

Telmisartan



$C_{33}H_{30}N_4O_2$ Mol. Wt. 514.6

Telmisartan is 4′ -{[4-methyl-6-(1-methyl-1*H*-benzimidazol-2-yl)-2-propyl-1*H*-benzimidazol-1-yl]methyl}-2-biphenylcarboxylic acid.

Telmisartan contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{33}H_{30}N_4O_2$, calculated on the dried basis.

Category. Antihypertensive.

Dose. 40 mg once a day.

Description. A white to off-white crystalline powder.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *telmisartan RS* or with the reference spectrum of telmisartan.

Tests

Appearance of solution. A 5 per cent w/v solution in *1 M sodium hydroxide* is not more intensely coloured than reference solution YS4 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 5 ml of *methanol*, add 100 µl of a 4 per cent w/v solution of *sodium hydroxide* and dilute to 50.0 ml with *methanol*.

Reference solution. Dilute 1.0 ml of the test solution to 10.0 ml with *methanol*. Dilute 1.0 ml of this solution to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Thermoquest),
- column temperature. 40°,
- mobile phase: A. dissolve 2.0 g of *potassium dihydrogen phosphate* and 3.8 g of *sodium pentanesulphonate monohydrate* in *water*, adjusted to pH 3.0 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

B. a mixture of 20 volumes of *methanol* and 80 volumes of *acetonitrile*,

- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 3	70	30
3 - 28	70→20	30→80
30	70	30

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 2000, tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 2 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). The sum of areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of buffer solution prepared by diluting 5.0 ml of *triethylamine* to 2000 ml with *water* and 20 volumes of *methanol.*

Test solution. Dissolve 40 mg of substance under examination in 100.0 ml of the solvent mixture. Dilute 5.0 ml of the solution to 50.0 ml with the solvent mixture.

Reference solution. A 0.004 per cent w/v solution of *telmisartan RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*; add 2 ml of *triethylamine* and adjust the pH to 2.4 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 298 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{33}H_{30}N_4O_2$.

Storage. Store protected from light and moisture.

Telmisartan Tablets

Telmisartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of telmisartan, $C_{33}H_{30}N_4O_2$.

Usual strengths. 20 mg; 40 mg; 80 mg.

Identification

In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 75 rpm and 60 minutes.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Solvent mixture. 80 volumes of buffer solution prepared by diluting 5.0 ml of *triethylamine* to 2000 ml with *water* and 20 volumes of *methanol.*

Test solution. Use the filtrate, dilute if necessary with the dissolution medium.

Reference solution. A 0.044 per cent w/v solution of *telmisartan RS* in the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{33}H_{30}N_4O_2$.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of buffer solution prepared by diluting 5.0 ml of *triethylamine* to 2000 ml with *water* and 20 volumes of *methanol.*

Test solution. Weigh and powder 20 tablets. Disperse a quantity of powder containing about 100 mg of Telmisartan in 100.0 ml of solvent mixture, sonicate for 45 minutes and filter.

Reference solution. A 0.0005 per cent w/v solution of *telmisartan RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: A. dissolve 0.5 g of *potassium dihydrogen phosphate* in 1000 ml of *water*; add 2 ml of *triethylamine,* adjusted to pH 3.2 with *orthophosphoric acid,*

  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 298 nm,
- injection volume. 20 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	78	22
6	80	20
7	70	30
15	60	40
25	60	40
26	40	60
35	20	80
40	78	22

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000, tailing factor is not more than 2.0 and relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent), the sum of the area of all the secondary peaks is not more than 4 times the area of the principal peak in the chromatogram obtained with reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of buffer solution prepared by diluting 5.0 ml of *triethylamine* to 2000 ml with *water* and 20 volumes of *methanol.*

Test solution. Shake 5 intact tablets with 100 ml of the solvent mixture and sonicate for 45 minutes. Dilute to 250 ml with the solvent mixture and filter. Further dilute to obtain 0.004 per cent w/v solution with the solvent mixture.

Reference solution. A 0.004 per cent w/v solution of *telmisartan RS* in the solvent mixture.

Chromatographic system

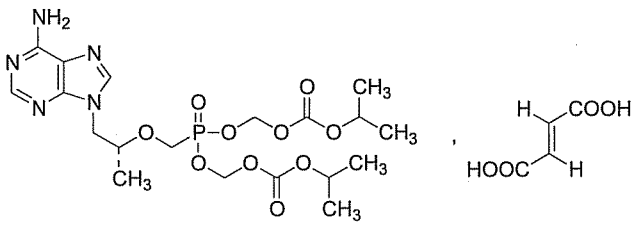
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Inertsil ODS-3),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen phosphate* in 1000 ml of *water*; add 2 ml of *triethylamine* and adjust the pH to 2.4 with *orthophosphoric acid* and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 298 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 3000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{33}H_{30}N_4O_2$ in the tablets.

Tenofovir Disoproxil Fumarate



$C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ Mol. Wt. 635.5

Tenofovir Disoproxil Fumarate salt of bis(isopropyloxy-carbonyloxymethyl ester of (*R*)-9-(2-phosphonomethoxy-propyl)adenine with fumaric acid.

Tenofovir Disoproxil Fumarate contain not less than 97.0 per cent and not more than 102. 0 per cent of $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$, calculated on the anhydrous basis.

Category. Antiviral.

Dose. 300 mg daily.

Description. A white to off- white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tenofovir disoproxil fumarate RS* or with the reference spectrum of tenofovir disoproxil fumarate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Dissolve 100 mg of the substance under examination in 50 ml of *methanol*.

Reference solution (a). A 0.2 per cent w/v solution of *tenofovir disoproxil fumarate RS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with *methanol*.

Reference solution (c). Dissolve 10 mg of the *fumaric acid* in 50 ml of *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as ODS 3V),
- column temperature. 30°,
- mobile phase: A. *0.1 M ammonium acetate solution* with the pH adjusted to 3.8 with *glacial acetic acid*, B. a mixture of 70 volumes of *methanol* and 30 volumes of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	95	5
10	50	50
25	50	50
50	20	80
60	95	5
65	95	5

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution, reference solution (b) and reference solution (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of all the secondary peaks is not more than 2.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (2.5 per cent). Ignore any peak corresponding to the peak obtained in the chromatogram in the reference solution (c) and any peak having area less than 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Fumaric Acid. 17.5 per cent to 19.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. Dissolve 25 mg of the *fumaric acid* in 50 ml of the mobile phase. Dilute 10 ml of the solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of a buffer solution prepared by adding 0.1 per cent v/v of *triethylamine* in *0.05M sodium dihydrogen phosphate* with the pH adjusted to 2.3 with *orthophosphoric acid* and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of fumaric acid.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use and carry out the test protected from light.

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.1 per cent w/v solution of *tenofovir disoproxil fumarate RS* in the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 40 volumes of *acetonitrile* and 60 volumes of a buffer solution prepared by adding 1 ml *triethylamine* to *0.05M sodium dihydrogen phosphate* with the pH adjusted to 2.3 with *orthophosphoric acid* and filtered,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

Storage. Store protected from light in a refrigerator (2° to 8°).

Tenofovir Disoproxil Fumarate Tablets

Tenofovir Disoproxil Fumarate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

Usual strength. 300 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range 200 nm to 400 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at the same wavelength as the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes

Withdraw a suitable volume of the medium and filter promptly. Dilute the filtrate, if necessary, with the same solvent. Measure the absorbance (2.4.7) of the resulting solution at the maximum at about 260 nm. Calculate the content of $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ in the medium from the absorbance obtained from a solution of known concentration of *tenofovir disoproxil fumarate RS*.

D. Not less than 80 per cent of the stated amount of $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Tenofovir Disoproxil Fumarate and disperse in 50 ml of *methanol* and filter.

Reference solution (a). A solution of *tenofovir disoproxil fumarate RS* containing 0.2 per cent of *tenofovir disoproxil* in *methanol*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with *methanol*.

Reference solution (c). Dissolve 10 mg of *fumaric acid* in 50 ml of *methanol*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dissolve 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjust the pH to 3.8 with *glacial acetic acid*,
  B. *methanol*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Time (in min.)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	95	5
10	50	50
25	50	50
50	20	80
60	95	5

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency in not less than 2000 theoretical plates.

Inject the test solution, reference solutions (b) and (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.5 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the peak in the chromatogram obtained with the reference solution (b) (6.0 per cent). Ignore the peak corresponding to the peak in the chromatogram obtained in the reference solution (c).

Other tests. Comply with the tests stated under Tablets.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Equal volumes of *water* and *methanol*.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 300 mg of Tenofovir Disoproxil Fumarate, dissolve in 100 ml of solvent mixture and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution. A 0.120 per cent w/v solution of *tenofovir disoproxil fumarate RS* in the solvent mixture. Dilute 5.0 ml of the solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 3V),
- mobile phase: a mixture of 60 volumes of a buffer solution prepared by dissolving 7.8 g of *sodium dihydrogen orthophosphate* in 1000 ml of *water*, adding 1 ml of *triethylamine* and adjusting the pH to 2.3 with *orthophosphoric acid (10 per cent v/v)*, and 40 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Tenofovir and Emtricitabine Tablets

Tenofovir Disoproxil Fumarate and Emtricitabine Tablets

Tenofovir and Emtricitabine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of tenofovir disoproxil fumarate, $C_{19}H_{30}N_5O_{10}P,C_4H_4O_4$ and emtricitabine, $C_8H_{10}FN_3O_3S$.

Usual strength. Tenofovir 300 mg and Emtricitabine 200 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1
Medium. 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14)

Test solution. The filtrate obtained as given above. Dilute the filtrate if necessary, with the dissolution medium.

Reference solution. A solution containing 0.32 per cent w/v of *tenofovir disoproxil fumarate RS* and 0.24 per cent w/v of *emtricitabine RS* in *methanol*. Dilute 5 ml of the solution to 50 ml with the dissolution medium.

Use the chromatographic system given under Assay.

Inject the test solution and the reference solution.

Calculate the contents of $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ and $C_8H_{10}FN_3O_3S$.

D. Not less than 75 per cent of the stated amounts of $C_{19}H_{30}N_5O_{10}P.C_4H_4O_4$ and $C_8H_{10}FN_3O_3S$.

Related substances. Determine by liquid chromatography (2.4.14).

For Emtricitabine

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of Emtricitabine, disperse in 5 ml of *methanol*, dilute to 50 ml with mobile phase A and filter.

Reference solution (a). A 0.1 per cent w/v solution of *emtricitabine RS* in mobile phase A.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase A.

Chromatographic system,

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- column temperature. 35°,
- mobile phase: A. a mixture of 95 volumes of a buffer solution prepared by dissolving 1.9 g of ammonium *acetate* in 1000 ml of *water*, adjusted the pH to 3.8 with *glacial acetic acid*, and 5 volumes of *methanol*,
  B. a filtered mixture of 30 volumes of the buffer solution and 70 volumes of *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 277 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
30	100	0
35	0	100
55	0	100
60	100	0
75	100	0

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 500 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent) and the sum of areas of all the secondary peaks is not more than 3 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.0 per cent).

For Tenofovir Disoproxil Fumarate

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 50 mg of Tenofovir Disoproxil Fumarate and disperse in 50 ml of *methanol.*

Reference solution (a). A 0.1 per cent w/v solution of *tenofovir disoproxil fumarate RS* in *methanol.*

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with *methanol.*

Reference solution (c). Dissolve 10 mg of *fumaric acid* in 50 ml of *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: A. a buffer solution prepared by dissolving 1.9 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 3.8 with *glacial acetic acid,*

  B. *methanol,*
- flow rate. 1.5 ml per minute,
- a linesr gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

Time (in mins)	Mobile phase A (per cent)	Mobile phase B (per cent)
0	95	5
10	50	50
25	50	50
50	20	80
60	95	5
75	95	5

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution, reference solutions (b) and (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 3.5 times the area of the peak in the chromatogram obtained with the reference solution (b) (3.5 per cent) and the sum of areas of all the secondary peaks is not more than 6 times the area of the peak in the chromatogram obtained with the reference solution (b) (6.0 per cent). Ignore the peak corresponding to the peak in the chromatogram obtained in the reference solution (c).

Other tests. Comply with the tests stated under the Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 100 mg of Tenofovir Disoproxil Fumarate, disperse in 10 ml of *water* and dilute to 250.0 ml with *methanol.* Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Reference solution. A solution containing 0.025 per cent w/v of *emtricitabine RS* and 0.04 per cent w/v of *tenofovir disoproxil fumarate RS* in *methanol.* Dilute 5.0 ml of the solution to 25.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 4.6 mm ξ 5 cm packed with octylsilane bonded to porous silica (3 µm),
- column temperature 40°,
- mobile phase: A. a buffer solution prepared by dissolving 1.35 g of *monobasic potassium phosphate* in 1000 ml of *water* and adjusting the pH to 3.0 with *orthophosphoric acid,*

  B. a mixture of 20 volumes of the buffer solution and 80 volumes of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Time (in mins)	Mobile phase A (per cent)	Mobile phase B (per cent)
0	94	6
3	94	6
5	50	50
8	35	65
9	94	6
12	94	6

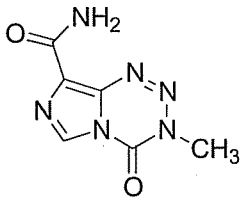
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 2000 theoretical plates for the peak due to tenofovir disoproxil, 500 theoretical plates for the peak due to emtricitabine and the relative standard deviation for replicate injections is not more than 2.0 per cent for each component.

Inject the test solution and the reference solution.

Calculate the contents of $C_{19}H_{30}N_5O_{10}P$,$C_4H_4O_4$ and $C_8H_{10}FN_3O_3S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Temozolomide



$C_6H_6N_6O_2$ Mol. Wt. 194.2

Temozolomide is 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-*d*]-1,2,3,5-tetrazine-8-carboxamide.

Temozolomide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_6N_6O_2$, calculated on the dried basis.

Category. Anticancer.

Dose. 75 mg daily.

Description. A white to almost white powder.

CAUTION — Temozolomide is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *temozolomide RS* or with the reference spectrum of temozolomide.

B. In the Assay, the principal peak in the chromatogram obtained with test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Hypersil BDS),
- mobile phase: a mixture of 90 volumes of 0.5 per cent w/v solution of *acetic acid* and 10 volumes of *acetronitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 329 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoritical plates is not less than 2000 and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 1.0 gm complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 gm by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *temozolomide RS* in the mobile phase.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_6H_6N_6O_2$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Temozolomide Capsules

Temozolomide Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of temozolomide, $C_6H_6N_6O_2$.

CAUTION —Temozolomide is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.

Usual strengths. 20 mg; 100 mg; 250 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution.

Other tests. Complies with tests stated under capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of mixed contents of 20 capsules containing 20 mg of Temozolomide in 100.0 ml of mobile phase.

Reference solution. A 0.02 per cent w/v solution of *temozolomide RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (such as Hypersil BDS),
- mobile phase: a mixture of 90 volumes of 0.5 per cent v/v *acetic acid* and 10 ml volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 329 nm,
- injection volume. 20 µl.

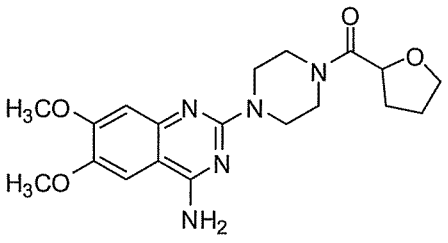
Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency is not less than 2000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_6H_6N_6O_2$ in the capsules.

Storage. Store protected from light and moisture.

Terazosin Hydrochloride



, HCl , $2H_2O$

$C_{19}H_{25}N_5O_4,HCl,2H_2O$ Mol. Wt. 459.9

Terazosin Hydrochloride is (*RS*)-6,7-dimethoxy-2-[4-(tetrahydrofuran-2-carbonyl)piperazin-1-yl}quinazolin-4-ylamine hydrochloride dihydrate.

Terazosin Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{19}H_{25}N_5O_4,HCl$, calculated on the anhydrous basis.

Category. Antihypertensive.

Description. A white or slightly yellow, crystalline powder.

Dose. 1 mg daily.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *terazosin hydrochloride RS* or with the reference spectrum of terazosin hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution in *carbon-dioxide free water* is clear (2.4.1) and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 3.0 to 5.0, determined in 2.0 per cent w/v solution in *carbon-dioxide free water.*

Impurities N and O. Determine by liquid chromatography (2.4.14).

Solvent mixture. 20 volumes of *acetonitrile* and 80 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in 50 ml of the solvent mixture.

Reference solution (a). Dissolve 5 mg each of *2-chloro-6,7-dimethoxyquinazolin-4-amine RS (terazosin impurity A RS)* and *1-[[(2RS)-tetrahydrofuran-2-yl]carbonyl]piperazine RS (terazosin impurity N RS)* in *acetonitrile*, add 5 ml of the test solution and dilute to 50 ml with *acetonitrile.* Dilute 10 ml of this solution to 100 ml with the solvent mixture.

Reference solution (b). Dilute 10 ml of reference solution (a) to 100 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 2.8 g of *sodium laurylsulphate* in 1000 ml of *water* and add 11 ml of a solution containing 20.24 per cent w/v of *triethylamine* and 23.0 per cent w/v of *orthophosphoric acid* and adjust the pH to 2.5 with *orthophosphoric acid*; mix 60 volumes of this solution with 40 volumes of *acetonitrile,*
- flow rate. 1.0 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject reference solution (b). The relative retention time with reference to terazosin for 1,4-bis[(tetrahydrofuran-2-yl)carbonyl]piperazine (terazosin impurity O) is about 0.2, for

terazosin impurity N is about 0.3 and for terazosin impurity A is about 0.4. The test is not valid unless the resolution between the peaks due to terazosin impurity A and N is not less than 1.5.

Inject the test solution and reference solution (b). Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of each peak corresponding to terazosin impurity N and terazosin impurity O is not more than the area of the peak due to terazosin in the chromatogram obtained with reference solution (b) (0.1 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 100 ml with the mobile phase. Dilute 5.0 ml of this solution to 100 ml with the mobile phase.

Reference solution (b). Dissolve the contents of a vial of *terazosin for system suitability RS* (containing terazosin impurity A, terazosin impurity B (1-(4-hydroxy-6,7-dimethoxyquinazolin-2-yl)-4-[[(2*RS*)- tetrahydrofuran-2-yl]carbonyl]piperazine), terazosin impurity C (6,7-dimethoxy-2-(piperazin-1-yl)quinazolin-4-amine), terazosin impurity J (1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-4-[(2*RS*)-2-hydroxypentanoyl]piperazine), terazosin impurity K (prazosin) and terazosin impurity M (1,4-bis(furan-2-ylcarbonyl) piperazine) in 10 ml of the mobile phase.

Reference solution (c). Dissolve 5 mg of *1-(furan-2-ylcarbonyl)piperazine RS (terazosin impurity L RS)* in 100 ml of the mobile phase. Dilute 1.0 ml of this solution to 100 ml with the mobile phase.

Reference solution (d). To 5 mg of *2,2-(piperazine-1,4-diyl)bis(6,7-dimethoxyquinazolin-4-amine) RS (terazosin impurity E RS*) add 70 ml of *methanol* and 30 ml of *water.* Allow to stand for at least 1 hour to dissolve the substance.

Chrmatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: mix 2 volumes of *triethylamine*, 350 volumes of *acetonitrile*, and 1650 volumes of a solution containing 0.6 per cent w/v of *sodium citrate* and 1.425 per cent w/v of *anhydrous citric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to terazosin impurity B and terazosin impurity J is not less than 1.5.

Inject the test solution, reference solution (a) and (c). Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of secondary peak corresponding to terazosin impurity A, C, E and K is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). The area of secondary peak corresponding to terazosin impurity L is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.1 per cent). The area of secondary peak corresponding to terazosin impurity B, J, M is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). Multiply the peak areas of the impuirites by the correction factor for calculating the contents, for impuirty C is 0.7 and for impuirty M is 1.6. The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent); The sum of the areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

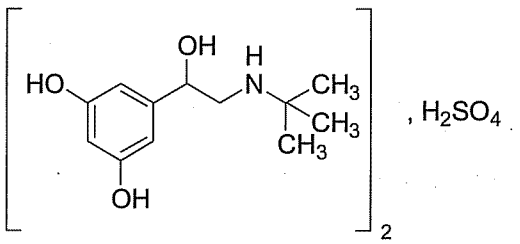
Water (2.3.43). 7.8 to 8.6 per cent, determined on 0.2 g.

Assay. Weigh accurately about 0.3 g , dissolve in a mixture of 5 ml of *0.01 M hydrochloric acid* and 50 ml of *methanol.* Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.04239 g of $C_{19}H_{26}ClN_5O_4$.

Storage. Store protected from light.

Terbutaline Sulphate



$(C_{12}H_{19}NO_3)_2,H_2SO_4$ Mol. Wt. 548.7

Terbutaline Sulphate is (*RS*)-2-(*tert*-butylamino)-1-(3,5-dihydroxyphenyl)ethanol sulphate.

Terbutaline Sulphate contains not less than 98.0 per cent and not more than 101.0 per cent of $(C_{12}H_{19}NO_3)_2, H_2SO_4$, calculated on the dried basis.

Category. Beta-adrenoceptor agonist.

Dose. Orally, upto 15 mg daily, in divided doses; by inhalation, for an adult, one to two inhalations, each of 250 µg, 3 or 4 times a day up to a maximum of 24 inhalations in 24 hours; for a child, one to two inhalations, each of 250 µg, 2 or 3 times a day up to a maximum of 10 inhalations in 24 hours; by subcutaneous injection, 250 to 500 µg, 4 times a day.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *terbutaline sulphate RS* or with the reference spectrum of terbutaline sulphate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.007 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 276 nm and 280 nm, which may be fused; absorbance at both 276 nm and 280 nm, 0.46 to 0.49.

C. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

D. A 2.0 per cent w/v solution in *carbon dioxide-free water* gives reaction A of sulphates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1); absorbance of a 2-cm layer at 400 nm, not more than 0.11 (2.4.7).

Acidity. Dissolve 0.2 g in 10 ml of *carbon dioxide-free water* and titrate with *0.01 M sodium hydroxide*, using *methyl red solution* as indicator. Not more than 1.2 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to yellow.

tert-Butylamino-3,5-dihydroxyacetophenone. Absorbance of a 2 per cent w/v solution in *0.01 M hydrochloric acid* at about 330 nm, not more than 0.50 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 75 mg of the substance under examination 50.0 ml of the mobile phase.

Reference solution (a). Dissolve 7.5 mg of *1-(3,5-dihydroxyphenyl)-2-[(1,1-dimethylethyl)amino]ethanone RS (terbutaline impurity C RS)* and 22.5 mg of *terbutaline sulphate RS* in 50.0 ml of the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with the mobile phase. Dilute 2.0 ml of this solution to 20.0 ml with the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase. dissolve 4.23 g of *sodium hexanesulphonate* in 770 ml of *0.05 M ammonium formate solution* prepared by dissolving 3.15 g of *ammonium formate* in about 980 ml of *water,* adjusted to pH 3.0 by *anhydrous formic acid* and dilute to 1000 ml with *water* and add 230 ml of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 276 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to terbutaline impurity C and terbutaline is not less than 2.0.

Inject the test solution, reference solution (a) and (b). Run the chromatogram 6 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to terbutaline impurity C is not more than twice area of the corresponding peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of each peak due to 3,5-dihydroxybenzoic acid (á-resorcylic acid) (terbutaline impurity A), (4*RS*)-2-(1,1-dimethylethyl)-1,2,3,4-tetrahydroisoquinoline-4,6,8-triol (terbutaline impurity B) and 2-[benzyl-(1,1-dimethylethyl)amino]-1-(3,5-dihydroxyphenyl) ethanone (terbutaline impurity D) is not more than area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent). The sum of all the secondary peaks other than terbutaline impurity C is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.02 per cent).

Heavy metals (2.3.13). Mix 1.6 g with 0.6 g of *anhydrous sodium sulphate* and ignite without melting the sodium sulphate. Cool, add 3 ml of *2 M hydrochloric acid*, boil and dilute to 50 ml with *water*. Cool and filter, 25 ml of the filtrate complies with the limit test for heavy metals, Method A (25 ppm).

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.5 g, dissolve in 60 ml of *anhydrous glacial acetic acid* with the aid of heat. Titrate

with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.05486 g of $(C_{12}H_{19}NO_3)_2, H_2SO_4$.

Storage. Store protected from light and moisture.

Terbutaline Inhalation

Terbutaline Sulphate Inhalation; Terbutaline Inhalation Aerosol; Terbutaline Sulphate Inhalation Aerosol

Terbutaline Inhalation is a suspension of Terbutaline Sulphate, as a superfine powder, in a suitable liquid in a suitable pressurised container. It may contain suitable pharmaceutical aids such as surfactants, stabilising agents, etc.

Terbutaline Inhalation delivers not less than 75.0 per cent and not more than 125.0 per cent of the stated amount of terbutaline sulphate $(C_{12}H_{19}NO_3)_2,H_2SO_4$, per inhalation, by actuation of the valve.

Usual strength. 250 µg in each metered dose.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 65 volumes of *2-propanol*, 25 volumes of *cyclohexane* and 5 volumes of *formic acid*.

Test solution. Remove the actuator from the pressurised container, shake the container for about 30 seconds and place it in an inverted position in a small beaker containing 5 ml of *water*. Discharge a sufficient number of deliveries containing 5 mg of Terbutaline Sulphate, under the surface of the solvent.

Reference solution. A 0.1 per cent w/v solution of *terbutaline sulphate RS* in *water*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 2 per cent w/v solution of *4-aminoantipyrine* in *methanol*. Examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the chromatogram obtained with reference solution.

Tests

Other tests. Complies with the tests stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Follow the procedure described under Assay wherever the amount of active substance is to be determined in any test.

Assay. Carry out the test for Content of active ingredient delivered per actuation stated under Inhalation Preparations (Pressurised metered-dose Preparations).

Use 35 ml of *water* and finally dilute to 50.0 ml with *water*. Dilute a suitable volume of this solution with *water* to produce a solution containing 50 µg of Terbutaline Sulphate per ml. To 10.0 ml of this solution in a 50-ml volumetric flask add 35 ml of *buffer solution pH 9.5* and 1.0 ml of *4-aminoantipyrine solution*. Mix, add 1.0 ml of *potassium ferricyanide solution* with vigorous swirling of the flask, dilute to volume with *water* and mix. Exactly 75 seconds after the addition of the *potassium ferricyanide solution* measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using as the blank a solution prepared in the same manner using 10.0 ml of *water* in place of the solution of the substance under examination.

Calculate the content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ in the solution from the absorbance obtained by repeating the operation using a solution containing 100 µg of *terbutaline sulphate RS* in place of the solution of the substance under examination.

Calculate the amount of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ delivered per actuation of the valve.

Determine the content of active ingredient a second and third time by repeating the procedure on the middle ten and on the last ten successive combined actuations of the valve. For each of the three determinations the average content of $(C_{12}H_{19}NO_3)_2,H_2SO_4$ delivered per actuation of the valve meets the requirements.

Storage. Store protected from moisture at a temperature not exceeding 30°.

Labelling. The label states the amount of active ingredient delivered per inhalation.

Terbutaline Injection

Terbutaline Sulphate Injection

Terbutaline Injection is a sterile solution of Terbutaline Sulphate in Water for Injections.

Terbutaline Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of terbutaline sulphate $(C_{12}H_{19}NO_3)_2, H_2SO_4$.

Usual strength. 500 µg per ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *2-propanol*, 25 volumes of *cyclohexane* and 5 volumes of *formic acid*.

Test solution. Use the injection.

Reference solution. A 0.1 per cent w/v solution of *terbutaline sulphate RS* in *saline solution.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with a 2 per cent w/v solution of *4-aminoantipyrine* in *methanol.* Dry the plate in air and spray with a freshly prepared 8.0 per cent w/v solution of *potassium ferricyanide* in a mixture of 4 volumes of *strong ammonia solution* and 1 volume of *water.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.0 to 5.0.

Other tests. Complies with the tests stated under Parenteral preparations (Injections).

Assay. Measure accurately a volume containing about 5 mg of Terbutaline Sulphate and add sufficient *water* to produce 50.0 ml. To 5.0 ml add 35 ml of a buffer solution prepared by dissolving 36.3 g of *tris (hydroxymethyl) aminomethane* in 900 ml of *water*, adjusting the pH to between 9.4 and 9.6 and adding sufficient *water* to produce 1000 ml. Add 1.0 ml of a freshly prepared 2.0 per cent w/v solution of *4-aminoantipyrine*, mix and add 1.0 ml of a freshly prepared 8.0 per cent w/v solution of *potassium ferricyanide* with vigorous swirling and sufficient of the buffer solution to produce 50.0 ml. Exactly 75 seconds after the addition of the *potassium ferricyanide solution* measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using *water* as the blank.

Calculate the content of $(C_{12}H_{19}NO_3)_2$, H_2SO_4 from the absorbance obtained by repeating the operation using a 0.01 per cent w/v solution of *terbutaline sulphate RS* and beginning at the words "To 5.0 ml add 35 ml.....".

Storage. Store protected from light, in single dose containers.

Labelling. The label states that the injection should not be used if the solution is discoloured.

Terbutaline Tablets

Terbutaline Sulphate Tablets

Terbutaline Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of terbutaline sulphate $(C_{12}H_{19}NO_3)_2$, H_2SO_4.

Usual strengths. 2.5 mg; 5 mg.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Terbutaline Sulphate with 50 ml of *0.1 M sodium hydroxide* for 10 minutes, dilute to 100 ml with *0.1 M sodium hydroxide* and filter. Dilute 20 ml of the filtrate to 50 ml with *0.1 M sodium hydroxide.*

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 296 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 65 volumes of *2-propanol*, 25 volumes of *cyclohexane* and 5 volumes of *formic acid.*

Test solution. Shake a quantity of the powdered tablets containing 10 mg of Terbutaline Sulphate with 4 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *water* for 10 minutes, centrifuge and use the clear supernatant liquid.

Reference solution (a). A 0.25 per cent w/v solution of *terbutaline sulphate RS* in *water.*

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. After development, dry the plate in air, allow to stand for a few minutes in an atmosphere saturated with *diethylamine* and spray with *diazotised nitroaniline solution.* The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and the principal spot in the chromatogram obtained with reference solution (b) appears as a single compact spot.

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 550 nm (2.4.7). Calculate the content of $(C_{12}H_{19}NO_3)_2.H_2SO_4$ in the medium from the absorbance obtained from a solution of known concentration of *terbutaline RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $(C_{12}H_{19}NO_3)_2.H_2SO_4$.

Uniformity of content. Comply with the test stated under Tablets.

Powder one tablet, transfer to a 25-ml volumetric flask, add 15 ml of *0.01 M hydrochloric acid* and shake for 10 minutes. Dilute to volume with *0.01 M hydrochloric acid* and filter, rejecting the first 5 ml of the filtrate. Dilute, if necessary, a suitable volume of the filtrate with *0.01 M hydrochloric acid* to produce a solution containing 0.01 per cent w/v solution of

Terbutaline Sulphate. Carry out the method as described under Assay beginning at the words "To 5.0 ml add 35 ml of a buffer solution....". Calculate the content of $(C_{12}H_{19}NO_3)_2$, H_2SO_4 in the tablet from the absorbance obtained by carrying out the Assay simultaneously using *terbutaline sulphate RS*.

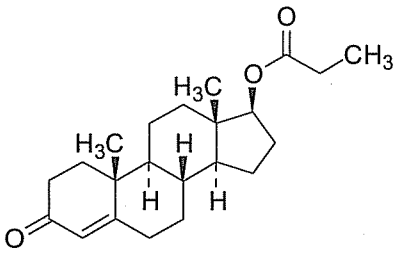
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of Terbutaline Sulphate, transfer to a 50-ml volumetric flask, add 30 ml of *0.01 M hydrochloric acid* and shake for 10 minutes. Dilute to volume with *0.01 M hydrochloric acid* and filter, rejecting the first 5 ml of the filtrate. To 5.0 ml add 35 ml of a buffer solution prepared by dissolving 36.3 g of *tris (hydroxymethyl) aminomethane* in 900 ml of *water*, adjusting the pH to between 9.4 and 9.6 and adding sufficient *water* to produce 1000 ml. Add 1.0 ml of a freshly prepared 2.0 per cent w/v solution of *4-aminoantipyrine*, mix and add 1.0 ml of a freshly prepared 8.0 per cent w/v solution of *potassium ferricyanide* with vigorous swirling and sufficient of the buffer solution to produce 50.0 ml. Exactly 75 seconds after the addition of the *potassium ferricyanide solution* measure the absorbance of the resulting solution at the maximum at about 550 nm (2.4.7), using *water* as the blank.

Calculate the content of $(C_{12}H_{19}NO_3)_2$, H_2SO_4 from the absorbance obtained by carrying out the Assay simultaneously using *terbutaline sulphate RS*.

Storage. Store protected from light and moisture.

Testosterone Propionate



$C_{22}H_{32}O_3$ Mol. Wt. 344.5

Testosterone Propionate is 3-oxoandrost-4-en-17β-yl propionate.

Testosterone Propionate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{22}H_{32}O_3$, calculated on the dried basis.

Category. Androgen; anabolic steroid.

Dose. By intramuscular injection, 5 to 25 mg, once or twice weekly.

Description. A white or almost white powder or colourless crystals; odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *testosterone propionate RS* or with the reference spectrum of testosterone propionate.

B. In the test for Related substances the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to testosterone propionate in the chromatogram obtained with reference solution (a).

C. Melting range. 119° to 123° (2.4.21).

Tests

Specific optical rotation (2.4.22). +83.0° to +90.0°, determined in a 1.0 per cent w/v solution in *ethanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in *methanol* and dilute to 50 ml with the same solvent.

Reference solution (a). Dissolve 2 mg of the substance under examination and 2 mg of *testosterone acetate RS* in *methanol* and dilute to 50 ml with the same solvent.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *water* and 80 volumes of *methanol*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solutions. Continue the chromatography for twice the retention time for testosterone propionate.

The relative retention time of about four impurities with reference to testosterone propionate (retention time, about 9 minutes) range from 0.5 to about 1.4.

The test is not valid unless in the chromatogram obtained with reference solution (a) the resolution between the peaks due to testosterone and testosterone acetate is not less than 4.0.

In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent); the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 25 mg, dissolve in sufficient *ethanol* to produce 250.0 ml, mix, dilute 5.0 ml to 50.0 ml with the same solvent and measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7).

Calculate the content of $C_{22}H_{32}O_3$ taking 490 as the specific absorbance at 241 nm.

Storage. Store protected from light and moisture.

Testosterone Propionate Injection

Testosterone Propionate Injection is a sterile solution of Testosterone Propionate in Ethyl Oleate or any other suitable ester, in a suitable fixed oil or in any mixture of these.

Testosterone Propionate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of testosterone propionate, $C_{22}H_{32}O_3$.

Usual strength. 25 mg per ml; 50 mg per ml.

Identification

Dissolve a volume containing 50 mg of Testosterone Propionate in 8 ml of *light petroleum (40° to 60°)* and extract with three quantities, each of 8 ml, of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water*. Wash the combined extracts with 10 ml of *light petroleum (40° to 60°)*, dilute with *water* until the solution becomes turbid, allow to stand for 2 hours in ice and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *testosterone propionate RS* or with the reference spectrum of testosterone propionate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *light petroleum (40° to 60°)* and 10 volumes of *liquid paraffin*.

Mobile phase. A mixture of 30 volumes of *water* and 20 volumes of *glacial acetic acid*.

Test solution. Dissolve 25 mg of the residue in 10 ml of the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *testosterone propionate RS* in the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot A.

Tests

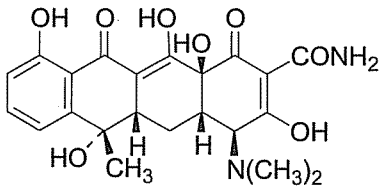
Other tests. Complies with the tests stated under Parenteral preparations (Injections).

Assay. To an accurately measured volume containing about 0.1 g of Testosterone Propionate add sufficient *chloroform* to produce 100.0 ml and mix. Dilute 3.0 ml to 50.0 ml with *chloroform* and to 5.0 ml of the solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank a solution prepared by treating 5 ml of *chloroform* in the same manner.

Calculate the content of $C_{22}H_{32}O_3$ from the absorbance obtained by repeating the operation using a 0.006 per cent w/v solution of *testosterone propionate RS* in *chloroform* and beginning at the words "to 5.0 ml of the solution.....".

Storage. Store protected from light.

Tetracycline



$C_{22}H_{24}N_2O_8$ Mol. Wt. 444.5

Tetracycline is (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide. It contains a variable quantity of water.

Tetracycline contains not less than 88.0 per cent of $C_{22}H_{24}N_2O_8$, calculated on the dried basis.

Category. Antibacterial.

Dose. Orally, 250 mg every 6 hours, increased in severe infections to 500 mg every 6 to 8 hours.

Description. A yellow, crystalline powder.

Identification

A. In the test for Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to tetracycline hydrochloride in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid*; a reddish violet colour develops. Add the solution to 2.5 ml of *water*; the colour changes to yellow.

C. Dissolve about 10 mg in a mixture of 1 ml of *2 M nitric acid* and 5 ml of *water*, shake well and add 1 ml of *silver nitrate solution*. Any opalescence produced is not more intense than that in a solution prepared in the same manner omitting the substance under examination.

Tests

pH (2.4.24). 3.5 to 6.0, determined in a 1.0 per cent w/v suspension in *carbon dioxide-free water*.

Specific optical rotation (2.4.22).–260° to –280°, determined at 20° in a 1.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14)

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of a mixture of 68 ml of *0.1 M ammonium oxalate* and 27 ml of *dimethylformamide* (diluting solvent).

Reference solution (a). A 0.0025 per cent w/v solution of *4-epitetracycline hydrochloride RS* in the diluting solvent.

Reference solution (b). A solution containing 0.0025 per cent w/v of *4-epitetracycline hydrochloride RS* and 0.01 per cent w/v of *tetracycline hydrochloride RS* in the diluting solvent.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: a mixture of 680 ml of *0.1 M ammonium oxalate*, 270 ml of *dimethylformamide* and 50 ml of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 1.5. In the chromatogram obtained with the test solution, the area of any peak corresponding to 4-epitetracycline is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) and the total area of all the peaks other than the principal peak is not greater than 5.0 per cent.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (50 ppm). Use 2.5 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 13.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 50 mg of the substance under examination and dissolve in 100.0 ml of a mixture of 68 ml of *0.1 M ammonium oxalate* and 27 ml of *dimethylformamide* (diluting solvent).

Reference solution (a). A 0.05 per cent w/v solution of *tetracycline hydrochloride RS* in the diluting solvent.

Reference solution (b). A solution containing 0.0025 per cent w/v of *4-epitetracycline hydrochloride RS* and 0.01 per cent w/v of *tetracycline hydrochloride RS* in the diluting solvent.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: a mixture of 680 ml of *0.1 M ammonium oxalate*, 270 ml of *dimethylformamide* and 50 ml of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

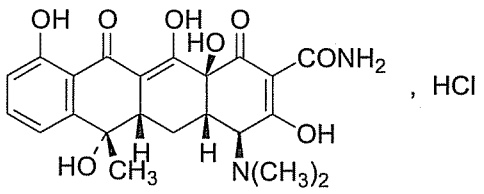
The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 1.5.

Calculate the content of $C_{22}H_{24}N_2O_8$.

1 mg of $C_{22}H_{24}N_2O_8$, HCl is equivalent to 0.92 mg of $C_{22}H_{24}N_2O_8$.

Storage. Store protected from light and moisture.

Tetracycline Hydrochloride



$C_{22}H_{24}N_2O_8$,HCl Mol. Wt. 480.9

Tetracycline Hydrochloride is (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4- dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-naphthacene-2- carboxamide hydrochloride.

Tetracycline Hydrochloride contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{22}H_{24}N_2O_8$, HCl, calculated on the dried basis.

Category. Antibacterial.

Dose. Orally, 250 mg every 6 hours, increased in severe infections to 500 mg every 6 to 8 hours; by intramuscular injection, 100 mg every 8 to 12 hours, increased in severe infections to every 4 to 6 hours; by intravenous infusion, 500 mg every 12 hours; maximum 2 g daily.

Description. A yellow, crystalline powder.

Identification

A. In the test for Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to tetracycline hydrochloride in the chromatogram obtained with reference solution (a).

B. To about 2 mg add 5 ml of *sulphuric acid*; a reddish violet colour develops. Add the solution to 2.5 ml of *water*; the colour changes to yellow.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 1.8 to 3.0, determined in a 1.0 per cent w/v suspension in *carbon dioxide-free water*.

Specific optical rotation (2.4.22). –239° to –255°, determined at 20° in a 1.0 per cent w/v solution in *0.1 M hydrochloric acid*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of a mixture of 68 ml of *0.1 M ammonium oxalate* and 27 ml of *dimethylformamide* (diluting solvent).

Reference solution (a). A 0.0025 per cent w/v solution of *4-epitetracycline hydrochloride RS* in the diluting solvent.

Reference solution (b). A solution containing 0.0025 per cent w/v of *4-epitetracycline hydrochloride RS* and 0.01 per cent w/v of *tetracycline hydrochloride RS* in the diluting solvent.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: 680 ml of *0.1 M ammonium oxalate*, 270 ml of *dimethylformamide* and 50 ml of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 1.5. In the chromatogram obtained with the test solution the area of any peak corresponding to 4-epitetracycline is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) and the total area of all peaks other than the principal peak is not greater than 5.0 per cent.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (50 ppm). Use 2.5 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 50 mg of the substance under examination and dissolve in about 50 ml of the diluting solvent described in the test for related substances and further add sufficient diluting solvent to produce 100.0 ml.

Reference solution (a). A 0.05 per cent w/v solution of *tetracycline hydrochloride RS* in the diluting solvent.

Reference solution (b). A solution containing 0.0025 per cent w/v of *4-epitetracycline hydrochloride RS* and 0.01 per cent w/v of *tetracycline hydrochloride RS* in the diluting solvent.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 to 10 µm),
- mobile phase: a mixture of 680 volumes of *0.1 M ammonium oxalate*, 270 volumes of *dimethylformamide* and 50 volumes of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 1.5.

Calculate the content of $C_{22}H_{24}N_2O_8$, HCl.

Tetracycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg.

Tetracycline Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture. If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude microorganisms.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Tetracycline Capsules

Tetracycline Hydrochloride Capsules

Tetracycline Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$, HCl.

Usual strength. 250 mg; 500 mg.

Identification

A. In the test for Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to tetracycline hydrochloride in the chromatogram obtained with reference solution (a).

B. To a quantity of the mixed contents of 20 capsules containing about 10 mg of Tetracycline Hydrochloride, add 20 ml of warm *ethanol (95 per cent)*, allow to stand for 20 minutes, filter and evaporate the filtrate to dryness on a water-bath. To 0.5 mg of the residue add *2 ml of sulphuric acid*; a reddish violet colour develops. Add the solution to 2.5 ml of *water*; the colour changes to yellow.

C. The residue obtained in test B gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the mixed contents of 20 capsules containing about 25 mg of Tetracycline Hydrochloride with 80 ml of *0.01 M methanolic hydrochloric acid* for 10 minutes, dilute to 100 ml with the same solvent, mix and filter if necessary.

Reference solution (a). A 0.002 per cent w/v solution of *4-epitetracycline hydrochloride RS* in *0.01 M methanolic hydrochloric acid.*

Reference solution (b). A solution containing 0.0015 per cent w/v each of *4-epitetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *0.01 M methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- column. temperature 40°,
- mobile phase: a mixture of 5 volumes of *dimethylformamide* and 95 volumes of *0.1 M oxalic acid*, the pH of the mixture being adjusted to 3.9 with *triethylamine*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 2.0. In the chromatogram obtained with the test solution the area of any peak corresponding to 4-epitetracycline hydrochloride is not greater than the area of the principal peak in the chromatogram obtained with reference solution (a) and the total area of all the peaks other than the principal peak is not greater than 10.0 per cent.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water* freshly prepared by distillation,
Speed and time. 75 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate. Measure the absorbance of the resulting solution, suitably diluted if necessary, at the maximum at about 276 nm (2.4.7). Calculate the content of $C_{22}H_{24}N_2O_8$, HCl in the medium from the absorbance obtained from a solution of known concentration of *tetracycline hydrochloride RS.*

D. Not less than 70 per cent of the stated amount of $C_{22}H_{24}N_2O_8$, HCl.

Loss on drying (2.4.19). Not more than 3.0 per cent, determined on 1.0 g of the contents of the capsules by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 50 mg of Tetracycline Hydrochloride, shake with about 50 ml of *0.01 M methanolic hydrochloric acid* and dilute with the same solvent to produce 100.0 ml, mix and filter. Discard the first few ml of the filtrate.

Reference solution (a). A 0.05 per cent w/v solution of *tetracycline hydrochloride RS* in *0.01 M methanolic hydrochloric acid.*

Reference solution (b). A solution containing 0.0025 per cent w/v of *4-epitetracycline hydrochloride RS* and 0.01 per cent w/v of *tetracycline hydrochloride RS* in *0.01 M methanolic hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 to10 µm),
- mobile phase: a mixture of 68 volumes of *0.1 M ammonium oxalate* and 27 volumes of *dimethylformamide* and 5 volumes of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The test is not valid unless the resolution between the two principal peaks in the chromatogram obtained with reference solution (b) is not less than 1.5.

Calculate the content of $C_{22}H_{24}N_2O_8$, HCl in the capsules.

Storage. Store protected from light and moisture.

Tetracycline Ointment

Tetracycline Hydrochloride Eye Ointment

Tetracycline Ointment contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of tetracycline hydrochloride, $C_{22}H_{24}N_2O_8$, HCl.

Usual strengths: 0.5 per cent w/w; 1 per cent w/w.

Identification

In the test for Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to tetracycline hydrochloride in the chromatogram obtained with the reference solution.

Tests

Water (2.3.43). Not more than 0.5 per cent, determined on 2.0 g dissolved in a mixture of 2 volumes of *carbon tetrachloride*, 2 volumes of *chloroform* and 1 volume of *methanol.*

Other tests. Complies with the tests stated under Eye Ointments.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity containing about 25 mg of Tetracycline Hydrochloride and transfer to a separating funnel with the aid of 15 ml of *cyclohexane.* Add 15 ml of a mixture of 68 volumes of *0.1 M ammonium oxalate* and 27 volumes of *dimethylformamide* (diluting solvent) and shake well. Collect the lower layer in a 50-ml volumetric flask. Repeat the extraction with two further quantities, each of 15 ml, of the diluting solvent, combining the extracts in the same 50-ml volumetric flask. Add sufficient diluting solvent to produce 50.0 ml, mix and filter.

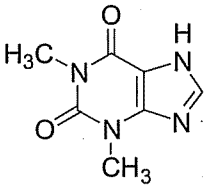
Reference solution. A 0.05 per cent w/v solution of *tetracycline hydrochloride RS* in the diluting solvent.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 to10 µm),
- mobile phase: a mixture of 68 volumes of *0.1 M ammonium oxalate* and 27 volumes of *dimethylformamide* and 5 volumes of *0.2 M dibasic ammonium phosphate*, the pH of the mixture being adjusted, if necessary, to 7.6 to 7.7 with *3 M ammonia* or *3 M phosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Calculate the content of $C_{22}H_{24}N_2O_8$, HCl in the eye ointment.

Storage. Store protected from moisture.

Theophylline



$C_7H_8N_4O_2$ Mol. Wt. 180.2 (anhydrous)
$C_7H_8N_4O_2,H_2O$ Mol. Wt. 198.2 (hydrate)

Theophylline is 1,3-dimethyl-3,7-dihydro-1*H*-purine-2, 6-dione. It contains one molecule of water or is anhydrous.

Theophylline contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_8N_4O_2$, calculated on the dried basis.

Category. Xanthine bronchodilator.

Dose. By intravenous infusion, 2.5 to 5.0 mg of anhydrous theophylline per kg body weight over a period of 20 minutes.

Description. A white, crystalline powder; odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and D may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *theophylline RS* or with the reference spectrum of theophylline.

B. Dissolve about 10 mg in 10 ml of *water*, add 0.5 ml of a 5 per cent w/v solution of *mercuric acetate* and allow to stand; a white, crystalline precipitate is produced.

C. The melting range, after drying at 105°, 270° to 274° (2.4.21).

D. Gives the reaction of xanthines (2.3.1).

Tests

Appearance of solution. Dissolve 0.5 g in 75 ml of *carbon dioxide-free water* with heating; the resulting solution (solution A), is clear (2.4.1), and colourless (2.4.1).

Acidity. To 50 ml of solution A add 0.1 ml of *methyl red solution*. The solution is red and not more than 1.0 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution to yellow.

Light absorption (2.4.7). Absorbance of a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* at the maximum at about 270 nm, not less than 0.53.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in 20.0 ml of the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b). Dissolve 10 mg of *theobromine* in the mobile phase, add 5 ml of the test solution and dilute to 100 ml with the mobile phase. Dilute 5 ml of this solution to 50.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (7 µm),
- mobile phase: a mixture of 7 volumes of *acetonitrile* and 93 volumes of 0.14 per cent w/v solution of *sodium acetate* containing 0.5 per cent v/v solution of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to theobromine and theophylline is not less than 2.0. The relative retention time with reference to theophylline for theophylline impurity C is about 0.3, for theophylline impurity B is about 0.4, for theophylline impurity D is about 0.5 and for theophylline impurity A is about 2.5.

Inject the test solution and reference solution (a). Run the chromatogram 3.5 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Heavy metals (2.3.13).1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent (for the anhydrous form) and 8.0 per cent to 9.5 per cent (for the hydrated form), determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, add 50 ml of *water* and gently warm the mixture on a water-bath until complete solution is effected. Cool, add 20.0 ml of *0.1 M silver nitrate* and 1.0 ml of *bromothymol solution* and titrate with *0.1 M sodium hydroxide* until a blue colour is obtained.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01802 g of $C_7H_8N_4O_2$.

Storage. Store protected from moisture.

Theophylline Injection

Theophylline in Dextrose Injection

Theophylline Injection is a sterile solution of Theophylline and Dextrose in Water for Injections.

Theophylline Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of anhydrous theophylline, $C_7H_8N_4O_2$, and not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dextrose, $C_6H_{12}O_6$, H_2O.

Usual strengths. Anhydrous theophylline, 0.4, 0.8, 1.6, 2, 3.2, 4 mg per ml in 5 per cent w/v Dextrose.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay for theophylline shows an absorption maximum at about 274 nm.

B. Add 0.2 ml of the injection to 5 ml of *potassium cupri-tartrate solution* and heat to boiling; a red to orange precipitate is formed.

Tests

pH (2.4.24). 3.5 to 6.5.

5-Hydroxymethylfurfural and Related substances. Use a glass chromatographic column (66 cm x 11 mm) with a sealed-in, coarse-porosity sintered disc or a glass wool plug and fitted with a stopcock. Mix 8 g of a 20- to 50-mesh styrenedivinyl-benzene anion-exchange resin in the hydroxide form with 25 ml of *water*, allow to settle and decant the supernatant liquid until a slurry of resin remains. Pour the slurry into the column and allow to settle as a homogeneous bed having a bed volume of about 15 ml. Wash the resin bed at a flow rate of about 3 ml per minute with 100 ml of a 5.7 per cent w/v solution of *ammonium carbonate* followed by washing with *water* until the eluate has a pH of 7.

Dilute an accurately measured volume of the injection containing 1.0 g of Dextrose, $C_6H_{12}O_6,H_2O$, to 250.0 ml with *water*. Pass this solution through the resin bed in the column at a flow rate of about 3.5 ml per minute, discarding the first 50 ml of the eluate. Measure the absorbance of the eluate at 284 nm, using *water* as the blank; the absorbance is not more than 0.25 (2.4.7).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For theophylline* — Dilute a suitable volume of the injection with sufficient *0.1 M sodium hydroxide* to produce a solution containing 0.008 per cent w/v of anhydrous theophylline. Measure the absorbance of the resulting solution at the maximum at about 274 nm (2.4.7), using as the blank a solution prepared in the same manner omitting the substance under examination.

Calculate the content of $C_7H_8N_4O_2$ from the absorbance obtained by repeating the operation using *theophylline RS* in place of the substance under examination.

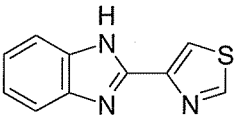
For dextrose — Transfer an accurately measured volume of the injection containing about 5 mg of Dextrose to a 100-ml volumetric flask, add 0.2 ml of *6 M ammonia*, dilute to volume with *water* and mix. Determine the optical rotation of the resulting solution in a suitable polarimeter tube at 25° (2.4.22). The observed rotation, in degrees multiplied by 1.0425*A*, represents the weight, in g, of $C_6H_{12}O_6,H_2O$ in the volume of the injection taken for the assay, where *A* is the ratio 200 divided by the length, in mm, of the polarimeter tube employed.

Storage. Store protected from light, in single dose containers.

Labelling. The label states the strength in terms of the amounts of anhydrous theophylline and Dextrose.

Thiabendazole

Tiabendazole



$C_{10}H_7N_3S$ Mol. Wt. 201.3

Thiabendazole is 2-(1,3-thiazol-4-yl)-1*H*-benzimidazole.

Thiabendazole contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_7N_3S$, calculated on the anhydrous basis.

Category. Anthelmintic.

Dose. In the treatment of nematode infestation, 1.5 g twice daily for three days.

Description. A white or almost white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiabendazole RS* or with the reference spectrum of thiabendazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 243 nm and 302 nm; ratio of the absorbance at the maximum at about 302 nm to that at about 243 nm, 1.8 to 2.1.

C. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

D. Dissolve 5 mg in 5 ml of *0.1 M hydrochloric acid*, add 3 mg of *4-phenylenediamine dihydrochloride* and shake until dissolved. Add 0.1 g of *zinc powder*, mix, allow to stand for 2 minutes and add 10 ml of *ferric ammonium sulphate solution*; a deep blue or bluish violet colour is produced.

Tests

Appearance of solution. Add 5 ml of *methanol* to 0.5 g in a flask fitted with a ground-glass stopper, stir for 5 minutes, with a magnetic stirrer and filter through a sintered-glass filter (1.6 µm to 4 µm). The solution is not more intensely coloured than reference solution BS6 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 62.5 volumes of *toluene*, 25 volumes of *glacial acetic acid*, 10 volumes of *acetone* and 2.5 volumes of *water*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Test solution (b). Dilute 2 ml of test solution (a) to 20 ml with *methanol*.

Reference solution (a). Dilute 1 ml of test solution (b) to 10 ml with *methanol*.

Reference solution (b). Dilute 1 ml of test solution (b) to 25 ml with *methanol*.

Reference solution (c). Dissolve 20 mg of *thiabendazole RS* in 20 ml of *methanol*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b)

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Weigh accurately about 0.15 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02013 g of $C_{10}H_7N_3S$.

Storage. Store protected from light and moisture.

Thiabendazole Tablets

Tiabendazole Tablets

Thiabendazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of thiabendazole, $C_{10}H_7N_3S$.

Usual strength. 500 mg.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum at about 302 nm.

B. Dissolve a quantity of the powdered tablets containing 30 mg of Thiabendazole in 5 ml of *0.1 M hydrochloric acid*, add 3 mg of *4-phenylenediamine dihydrochloride* and shake until dissolved. Add 0.1 g of *zinc powder*, mix, allow to stand for 2 minutes and add 10 ml of *ferric ammonium sulphate solution*; a deep bluish violet colour is produced.

Tests

Disintegration. The test does not apply.

Other tests. Comply with the tests stated under Tablets.

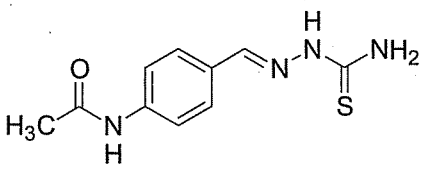
Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Thiabendazole, add 75 ml of *0.1 M hydrochloric acid*, warm on a water-bath for 15 minutes, shaking occasionally, cool, dilute to 100.0 ml with *0.1 M hydrochloric acid* and filter. Dilute 5.0 ml of the filtrate to 1000.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7).

Calculate the content of $C_{10}H_7N_3S$ taking 1230 as the specific absorbance at 302 nm.

Storage. Store protected from light and moisture.

Labelling. The label states that the tablets should be chewed before swallowing.

Thiacetazone



$C_{10}H_{12}N_4OS$ Mol. Wt. 236.3

Thiacetazone is 4-acetamidobenzaldehyde thiosemicarbazone.

Thiacetazone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{10}H_{12}N_4OS$, calculated on the dried basis.

Category. Antitubercular.

Dose. 150 mg daily.

Description. Pale yellow crystals or a crystalline powder; almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiacetazone RS* or with the reference spectrum of thiacetazone.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.0003 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 328 nm absorbance at about 328 nm, about 0.55.

C. Boil 10 mg with 5 ml of *1 M hydrochloric acid* for 3 minutes, cool and add sufficient *water* to produce 200 ml. Mix 5 ml of this solution with 0.25 ml of *sodium nitrite solution* and add the mixture to 0.5 ml of *2-naphthol solution*; a red colour is produced.

Tests

Thiosemicarbazide. To 2.0 g, finely powdered, add sufficient *water* to produce 50 ml, shake, allow to stand for 1 hour with occasional shaking and filter, discarding the first few ml of the filtrate. Acidify 25 ml of the clear filtrate with *dilute sulphuric acid*, add 0.1 ml of *o-phenanthroline-ferrous complex solution* and titrate with *0.1 M ceric ammonium sulphate* to a blue end-point which persists for 1 minute; not more than 0.8 ml is required.

4-acetamidobenzalazine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Ethyl acetate.

Test solution. Dissolve 0.4 g of the substance under examination in 100 ml of *methanol*.

Reference solution. Dissolve with the aid of heat 0.016 g of *4-acetamidobenzalazine RS* in 150 ml of *methanol*, cool and dilute to 200 ml with *methanol* and further dilute 5 ml of this solution to 50 ml with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *2 M nitric acid* and within 2 minutes examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.1 g, dissolve in 60 ml of *methanol* by heating at 60° in a water-bath, add slowly 20 ml of hot *methanolic silver nitrate solution*, maintain the solution at 60° until the precipitate coagulates and leaves a clear supernatant liquid. Cool, filter through a sintered-glass crucible (porosity No. 4), wash the residue with *methanol* until the washings are free from silver nitrate and dry to constant weight at 105°.

1 g of residue is equivalent to 0.4606 g of $C_{10}H_{12}N_4OS$.

Storage. Store protected from light and moisture.

Thiacetazone and Isoniazid Tablets

Thiacetazone and Isoniazid Tablets contain one part by weight of Thiacetazone and two parts by weight of Isoniazid.

Thiacetazone and Isoniazid Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amounts of thiacetazone, $C_{10}H_{12}N_4OS$, and isoniazid, $C_6H_7N_3O_2$.

Category. Antitubercular.

Dose. Thiacetazone, 150 mg and Isoniazid, 300 mg daily, in divided doses.

Usual strengths. Thiacetazone, 37.5 mg and Isoniazid, 75 mg; Thiacetazone, 75 mg and Isoniazid, 150 mg; Thiacetazone, 150 mg and Isoniazid, 300 mg.

Identification

A. Extract a quantity of the powdered tablets containing about 30 mg of Thiacetazone with 70 ml of *ethanol (95 per cent)* with the aid of heat for 15 minutes with occasional shaking. Cool, dilute to 100 ml with *ethanol (95 per cent)* and filter. Dilute 1 ml to 100 ml with *ethanol (95 per cent)*. The solution complies with the following test.

When examined in the range 230 nm to 360 nm (2.4.7), a 0.0005 per cent w/v solution in *0.1 M hydrochloric acid* shows absorption maxima at about 243 nm and 302 nm; ratio of the absorbance at the maximum at about 302 nm to that at about 243 nm, 1.8 to 2.1.

B. Shake a quantity of the powdered tablets containing 1 mg of Isoniazid with 50 ml of *ethanol (95 per cent)* and filter. To 5 ml of the filtrate add 0.1 g of *borax* and 5 ml of a 5 per cent w/v solution of *1-chloro-2,4-dinitrobenzene* in *ethanol (95 per cent)*, evaporate to dryness on a water-bath and continue heating for a further 10 minutes. To the residue add 10 ml of *methanol* and mix; a reddish purple colour is produced.

Tests

Disintegration (2.5.1). Not more than 30 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. *For thiacetazone* — Weigh and finely powder 20 tablets. Weigh accurately a quantity of the powder containing about 30 mg of Thiacetazone, add 70 ml of *ethanol (95 per cent)* and heat on a water-bath for 15 minutes with intermittent shaking. Cool, add sufficient *ethanol (95 per cent)* to produce 100.0 ml and filter. To 1.0 ml of the filtrate add sufficient *ethanol (95 per cent)* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 328 nm (2.4.7), using *ethanol (95 per cent)* as the blank.

Calculate the content of $C_{10}H_{12}N_4OS$ from the absorbance obtained by repeating the operation using *thiacetazone RS* in place of the tablets under examination.

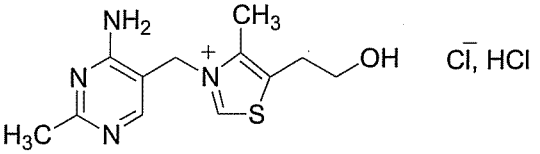
For isoniazid — Weigh accurately a quantity of the powdered tablets containing about 0.2 g of Isoniazid, dissolve as completely as possible in 100 ml of *water* and filter. Wash the residue with *water*, combine the filtrate and washings and dilute to 250.0 ml with *water*. To 50.0 ml of the resulting solution add 50 ml of water, 20 ml of *hydrochloric acid* and 0.2 g of *potassium bromide*. Titrate with *0.0167 M potassium bromate*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.0167 M potassium bromate* is equivalent to 0.003429 g of $C_6H_7N_3O_2$.

Storage. Store protected from light and moisture.

Thiamine Hydrochloride

Aneurine Hydrochloride; Vitamin B_1



$C_{12}H_{17}ClN_4OS$, HCl Mol. Wt. 337.3

Thiamine Hydrochloride is 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride.

Thiamine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{12}H_{17}ClN_4OS$, HCl, calculated on the dried basis.

Category. B-group vitamin.

Dose. Prophylactic, orally, 2 to 5 mg once daily; therapeutic, orally or by subcutaneous or intramuscular injection, 25 to 100 mg daily. In multivitamin preparations, prophylactic, orally, 1 to 2 mg daily; therapeutic, orally, 4.5 to 10 mg daily.

Description. A white or almost white, crystalline powder or small colourless crystals; odour, slight and characteristic.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiamine hydrochloride RS* or with the reference spectrum of thiamine hydrochloride.

B. Dissolve about 20 mg in 10 ml of *water*, add 1 ml of *2 M acetic acid* and 1.6 ml of *1 M sodium hydroxide*, heat on a water-bath for 30 minutes and allow to cool. Add 5 ml of *2 M sodium hydroxide*, 10 ml of *potassium ferricyanide solution* and 10 ml of *1-butanol* and shake vigorously for 2 minutes. The upper layer exhibits an intense light blue fluorescence, particularly in ultraviolet light at 365 nm. Repeat the test but adding 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of *1 M sodium hydroxide*; practically no fluorescence is produced.

C. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and not more intensely coloured than reference solution YS7 or GYS6 (2.4.1).

pH (2.4.24). 2.7 to 3.3, determined in a 2.5 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 volumes of *glacial acetic acid* and 95 volumes of *water*.

Test solution. Dissolve 0.35 g of the substance under examination in 15.0 ml of the solution mixture and dilute to 100.0 ml with *water*.

Reference solution (a). Dissolve 5 mg each of the substance under examination and *thioxothiamine RS (thiamine impurity A RS)* in 4 ml of the solvent mixture and dilute to 25.0 ml with *water*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 50.0 ml with *water*. Dilute 5.0 ml of this solution to 25.0 ml with *water*.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (7 µm),
- column temperature. 45°,
- mobile phase: A. 0.38 per cent w/v solution of *sodium hexanesulphonate,* adjusted to pH 3.1 with *orthophosphoric acid,*
  B. *methanol,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 25 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 25	90 →70	10 →30
25 – 33	70 →50	30 →50
33 - 40	50	50
40 – 45	50 →90	50 →10

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to thiamine impurity A and thiamine is not less than 1.6.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.4 per cent). The sum of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any peak with an area less than 0.125 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Nitrates. To 2 ml of a 2.0 per cent w/v solution add 2 ml of *sulphuric acid*, cool and superimpose 2 ml of *ferrous sulphate solution*; no brown ring is produced at the junction of the two layers.

Sulphates (2.3.17). 0.5 g complies with the limit test for sulphates (300 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 5 ml of *anhydrous formic acid*, add 65 ml of *anhydrous glacial acetic acid* and 10 ml of *mercuric acetate solution*, with stirring. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01686 g of $C_{12}H_{17}ClN_4OS, HCl$.

Storage. Store protected from light and moisture, non-metallic containers.

Thiamine Injection

Thiamine Hydrochloride Injection; Aneurine Hydrochloride Injection; Vitamin B_1 Injection

Thiamine Injection is a sterile solution of Thiamine Hydrochloride in Water for Injection.

Thiamine Injection contains not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS, HCl$.

Usual strength. 100 mg per ml.

Identification

A. To a volume containing 20 mg of Thiamine Hydrochloride in 10 ml of *water*, add 1 ml of *2 M acetic acid* and 1.6 ml of *1 M sodium hydroxide*, heat on a water-bath for 30 minutes and allow to cool. Add 5 ml of *2 M sodium hydroxide*, 10 ml of *potassium ferricyanide solution* and 10 ml of *1-butanol* and shake vigorously for 2 minutes. The upper layer exhibits an intense light blue fluorescence, particularly in ultraviolet light at 365 nm. Repeat the test but adding 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of *1 M sodium hydroxide*; practically no fluorescence is produced.

B. To a mixture of 0.1 ml of *nitrobenzene* and 0.2 ml of *sulphuric acid* add a volume containing 5 mg of Thiamine Hydrochloride.

Allow to stand for 10 minutes, cool in ice and add slowly with stirring 5 ml of *water* followed by 5 ml of *10 M sodium hydroxide*. Add 5 ml of *acetone* and allow to stand; no violet colour is produced in the upper layer.

Tests

pH (2.4.24). 2.5 to 4.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a volume of the injection containing about 0.1 g of Thiamine Hydrochloride to 100.0 ml with *0.1 M hydrochloric acid* and further dilute 5.0 ml to 100.0 ml with *water*.

Reference solution. A 0.005 per cent w/v solution of *thiamine mononitrate RS* in *0.005 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by dissolving 1 g of *sodium heptanesulphonate* in a mixture of 180 ml of *methanol* and 10 ml of *triethylamine*, diluting to 1000 ml with *water* and adjusting the pH to 3.2 with *orthophosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 244 nm,
- injection volume. 20 µl.

Calculate the content of $C_{12}H_{17}ClN_4OS$.

1 mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS$, HCl.

Storage. Store protected from light.

Thiamine Tablets

Thiamine Hydrochloride Tablets; Aneurine Hydrochloride Tablets; Vitamin B_1 Tablets

Thiamine Tablets contain not less than 92.5 per cent and not more than 110.0 per cent of the stated amount of thiamine hydrochloride, $C_{12}H_{17}ClN_4OS$, HCl.

Usual strengths. 5 mg; 10 mg; 50 mg.

Identification

A. Dissolve a quantity of the powdered tablets containing 20 mg of Thiamine Hydrochloride as completely as possible in 10 ml of *water* and 2 ml of *1 M acetic acid* and filter. Add 5 ml of *2 M sodium hydroxide*, 10 ml of *potassium ferricyanide solution* and 10 ml of *1-butanol* and shake vigorously for 2 minutes. The upper layer exhibits an intense light blue fluorescence, particularly in ultraviolet light at 365 nm. Repeat the test but adding 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of *1 M sodium hydroxide*; practically no fluorescence is produced.

B. To a mixture of 0.1 ml of *nitrobenzene* and 0.2 ml of *sulphuric acid* add the powdered tablets containing 5 mg of Thiamine Hydrochloride. Allow to stand for 10 minutes, cool in ice and add slowly with stirring 5 ml of *water* followed by 5 ml of *10 M sodium hydroxide*. Add 5 ml of *acetone* and allow to stand; no violet colour is produced in the upper layer.

C. The powdered tablets give the reactions of chlorides (2.3.1).

Tests

Uniformity of content. *(For tablets containing 10 mg or less)* — Comply with the test stated under Tablets.

Finely crush one tablet, add 20 ml of *ethanol (95 per cent)*, stir the mixture for 30 minutes and centrifuge. Repeat the extraction with three further quantities, each of 15 ml, of *ethanol (95 per cent)*. Combine the extracts, add sufficient *ethanol (95 per cent)* to produce 100.0 ml and mix. Dilute a suitable volume of the resulting solution containing 1 mg of Thiamine Hydrochloride with sufficient *ethanol (95 per cent)* to produce 100.0 ml. Measure the absorbance of the resulting solution at the maximum at about 233 nm (2.4.7).

Calculate the content of $C_{12}H_{17}ClN_4OS$, HCl in the tablet taking 380 as the specific absorbance at 233 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. For tablets containing less than 10 mg of Thiamine Hydrochloride, add 5 ml of *0.1 M hydrochloric acid* and 50 ml of *water* to a quantity of the powdered tablets containing 6 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 100.0 ml with *water* and filter. For tablets containing 10 mg or more of Thiamine Hydrochloride, add 50 ml of *0.1 M hydrochloric acid* and 500 ml of *water* to a quantity of the powdered tablets containing 60 mg of Thiamine Hydrochloride, shake for 20 minutes, dilute to 1000.0 ml with *water* and filter.

Reference solution. A 0.006 per cent w/v solution of *thiamine mononitrate RS* in *0.005 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a solution prepared by dissolving 1 g of *sodium heptanesulphonate* in a mixture of 180 ml of *methanol* and 10 ml of *triethylamine*, diluting to 1000 ml with *water* and adjusting the pH to 3.2 with *orthophosphoric acid*,

- flow rate. 2 ml per minute,
- spectrophotometer set at 244 nm,
- injection volume. 20 µl.

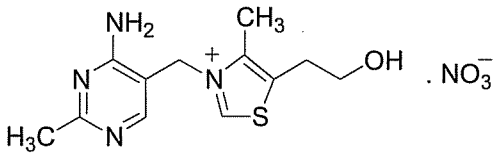
Calculate the content of $C_{12}H_{17}ClN_4OS$ in the tablets.

1 mg of $C_{12}H_{17}N_5O_4S$ is equivalent to 1.030 mg of $C_{12}H_{17}ClN_4OS$, HCl.

Storage. Store protected from light and moisture in non-metallic containers.

Thiamine Mononitrate

Thiamine Nitrate



$C_{12}H_{17}N_5O_4S$ Mol. Wt. 327.4

Thiamine Mononitrate is 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium nitrate.

Thiamine Mononitrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{17}N_5O_4S$, calculated on the dried basis.

Category. B-group vitamin.

Dose. Prophylactic, 2 to 5 mg daily; therapeutic, 25 to 100 mg daily. In multivitamin oral preparations, prophylactic, 1 to 2 mg daily; therapeutic, 4.5 to 10 mg daily.

Description. A white or almost white, crystalline powder or small, colourless crystals.

Identification

Test A may be omitted if tests B and C are carried out. Test B may be omitted if tests A and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiamine mononitrate RS* or with the reference spectrum of thiamine mononitrate.

B. Dissolve about 20 mg in 10 ml of *water*, add 1 ml of *2 M acetic acid* and 1.6 ml of *1 M sodium hydroxide*, heat on a water-bath for 30 minutes and allow to cool. Add 5 ml of *2 M sodium hydroxide*, 10 ml of *potassium ferricyanide solution* and 10 ml of *1-butanol* and shake vigorously for 2 minutes. The upper layer exhibits an intense light blue fluorescence, particularly in ultraviolet light at 365 nm. Repeat the test but adding 0.9 ml of *1 M sodium hydroxide* and 0.2 g of *sodium sulphite* in place of the 1.6 ml of *1 M sodium hydroxide*; practically no fluorescence is produced.

C. Gives reaction A of nitrates (2.3.1).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 6.8 to 7.6, determined in a 2.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 5 volumes of *glacial acetic acid* and 95 volumes of *water*.

Test solution. Dissolve 0.35 g of the substance under examination in 15.0 ml of the solution mixture and dilute to 100.0 ml with *water*.

Reference solution (a). Dissolve 5 mg each of the substance under examination and *thioxothiamine RS (thiamine impurity A RS)* in 4 ml of the solvent mixture and dilute to 25.0 ml with *water*. Dilute 5.0 ml of the solution to 25.0 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: A. 0.38 per cent w/v solution of *sodium hexanesulphonate*, adjusted to pH 3.1 with *orthophosphoric acid*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 248 nm,
- injection volume. 25 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 25	90 →70	10 →30
25 – 33	70 →50	30 →50
33 - 40	50	50
40 – 45	50 →90	50 →10

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to thiamine impurity A and thiamine is not less than 1.6.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any

secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). The sum of all the secondary peaks is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.5 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). 1.0 g complies with the limit test for chlorides (250 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

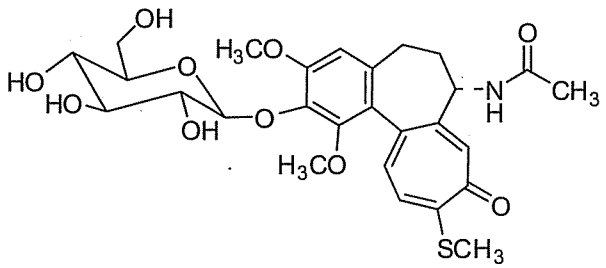
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 5 ml of *anhydrous formic acid*, add 70 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01637 g of $C_{12}H_{17}N_5O_4S$.

Storage. Store protected from light and moisture, non-metallic containers.

Thiocolchicoside



$C_{27}H_{33}NO_{10}S$ Mol. Wt. 563.5

Thiocolchicoside is 2-demethoxy-2-glucosidoxythiocolchicine.

Thiocolchicoside contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{27}H_{33}NO_{10}S$, calculated on the dried basis.

Description. A yellow crystalline powder.

Category. Muscle relaxant.

Dose. 4 mg to 8 mg daily.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiocolchicoside RS* or with the reference spectrum of thiocolchicoside.

B. When examined in the range of 220 nm to 440 nm (2.4.7), a 0.0015 per cent w/v solution in *water* shows absorption maxima at the same wavelength as obtained with the reference solution.

Tests

pH (2.4.24). 6.0 to 7.5, determined in a 0.5 per cent w/v solution in *carbon-dioxide free water*.

Specific optical rotation (2.4.22). - 550° to - 580° at 23°, determined on 0.5 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the substance under examination in 50.0 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v solution of *colchicoside RS* in *methanol*.

Reference solution (b). A 0.05 per cent w/v solution of *thiocolchicoside RS* in *methanol*.

Reference solution (c). Dilute 1.0 ml each of reference solution (a) and (b) to 100.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica (5 µm),
- mobile phase: A. *n- heptane* containing *2 per cent acetic acid*,
  B. *chloroform*,
  C. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 360 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	86	10	4
50	0	65	35
51	86	10	4
60	86	10	4

Inject reference solution (c). The test is not valid unless the theoretical plates is not less than 80000, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the peak due to thiocolchicoside.

The relative retention time with reference to thiocolchicoside for colchicine is about 0.55, for N- deacetyl N- formyl thiocolchicoside is about 1.05 and for colchicoside is about 1.10.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution the area of each peak corresponding to N-deacetyl N-formyl thiocolchicoside and colchicoside is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of the areas of all other secondary peaks is not more than the area of peak due to thiocolchicoside in the chromatogram obtained with reference solution (c) (0.5 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 4.0 per cent, determined on 1.0 g by drying in oven at 105° for 3 hours.

Microbial contamination (2.2.9). Total microbial count not more than 1000 CFU per g, total yeast and mould count not more than 100 CFU per g, 1 g is free from *Escherichia coli, Salmonella, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterobacteriaceae.*

Bacterial endotoxins (2.2.3). Not more than 87.5 Endotoxin Units per mg of Thiocolchicoside.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of substance under examination in 100.0 ml of *water.* Dilute 10.0 ml of this solution to 100.0 ml with *water.*

Reference solution. A 0.1 per cent w/v solution of *thiocolchicoside RS* in *water.* Dilute 10.0 ml of this solution to 100.0 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 370 nm,
- injection volume. 20 µl.

Time (in min.)	Flow (ml/min.)	Mobile Phase A (per cent /v/v)	Mobile Phase B (per cent v/v)
0	1.0	90	10
10	1.0	90	10
11	1.0	75	25
12	1.8	75	25
15	1.8	75	25
17	1.8	70	30
18	1.8	70	30
20	1.8	90	10
22	1.0	90	10

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate content of $C_{27}H_{33}NO_{10}S$.

Storage: Store protected from light and at a temperature not exceeding 30°.

Thiocolchicoside Capsules

Thiocolchicoside Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of thiocolchicoside, $C_{27}H_{33}NO_{10}S$.

Dose. 4 mg; 8 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

B. Examine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 70 volumes of *ethyl acetate,* 20 volumes of *ethanol* and 10 volumes of *water.*

Test solution. Dissolve 50 mg of the substance under examination in 5.0 ml of *methanol.*

Reference solution. A 1.0 per cent w/v solution of *thiocolchicoside RS* in *methanol.*

Apply to the plate 10 ml of each solution. After development, dry the plate in a current of air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 7.5,*
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate.

Reference solution. A 0.001 per cent w/v solution of *thiocolchicoside RS* in *water.*

Use the chromatographic condition as described under Assay.

Calculate the content of $C_{27}H_{33}NO_{10}S$.

D. Not less than 80 per cent of the stated amount of $C_{27}H_{33}NO_{10}S$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse the content of the capsules containing about 50 mg of Thiocolchicoside in 50.0 ml of *methanol.*

Reference solution (a). A 0.05 per cent w/v solution of *colchicoside RS* in *methanol.*

Reference solution (b). A 0.05 per cent w/v solution of *thiocolchicoside RS* in *methanol.*

Reference solution (c). Dilute 1.0 ml each of reference solution (a) and (b) to 100.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with silica (5 µm),
- mobile phase: A. *n- heptane* containing 2 per cent *acetic acid,*
  B. *chloroform,*
  C. *methanol,*
- a linear gradient programme using the conditions given below,
- flow rate. 0.8 ml per minute,
- spectrophotometer set at 360 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
0	86	10	4
50	0	65	35
51	86	10	4
60	86	10	4

Inject reference solution (c). The test is not valid unless the theoretical plate is not less than 80000, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent for the peak due to thiocolchicoside.

The relative retention time with reference to thiocolchicoside for colchicine is about 0.55, for N- deacetyl N- formyl thiocolchicoside is about 1.05 and for colchicoside is about 1.10.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution the area of each peak corresponding to N-deacetyl N-formyl thiocolchicoside and colchicoside is not more than the area of corresponding peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of the areas of all other secondary peaks is not more than the area of peak due to thiocolchicoside in the chromatogram obtained with reference solution (c) (0.5 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the content of 20 capsules containing about 10 mg of Thocolchicoside with 100.0 ml of *water.*

Reference solution. A 0.01 per cent w/v solution of *thiocolchicoside RS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 370 nm,
- injection volume. 20 µl.

Time (in min.)	Flow (ml/min.)	Mobile Phase A (per cent /v/v)	Mobile Phase B (per cent v/v)
0.01	1.0	90	10
10	1.0	90	10
11	1.0	75	25
12	1.8	75	25
15	1.8	75	25
17	1.8	70	30
18	1.8	70	30
20	1.8	90	10
22	1.0	90	10

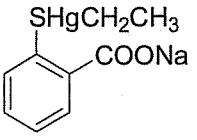
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{27}H_{33}NO_{10}S$ in the capsules.

Thiomersal

Thimerosal



$C_9H_9HgNaO_2S$ Mol. Wt. 404.8

Thiomersal is the sodium salt of [(2-carboxyphenyl)thio] ethylmercury.

Thiomersal contains not less than 97.0 per cent and not more than 101.0 per cent of $C_9H_9HgNaO_2S$, calculated on the dried basis.

Category. Antiseptic; pharmaceutical aid (antimicrobial preservative).

Description. A light cream, crystalline powder; odour, slight and characteristic.

Identification

A. Dissolve 0.1 g in 10 ml of *water* and add 2 ml of *silver nitrate solution*; a pale yellow precipitate is produced.

B. Dissolve 0.5 g in 10 ml of *water* and add 2 ml of *2 M hydrochloric acid*; a white precipitate is produced which, after washing with *water* and drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa, melts at about 110° (2.4.21).

Tests

pH (2.4.24). 6.0 to 8.0, determined in a 1.0 per cent w/v solution.

Ether-soluble matter. Shake 0.5 g with 20 ml of *ether* for 10 minutes, filter and evaporate to dryness. The residue, after drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours, weighs not more than 3 mg.

Inorganic mercury compounds. Not more than 0.7 per cent, determined by the following method.

NOTE—Protect the solutions from light.

Label five 10-ml volumetric flasks A, B, C, D and E. Place 5 ml of a 0.1 per cent w/v solution of the substance under examination in each of flasks A, B, C and D. To each of flasks C and D add 0.5 ml of a 0.0095 per cent w/v solution of *mercuric chloride*. Add sufficient *water* to flasks A and C to produce 10.0 ml and add sufficient of a freshly prepared 33.2 per cent w/v solution of *potassium iodide* to flasks B and D to produce 10.0 ml. Place 5 ml of the *potassium iodide solution* in flask E and add sufficient *water* to produce 10.0 ml. Measure the absorbances of each of the solutions (Aa, Ab, Ac, Ad, Ae) at about 323 nm (2.4.7), using *water* as the blank.

Calculate the content of inorganic mercury compounds, expressed as Hg, in the substance under examination from the expression $0.7019\ (Ab - Aa - Ae)/(Aa + Ad - Ab - Ac)$, where the figure 0.7019 is a constant obtained from the formula

$$\frac{\text{atomic weight of mercury}}{\text{molecular weight of } HgCl_2} \times \frac{\text{concentration of } HgCl_2}{100}$$

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

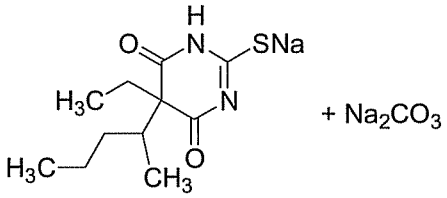
Assay. Weigh accurately about 0.5 g, transfer to a 100-ml long-necked flask, add 5 ml of *sulphuric acid* and heat gently until charring occurs; continue to heat and add *hydrogen peroxide solution (100 vol)* dropwise, until the mixture is colourless. Dilute with *water*, evaporate until slight fuming occurs, dilute to 10 ml with *water*, cool and titrate with *0.1 M ammonium thiocyanate* using *ferric ammonium sulphate solution* as indicator.

1 ml of *0.1 M ammonium thiocyanate* is equivalent to 0.02024 g of $C_9H_9HgNaO_2S$.

Storage. Store protected from light and moisture.

Thiopentone Sodium

Thiopental Sodium



$C_{11}H_{17}N_2NaO_2S$ Mol. Wt. 264.3

Thiopentone Sodium is a mixture of sodium (*RS*)-5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate and anhydrous sodium carbonate.

Thiopentone Sodium contains not less than 84.0 per cent and not more than 87.0 per cent of $C_{11}H_{18}N_2O_2S$ and not less than 10.2 per cent and not more than 11.2 per cent of Na, both calculated on the dried basis.

Category. General anaesthetic.

Dose. By intravenous injection, initially, 100 to 500 mg, repeated if necessary after 20 to 30 seconds; maximum dose, 2 g.

Description. A yellowish white powder; odour, faintly resembling garlic; hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Acidify 10 ml of a 10 per cent w/v solution in *carbon dioxide-free water* with *2 M hydrochloric acid*; the solution effervesces. Shake the solution with 20 ml of *ether*, separate the ether layer, wash with 10 ml of *water* and dry over *anhydrous sodium sulphate*. Filter, evaporate the filtrate to dryness and dry the residue at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiopentone RS* or with the reference spectrum of thiopentone.

B. Complies with the test for identification of barbiturates (2.3.2), but using the following solutions.

Test solution. A 0.1 per cent w/v solution of the substance under examination in *water*.

Reference solution. Dissolve 85 mg of *thiopentone RS* in 10 ml of *2 M sodium hydroxide* and dilute to 100 ml with *water*.

C. Acidify 5 ml of a 5 per cent w/v solution with *dilute acetic acid* and filter. Wash the precipitate with *water*, recrystallise from *water* and dry at 70°; the crystals melt at about 160° (2.4.21).

D. Dissolve 1 mg of the crystals obtained in test A in 1 ml of *0.1 M sodium hydroxide*. Add about 1 mg of *sodium nitroprusside* and, after 15 minutes, 1 ml of *dilute hydrochloric acid*; a reddish violet colour is produced.

E. Gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than reference solution GYS3 (2.4.1).

Related substances (2.3.4). Complies with the test, but using *water* as the solvent for the test solution and the reference solution. After development, examine the plate in ultraviolet light at 254 nm but do not spray it with the *diphenylcarbazone-mercury reagent*.

Chlorides (2.3.12). To 10 ml of solution A add 30 ml of *water* and 10 ml of *2 M nitric acid*. Shake successively with three quantities, each of 25 ml, of *ether*, discard the ether layers and heat the aqueous solution on water-bath to remove any residual ether. 30 ml of the aqueous layer complies with the limit test for chlorides (330 ppm).

Loss on drying (2.4.19). Not more than 2.5 per cent, determined on 0.5 g by drying in an oven at 100° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. *For thiopentone* — Weigh accurately about 0.15 g, dissolve in 5 ml of water, add 2 ml of *1 M sulphuric acid* and extract with four quantities, each of 10 ml, of *chloroform*. Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 30 ml of *dimethylformamide*, previously neutralised with *0.1 M lithium methoxide*. Titrate immediately with *0.1 M lithium methoxide*, using 1 drop of a 0.2 per cent w/v solution of *thymol blue* in *methanol* as indicator, until a blue colour is obtained. Protect the solution from absorption of carbon dioxide during the titration.

1 ml of *0.1 M lithium methoxide* is equivalent to 0.02423 g of $C_{11}H_{18}N_2O_2S$.

For sodium — Weigh accurately about 0.4 g, dissolve in 30 ml of *water*, add 1 drop of *methyl red solution* and titrate with *0.05 M sulphuric acid* until the yellow colour changes to red. Boil gently for 2 minutes, cool and, if necessary, continue the titration with *0.05 M sulphuric acid* until the red colour is restored.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.002299 g of Na.

Storage. Store protected from light and moisture.

Thiopentone Injection

Thiopentone Sodium Injection; Thiopental Injection

Thiopentone Injection is a sterile material consisting of Thiopentone Sodium with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Thiopentone Injection contains thiopentone, $C_{11}H_{18}N_2O_2S$ that is not less than 77.0 per cent and not more than 92.0 per cent and sodium, Na that is not less than 9.4 per cent and not more than 11.8 per cent of the stated amount of thiopentone sodium.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strengths. 250 mg; 500 mg; 1 g; 5 g.

Description. A yellowish white powder; hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Acidify 10 ml of a 10 per cent w/v solution in *carbon dioxide-free water* with *2 M hydrochloric acid*; the solution effervesces. Shake the solution with 20 ml of *ether*, separate the ether layer, wash with 10 ml of *water* and dry over

anhydrous sodium sulphate. Filter, evaporate the filtrate to dryness and dry the residue at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiopentone RS* or with the reference spectrum of thiopentone.

B. Complies with the test for identification of barbiturates (2.3.2), but using the following solutions.

Test solution. 0.1 per cent w/v solution of the substance under examination in *water*.

Reference solution. Dissolve 85 mg of *thiopentone RS* in 10 ml of *2 M sodium hydroxide* and dilute to 100 ml with *water*.

C. Acidify 5 ml of a 5 per cent w/v solution with *dilute acetic acid* and filter. Wash the precipitate with *water*, recrystallise from *water* and dry at 70°; the crystals melt at about 160° (2.4.21).

D. Dissolve 1 mg of the crystals obtained in test A in 1 ml of *0.1 M sodium hydroxide*. Add about 1 mg of *sodium nitroprusside* and, after 15 minutes, 1 ml of *dilute hydrochloric acid*; a reddish violet colour is produced.

E. Gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* (solution A) is clear (2.4.1), and not more intensely coloured than reference solution GYS3 (2.4.1).

Related substances (2.3.4). Complies with the test, but using *water* as the solvent for the test solution and the reference solution. After development, examine the plate in ultraviolet light at 254 nm but do not spray it with the *diphenylcarbazone-mercury reagent*.

Loss on drying (2.4.19). Not more than 2.5 per cent, determined on 0.5 g by drying in an oven at 100° at a pressure of 1.5 to 2.5 kPa for 4 hours.

Assay. *For thiopentone* — Mixed the contents of 10 containers and weigh accurately about 0.15 g, of the contents, dissolve in 5 ml of *water*, add 2 ml of *1 M sulphuric acid* and extract with four quantities, each of 10 ml, of *chloroform*. Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water-bath and dissolve the residue in 30 ml of *dimethylformamide*, previously neutralised with *0.1 M lithium methoxide*. Titrate immediately with *0.1 M lithium methoxide*, using 1 drop of a 0.2 per cent w/v solution of *thymol blue* in *methanol* as indicator, until a blue colour is obtained. Protect the solution from absorption of carbon dioxide during the titration.

1 ml of *0.1 M lithium methoxide* is equivalent to 0.02423 g of $C_{11}H_{18}N_2O_2S$.

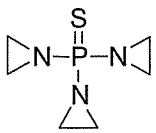
For sodium — Mixed the contents of 10 containers and weigh accurately about 0.4 g of the contents, dissolve in 30 ml of *water*, add 1 drop of *methyl red solution* and titrate with *0.05 M sulphuric acid* until the yellow colour changes to red. Boil gently for 2 minutes, cool and, if necessary, continue the titration with *0.05 M sulphuric acid* until the red colour is restored

1 ml of *0.05 M sulphuric acid* is equivalent to 0.002299 g of Na.

Storage. Store in single dose containers.

Labelling. The label states the amount of active ingredient in terms of Thiopentone Sodium.

Thiotepa



$C_6H_{12}N_3PS$ Mol. Wt. 189.2

Thiotepa is tris(1-aziridinyl)phosphine sulphide.

Thiotepa contains not less than 97.0 per cent and not more than 102.0 per cent of $C_6H_{12}N_3PS$, calculated on the anhydrous basis.

Category. Anticancer.

Dose. By injection, 300 to 400 µg per kg of body weight at intervals of 1 to 4 weeks.

Description. Fine, white, crystalline flakes; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thiotepa RS* or with the reference spectrum of thiotepa.

B. Determine on 20 mg by the oxygen-flask method (2.3.34), using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 ml with *water* (solution A). To 5 ml of solution A add 0.1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*; a turbidity is produced.

C. To 2 ml of solution A add 40 ml of *water* and 4 ml of *ammonium molybdate-sulphuric acid solution*, mix, add 0.1 g of *L-ascorbic acid* and boil for 1 minute; a blue colour is produced.

Tests

Appearance of solution. A 2.0 per cent w/v solution is clear (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.35 g of the substance under examination 100.0 ml of the *water*.

Reference solution (a). Dissolve 0.35 mg of the substance under examination in 100.0 ml of the *water*.

Reference solution (b). Dissolve 10 mg of the substance under examination in 2 ml of *methanol* in a ground-glass-stoppered tube, add 50 µl of a 0.1 per cent v/v solution of *orthophosphoric acid*, stopper the tube and heat in a water bath at 65° for 50 seconds (generation of methoxythiotepa). Allow the solution to cool and add 1 ml of *methanol*.

Reference solution (c). Dissolve 15 mg of the substance under examination in 10 ml of *water*, add 1 g of *sodium chloride*, boil in a water bath for 10 minutes and cool (generation of chloro- adduct).

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- mobile phase: a mixture of 15 volumes of *acetonitrile* and 85 volumes of *0.1 M phosphate buffer pH 7.0*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the two principal peaks is not less than 3. The relative retention time with reference to thiotepa for methoxythiotepa is about 1.3 and for chloro-adduct (thiotepa impurity A) is about 3.75.

Inject the test solution and reference solution (a). Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any peak corresponding to the chloro-adduct is not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent).

Water (2.3.43). Not more than 2.0 per cent, determined on 1.2 g.

Assay. Weigh accurately about 0.2 g into a stoppered flask, add 50 ml of a 20 per cent w/v solution of *sodium thiosulphate* and titrate immediately with *0.1 M hydrochloric acid*, using *methyl orange solution* as indicator, until a faint red colour persists for 10 seconds. Stopper the flask, allow to stand for 30 minutes and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator. Subtract the volume of *0.1 M sodium hydroxide* used from the volume of *0.1 M hydrochloric acid* used. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *hydrochloric acid* required.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.006307 g of $C_6H_{12}N_3PS$.

Storage. Store protected from light and moisture. At higher temperatures it tends to polymerise with loss of activity.

Thiotepa Injection

Thiotepa Injection is a sterile material consisting of Thiotepa with or without auxiliary agents. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Thiotepa Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of thiotepa, $C_6H_{12}N_3PS$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strength. 15 mg.

Description. A white powder.

Identification

A. Determine on 20 mg by the oxygen-flask method (2.3.34), using 5 ml of *1.25 M sodium hydroxide* as the absorbing liquid. When the process is complete, dilute to 25 ml with *water* (solution A). To 5 ml of solution A add 0.1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *1 M hydrochloric acid*, mix and add 0.05 ml of *barium chloride solution*; a turbidity is produced.

B. To 2 ml of solution A add 40 ml of *water* and 4 ml of *ammonium molybdate-sulphuric acid solution*, mix, add 0.1 g of *L-ascorbic acid* and boil for 1 minute; a blue colour is produced.

Tests

Appearance of solution. Dissolve a quantity containing 15 mg of Thiotepa in 4 ml of *water*; the solution is clear (2.4.1).

pH (2.4.24). 5.5 to 7.5, determined in a 1.0 per cent w/v solution in *carbon dioxide-free water*.

Bacterial endotoxins (2.2.3). Not more than 6.25 Endotoxin Units per mg of Thiotepa.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute a suitable quantity of the injection containing 0.15 g of Thiotepa in 100 ml of *water*.

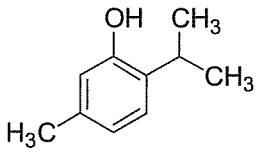
Reference solution. A 0.15 per cent w/v solution of *thiotepa RS* in *water*.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *water* and 30 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Calculate the content of $C_6H_{12}N_3PS$ in the injection.

Storage. Store protected from light. If solid matter separates from the constituted injection, the solution should not be used.

Thymol



$C_{10}H_{14}O$ Mol. Wt. 150.2

Thymol is 2-isopropyl-5-methylphenol.

Thymol contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{10}H_{14}O$.

Category. Pharmaceutical aid (antimicrobial preservative; antiseptic).

Description. Colourless crystals; odour, characteristic.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *thymol RS* or with the reference spectrum of thymol.

B. Dissolve 0.2 g with heating in 2 ml of *2 M sodium hydroxide*, add 0.2 ml of *chloroform* and heat on a water-bath; a violet colour develops.

C. Dissolve about 2 mg in 1 ml of *anhydrous acetic acid*, add 0.15 ml of *sulphuric acid* and 0.05 ml of *nitric acid*; a bluish green colour develops.

D. Melting range (2.4.21). 48° to 52°.

Tests

Appearance of solution. Dissolve 1.0 g in 10 ml of *2 M sodium hydroxide*. The solution is not more opalescent than opalescence standard OS4 (2.4.1), and not more intensely coloured than reference solution RS6 (2.4.1).

Acidity. To 1.0 g in a glass-stoppered flask add 20 ml of *water*, boil until dissolution is complete, cool, stopper the flask and shake vigorously for 1 minute. Add a few crystals of the substance under examination to induce crystallisation, shake vigorously for 1 minute and filter. To 5 ml of the filtrate add 0.05 ml of *methyl red solution* and 0.05 ml of *0.01 M sodium hydroxide*; the solution is yellow.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.1 g in sufficient *ethanol (95 per cent)* to produce 10 ml.

Reference solution (a). Dilute 1 ml of the test solution to 100 ml with *ethanol (95 per cent)*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 10 ml with *ethanol (95 per cent)*.

Reference solution (c). Dilute 5 ml of reference solution (b) to 10 ml with *ethanol (95 per cent)*.

Chromatographic system
- a glass column 4 m × 2 mm, packed with diatomaceous support (125 to 180 mesh) impregnated with a mixture suitable for the separation of free fatty acids (such as FFAP),
- temperature:
  column 80° for 2 minutes, increase @ 8° to 240° stand for 15 minutes,
  inlet port at 250° and detector at 300°,
- flow rate. 30 ml per minute of the carrier gas.

Inject test solution, reference solution (a), (b) and (c).

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not more than the area of the principal peak in the chromatogram obtained with

reference solution (a). Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with reference solution (c).

Residue on evaporation. Evaporate 2.0 g on a water-bath and heat at 105° for 1 hour. The residue weighs not more than 1.0 mg (0.05 per cent).

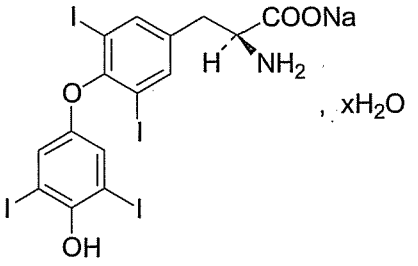
Assay. Weigh accurately about 0.1 g, transfer to an iodine flask and dissolve in 25 ml of *1 M sodium hydroxide.* Add 20 ml of hot *dilute hydrochloric acid* and immediately titrate with *0.05 M bromine* to within 1 to 2 ml of the calculated end-point. Warm the solution to about 75°, add 0.1 ml of *methyl orange solution* and shake vigorously. If the solution is red, continue the titration, dropwise and with shaking until the red colour is discharged. Repeat the alternate addition of *0.05 M bromine* and *methyl orange solution* until the red colour is discharged after the addition of the methyl orange solution.

1 ml of *0.05 M bromine* is equivalent to 0.003755 g of $C_{10}H_{14}O$.

Storage. Store protected from light and moisture.

Thyroxine Sodium

Levothyroxine Sodium; L-Thyroxine Sodium



$C_{15}H_{10}I_4NNaO_4,xH_2O$ Mol. Wt. 798.9 (anhydrous)

Thyroxine Sodium is sodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-L-tyrosinate and contains a variable quantity of water of crystallisation.

Thyroxine Sodium contains not less than 97.0 per cent and not more than 101.0 per cent of $C_{15}H_{10}I_4NNaO_4$, calculated on the dried basis.

Category. Thyroid hormone.

Dose. 50 to 300 µg daily.

Description. A white or slightly brownish yellow powder, slightly coloured, crystalline powder.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M sodium hydroxide* shows an absorption maximum at about 325 nm; absorbance at about 325 nm, 0.73 to 0.79.

B. In the test for Liothyronine, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

C. To about 50 mg in a porcelain dish add a few drops of *sulphuric acid (96 per cent w/w)*; violet vapors are evolved.

D. To 20 mg add 2 ml of *1 M sulphuric acid.* Heat on a water-bath followed by heating carefully over a naked flame, increasing the temperature to about 600°. Continue ignition until most of the black particles have disappeared. Dissolve the residue in 2 ml of *water*; the solution gives reaction A of sodium salts (2.3.1).

Tests

Appearance of solution. Dissolve 0.5 g in 23 ml of a gently boiling mixture of 4 volumes of *ethanol (95 per cent)* and 1 volume of *1 M hydrochloric acid.* Cool and dilute to 25 ml with the same mixture of solvents (solution A). The freshly prepared solution is not more intensely coloured than reference solution BYS3 (2.4.1).

Specific optical rotation (2.4.22).+16.0° to +20.0°, determined in solution A.

Liothyronine. Determine by thin-layer chromatography (2.4.17), coating the plate with a slurry of 30 g of *silica gel H* in 60 ml of a 0.75 per cent w/v solution of *soluble starch.*

Solvent mixture. 70 volumes of *methanol* and 5 volumes of *strong ammonia solution.*

Mobile phase. A mixture of 55 volumes of *ethyl acetate,* 35 volumes of *2-propanol* and 20 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). A solution containing 1.0 per cent w/v of the substance under examination and 0.01 per cent w/v of the *liothyronine RS* in the solvent mixture.

Reference solution (b). A 1.0 per cent w/v solution of *levothyroxine sodium RS* in the solvent mixture.

Reference solution (c). A 0.01 per cent w/v solution of *liothyronine RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray lightly with *ferric chloride-ferricyanide-arsenite solution.* Any spot corresponding to liothyronine in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (c). The test is not valid unless

the chromatogram obtained with reference solution (a) shows two clearly separated spots.

Loss on drying (2.4.19). 6.0 to 12.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 40 ml of *0.1 M sodium hydroxide*. Shake for about 30 minutes, add 40 ml of *methanol*, cool, mix well and dilute to 100 ml with a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol*. Dilute 5.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution. A 0.001 per cent w/v solution of *levothyroxine sodium RS* in a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with a stationary phase consisting of porous silica particles (5 to 10 µm) to which nitrile groups are chemically bonded,
- mobile phase: a mixture of 65 volumes of *water*, 1 volume of *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{10}I_4NNaO_4$.

Storage. Store protected from light and moisture.

Thyroxine Tablets

Thyroxine Sodium Tablets; Levothyroxine Tablets; Levothyroxine Sodium Tablets; L-Thyroxine Sodium Tablets

Thyroxine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of anhydrous thyroxine sodium, $C_{15}H_{10}I_4NNaO_4$.

Usual strength. 50 µg.

Identification

A. To a quantity of the powdered tablets containing 500 µg of *anhydrous thyroxine sodium* add a mixture of 3 ml of *ethanol (50 per cent)* and 0.2 ml of *hydrochloric acid*, boil gently for 30 seconds, cool, filter, add 0.1 ml of a 10 per cent w/v solution of *sodium nitrite* and boil; a yellow colour is produced. Cool and make alkaline with *5 M ammonia*; the solution becomes orange.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to thyroxine sodium in the chromatogram obtained with the reference solution.

Tests

Uniformity of content. Comply with the test stated under Tablets. using the following solutions.

Test solution. To one tablet, add 10 ml of *0.1 M sodium hydroxide* and shake for about 30 minutes. Add 10 ml of *methanol*, cool and dilute with sufficient of a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol* to produce 25.0 ml, mix well and filter. Dilute, if necessary, with the same solvent mixture to produce a solution containing 0.0002 per cent w/v of Thyroxine Sodium.

Reference solution. A 0.0002 per cent w/v solution of *levothyroxine sodium RS* in a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol*.

Follow the procedure described under Assay.

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 or more tablets. Weigh accurately a quantity of the powder containing about 1 mg of Thyroxine Sodium, add 40 ml of *0.1 M sodium hydroxide* and shake for about 30 minutes. Add 40 ml of *methanol*, cool, mix well and dilute with sufficient of a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol* to produce 100.0 ml, mix well and filter.

Reference solution. A 0.001 per cent w/v solution of *levothyroxine sodium RS* in a mixture of equal volumes of *0.1 M sodium hydroxide* and *methanol*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with a stationary phase consisting of porous silica particles (5 to 10 µm) to which nitrile groups are chemically bonded,
- mobile phase: a mixture of 65 volumes of *water*, 1 volume of *orthophosphoric acid* and 35 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{10}I_4NNaO_4$ in the tablets.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of anhydrous thyroxine sodium.

Ticarcillin and Clavulanic Acid Injection

Clavulanic Acid and Ticarcillin Injection

Ticarcillin and Clavulanic Acid Injection is a sterile iso-osmotic solution of Ticarcillin Monosodium and Clavulanate Potassium in Water for Injections. It contains one or more suitable buffering agents and a tonicity adjusting agent.

Ticarcillin and Clavulanic Acid Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of ticarcillin, $C_{15}H_{16}N_2O_6S_2$ and not less than 85.0 per cent and not more than 120.0 per cent of the stated amount of clavulanic acid, $C_8H_9NO_5$.

Ticarcillin and Clavulanic Acid for Injection

Ticarcillin and Clavulanic acid for Injection is a sterile, dry mixture of Ticarcillin Monosodium and Clavulanate Potassium.

The constituted solution complies with the requirements for clarity of solution and particulate matter stated under Parenteral Preparations (Injections).

Usual Strength. Ticarcillin 3 g and Clavullanic acid 100 mg.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Identification

In the Assay, the principal peak due to ticarcillin in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 5.5 to 7.5.

Bacterial endotoxins (2.2.3). Not more than 0.07 Endotoxin Unit per mg of ticarcillin.

Sterility (2.2.11). Complies with the test for sterility.

Water (2.3.43). Not more than 4.2 per cent.

Other tests. Complies with the tests stated under Parenteral Preparations (Powders for Injections).

Assay. Determine by liquid chromatography (2.4.14).

Sodium phosphate buffer pH 4.3. Dissolve 13.8 g of *monobasic sodium phosphate* in 900 ml of *water*, adjust the pH to 4.3 with *orthophosphoric acid* or *10 M sodium hydroxide,* dilute to 1000 ml with *water*.

Sodium phosphate buffer pH 6.4. Dissolve 6.9 g of *monobasic sodium phosphate* in 900 ml of *water*, adjust the pH to 6.4 with *10 M sodium hydroxide*, dilute 1000 ml with *water*.

Clavulanate lithium stock solution. A 0.06 per cent w/v solution of *clavulanate lithium RS* in *sodium phosphate buffer pH 6.4*.

Test solution. Dissolve a quantity of powder in sufficient *water* and dilute with *sodium phosphate buffer pH 6.4* to obtain a 0.09 per cent w/v solution of Ticarcillin.

Reference solution. Transfer about 100 mg of *ticarcillin monosodium monohydrate RS* to a 100-ml volumetric flask and add 150/J ml of *clavulanate lithium stock solution* (J is the ratio of the stated amount of ticarcillin to the stated amount of clavulanic acid in the powder) and dilute to100.0 ml with *sodium phosphate buffer pH 6.4*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 95 volumes of *sodium phosphate buffer pH 4.3* and 5 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency of the principal peaks is not less than 1000 theoretical plates; the tailing factor for the principal peaks are not more than 2.0; the resolution between the peaks due to ticarcillin and clavulanic acid is not less than 5.0; and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to ticarcillin for clavulanic acid is about 0.2.

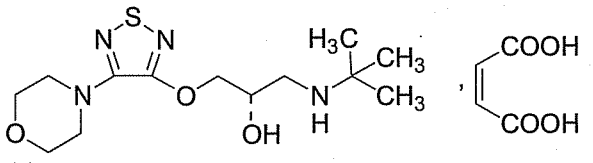
Inject the test solution and the reference solution.

Calculate the content of $C_{15}H_{16}N_2O_6S_2$ and $C_8H_9NO_5$ in the injection.

Storage. Store in single dose or multiple dose containers.

Labelling. The label states (1) that the injection is to be thawed just prior to use; (2) conditions for proper storage of the resultant solution; (3) directs that the solution is not to be refrozen.

Timolol Maleate



$C_{13}H_{24}N_4O_3S,C_4H_4O_4$ Mol. Wt. 432.5

Timolol Maleate is (*S*)-1-*tert*-butylamino-3-(4-morpholino-1,2,5-thiadiazol-3-yloxy)propan-2-ol hydrogen maleate.

Timolol Maleate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$, calculated on the dried basis.

Category. Beta-adrenoceptor antagonist (Antiglaucoma).

Dose. In hypertension, initially 5 mg twice daily or 10 mg once daily; maximum 60 mg daily. In angina, initially 5 mg 2 to 3 times daily; maintenance dose, 15 to 45 mg daily. For prophylaxis after infarction, initially 5 mg twice daily, started 1 to 4 weeks after infarction. For migraine prophylaxis, 10 to 20 mg daily.

Description. A white or almost white, crystalline powder or colourless crystals.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *timolol maleate RS* or with the reference spectrum of timolol maleate.

B. In the test for Related substances, after exposure to iodine vapour, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b).

C. Triturate 0.1 g with a mixture of 1 ml of *2 M sodium hydroxide* and 3 ml of *water* and shake with three quantities, each of 5 ml, of *ether*. To 0.1 ml of the aqueous layer add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat on a water-bath for 15 minutes; no violet-red colour is produced. Neutralise the remainder of the aqueous layer with *1 M sulphuric acid*, add 1 ml of *bromine water*, heat on a water-bath for 15 minutes, then heat to boiling and cool. To 0.2 ml of this solution add a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat on a water-bath for 15 minutes; a violet-red colour is produced. Add 0.2 ml of a 10 per cent w/v solution of *potassium bromide* and heat on a water-bath for 5 minutes; the colour changes to violet-blue.

Tests

Appearance of solution. A 2.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution BS8 (2.4.1).

pH (2.4.24). 3.8 to 4.3, determined in a 2.0 per cent w/v solution.

Specific optical rotation (2.4.22). –5.7° to –6.2°, determined in a 10.0 per cent w/v solution in *1 M hydrochloric aeid.*

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *dichloromethane*, 20 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 10 ml of *methanol.*

Test solution (b). Dissolve 0.1 g of the substance under examination in 100 ml of *methanol.*

Reference solution (a). A 0.02 per cent w/v solution of the substance under examination in *methanol.*

Reference solution (b). A 0.1 per cent w/v solution of *timolol maleate RS* in *methanol.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm and then expose to iodine vapour for 2 hours and examine in daylight. By both methods of visualisation, any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.35 g, dissolve in 60 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04325 g of $C_{13}H_{24}N_4O_3S, C_4H_4O_4$.

Storage. Store protected from light and moisture.

Timolol Eye Drops

Timolol Maleate Eye Drops

Timolol Eye Drops are a sterile solution of Timolol Maleate in Purified Water. They may contain suitable antimicrobial preservatives, buffering agents, stabilisers and suitable substances to increase the viscosity of the solution.

Timolol Eye Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of timolol, $C_{13}H_{24}N_4O_3S$.

Usual strengths. The equivalent of 0.25 per cent w/v and 0.5 per cent w/v of timolol.

Identification

A. Add a volume containing 50 mg of timolol to an equal volume of *carbonate buffer pH 9.7* and extract with two quantities, each of 40 ml, of *dichloromethane*. Reserve the aqueous layer for test B. Dry the combined dichloromethane extracts with *anhydrous sodium sulphate* and evaporate to dryness. Dry the residue at 60° at a pressure of 2 kPa for 15 minutes.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *timolol RS* or with the reference spectrum of timolol.

B. Filter the aqueous layer reserved in test A and evaporate to about 1 ml. Add 1 ml of *bromine solution*, heat in a water-bath for 10 minutes, boil, cool and add 0.1 ml of the solution to a solution of 10 mg *resorcinol* in 3 ml of *sulphuric acid*; a bluish black colour is produced on heating in a water-bath for 15 minutes.

Tests

pH (2.4.24). 6.5 to 7.5.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Use the undiluted eye drops.

Reference solution (a). Dilute 1 volume of the eye drops to 250 volumes with the mobile phase.

Reference solution (b). Dilute 1 volume of the eye drops to 500 volumes with the mobile phase.

Reference solution (c). A 0.3 per cent w/v solution of *maleic acid* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 57.5 volumes of *methanol* and 42.5 volumes of *0.02 M sodium octanesulphonate*, adjusted to pH 3.0 with *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume. 20 µl.

Record the chromatogram of the test solution for 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak, other than the peak corresponding to maleic acid, is not greater than the area of the peak in the chromatogram obtained with reference solution (a) and not more than two such peaks have an area greater than that of the peak obtained with reference solution (b).

Other tests. Comply with the tests stated under Eye Drops.

Assay. To a volume containing about 25 mg of timolol add *water* to produce 50.0 ml and mix. To 5.0 ml add 15 ml of *carbonate buffer pH 9.7* and extract with three quantities, each of 20 ml, and one quantity of 10 ml of *toluene*. Wash each extract successively with the same 10-ml volume of *carbonate buffer pH 9.7*. Combine the toluene extracts and extract with four quantities, each of 20 ml, of *0.05 M sulphuric acid*. Combine the extracts, dilute to 100.0 ml, filter and measure the absorbance of the filtrate at the maximum at about 295 nm (2.4.7), using as the blank a solution prepared by treating 5.0 ml of *water* in the same manner beginning at the words "add 15 ml of *carbonate buffer pH 9.7...*".

Calculate the content of $C_{13}H_{24}N_4O_3S$ taking 279 as the specific absorbance at 295 nm.

Storage. Store protected from light and moisture.

Labelling. The label states (1) the strength in terms of the equivalent amount of timolol; (2) the names and concentration of any added antimicrobial preservatives.

Timolol Tablets

Timolol Maleate Tablets

Timolol Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of timolol maleate, $C_{13}H_{24}N_4O_3S,C_4H_4O_4$.

Usual strength. 10 mg.

Identification

A. To a quantity of the powdered tablets containing 70 mg of Timolol Maleate add 20 ml of *carbonate buffer pH 9.7* and extract with two quantities, each of 40 ml, of *dichloromethane*. Reserve the aqueous layer for test B. Dry the extracts with *anhydrous sodium sulphate*, evaporate to dryness using a rotary evaporator, dry the residue at 60° at a pressure of 2 kPa for 15 minutes.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *timolol RS* or with the reference spectrum of timolol.

B. Filter the aqueous layer reserved in test A and evaporate to about 1 ml. Add 1 ml of *bromine water*, heat on a water-bath

for 15 minutes, then heat to boiling and cool. Add 0.1 ml of this solution to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid* and heat on a water-bath for 15 minutes; a bluish black colour is produced.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 0.1 g of Timolol Maleate with 10 ml of the mobile phase for 10 minutes and filter.

Reference solution (a). Dilute 1 volume of the test solution to 250 volumes with the mobile phase.

Reference solution (b). Dilute 1 volume of the test solution to 500 volumes with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm × 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 57.5 volumes of *methanol* and 42.5 volumes of *0.02 M sodium octanesulphonate*, adjusted to pH 3.0 with *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 295 nm,
- injection volume. 20 µl.

Run the chromatogram of the test solution for 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak, other than the peak corresponding to maleic acid, is not more than the area of the peak in the chromatogram obtained with reference solution (a) and not more than two such peaks have an area more than that of the peak obtained with reference solution (b).

Uniformity of content. Comply with the test stated under Tablets.

To one tablet, add 25.0 ml of *0.05 M sulphuric acid*, shake for 20 minutes and complete the Assay beginning at the words "and centrifuge....".

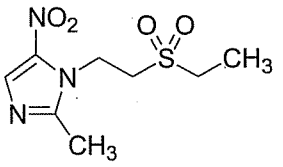
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 15 mg of Timolol Maleate, add 25.0 ml of *0.05 M sulphuric acid*, shake for 20 minutes and centrifuge until clear. Add 5.0 ml of the resulting supernatant liquid to 15 ml of *carbonate buffer pH 9.7* and extract with three quantities, each of 20 ml, and one quantity of 10 ml of *toluene*. Wash each extract successively with the same 10-ml volume of *carbonate buffer pH 9.7*, combine the toluene extracts and extract with four quantities, each of 20 ml, of *0.05 M sulphuric acid*. Combine the acid extracts, dilute to 100.0 ml, filter and measure the absorbance of the filtrate at the maximum at about 295 nm (2.4.7), using as the blank a solution prepared by treating a mixture of 5 ml of *water* and 15 ml of *carbonate buffer pH 9.7* in the same manner beginning at the words "and extract with three quantities,..."

Calculate the content of $C_{13}H_{24}N_4O_3S,C_4H_4O_4$ taking 204 as the specific absorbance at 295 nm.

Storage. Store protected from moisture.

Tinidazole



$C_8H_{13}N_3O_4S$ Mol. Wt. 247.3

Tinidazole is 1-[2-(ethylsulphonyl)ethyl]-2-methyl-5-nitroimidazole.

Tinidazole contains not less than 98.0 per cent and not more than 100.5 per cent of $C_8H_{13}N_3O_4S$, calculated on the dried basis.

Category. Antiprotozoal.

Dose. For trichomoniasis, 2 g as a single dose and repeat after 3 to 5 days rest, if necessary. For giardiasis, 150 mg twice daily for seven days or as a single dose of 2 g. For intestinal amoebiasis, 600 mg as a single dose daily for 2 to 6 days. For extraintestinal amoebiais, 2 g as a single daily dose for 3 days.

Description. Pale yellow crystals or a crystalline powder; odour, slight and characteristic.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tinidazole RS* or with the reference spectrum of tinidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 310 nm; absorbance at about 310 nm, about 0.35.

C. To about 5 mg, add 5 ml of *0.1 M hydrochloric acid*, 50 mg of *zinc powder*, 4 ml of *hydrochloric acid* and allow to stand for 30 minutes. Add 4 ml of a 1 per cent w/v solution of *vanillin*, heat on a boiling water-bath for 20 minutes, allow to cool to room temperature and dilute to 20 ml with *water*; a greenish yellow colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 95 volumes of *ethyl acetate*, 5 volumes of *methanol* and 5 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of a mixture of equal volumes of *chloroform* and *methanol*.

Reference solution. A 0.02 per cent w/v solution of the substance under examination in a mixture of equal volumes of *chloroform* and *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 105° for 4 hours.

Assay. Weigh accurately about 0.5 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using 0.15 ml of *nile blue A solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02473 g of $C_8H_{13}N_3O_4S$.

Storage. Store protected from light and moisture.

Tinidazole Tablets

Tinidazole Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tinidazole, $C_8H_{13}N_3O_4S$.

Usual strengths. 150 mg; 300 mg; 500 mg.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum at about 310 nm.

Extract a quantity of the powdered tablets containing 0.1 g of Tinidazole with 10 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following tests.

B. To about 5 mg add 5 ml of *0.1 M hydrochloric acid*, 50 mg of *zinc powder*, 4 ml of *hydrochloric acid* and allow to stand for 30 minutes. Add 4 ml of a 1 per cent w/v solution of *vanillin*, heat on a boiling water-bath for 20 minutes, allow to cool to room temperature and dilute to 20 ml with *water*; a greenish yellow colour is produced.

C. Melting range (2.4.21). 125° to 128°.

Tests

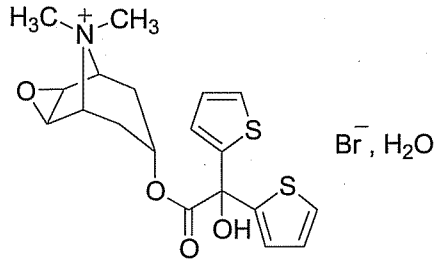
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.15 g of Tinidazole, add 20 ml of *methanol*, shake well and add sufficient *methanol* to produce 100.0 ml. Mix well and filter. Dilute 10.0 ml of the solution to 100.0 ml with *methanol* and further dilute 10.0 ml of this solution to 100.0 ml with *methanol*. Measure the absorbance of the resulting solution at the maximum at about 310 nm (2.4.7).

Calculate the content of $C_8H_{13}N_3O_4S$ taking 356 as the specific absorbance at 310 nm.

Storage. Store protected from light and moisture.

Tiotropium Bromide Monohydrate



$C_{19}H_{22}BrNO_4S_2, H_2O$ Mol. Wt. 490.2

Tiotropium Bromide Monohydrate is 6β,7β-epoxy-3β-hydroxy-8-methyl-1α*H*-5α*H*-tropanium bromide monohydrate.

Tiotropium Bromide Monohydrate contains not less than 98.0 per cent and not more than 102.0 per cent of tiotropium bromide, $C_{19}H_{22}NO_4S_2Br$, calculated on the anhydrous basis.

Category. Bronchodilator.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tiotropium bromide monohydrate RS* or with the reference spectrum of tiotropium bromide monohydrate.

B. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. A 0.002 per cent w/v solution of *tiotropium bromide monohydrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *methanol* and 55 volumes of a buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 5.5 with *dilute acetic acid (10 per cent v/v)*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 3000 theoretical plates.

Inject the test solution. Any individual impurity is not more than 0.5 per cent and the sum of all the impurities is not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 4.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 20 mg of the substance under examination in 100.0 ml of the mobile phase. Dilute 5.0 ml of the resulting solution to 50.0 ml with the mobile phase.

Reference solution. A 0.002 per cent w/v solution of *tiotropium bromide monohydrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 45 volumes of *methanol* and 55 volumes of a buffer solution prepared by dissolving 3.85 g of *ammonium acetate* in 1000 ml of *water* and adjusting the pH to 5.5 with *dilute acetic acid (10 per cent v/v)*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0, the column efficiency in not less than 3000 theoretical plates and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{22}NO_4S_2Br$.

Storage. Store protected from light and moisture.

Tiotropium Bromide Powder for Inhalation

Tiotropium Powder for Inhalation consists of Tiotropium Bromide in microfine powder either alone or admixed with Lactose in a pre-metered unit for use in a suitable powder inhaler.

Tiotropium Powder for Inhalation contains not less than 90.0 per cent and not more than 125.0 per cent of the stated amount of tiotropium, $C_{19}H_{22}NO_4S_2$ per unit dose.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Inhalation Preparations (Powders for Inhalation).

Follow the procedure described under Assay with suitable dilution of the reference solution wherever the amount of active substance is to be determined in any test.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 90 volumes of 0.05 per cent v/v *orthophosphoric acid* and 10 volumes of *acetonitrile*.

Test solution. Dissolve a quantity of the mixed contents of 20 capsules in sufficient of the solvent mixture to get a solution containing 1.8 µg per ml of Tiotropium.

Reference solution. A solution of *tiotropium bromide RS* equivalent to tiotropium 1.8 µg per ml in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of a buffer solution prepared by dissolving 2 ml *triethylamine* in 1000 ml of

water and adjusting the pH to 2.5 with *orthophosphoric acid*, and 20 volumes of *acetonitrile*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 237 nm,
- inject 200 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 4500 theoretical plates, the tailing factor is not more than 1.7 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{22}NO_4S_2$ per unit.

Storage. Store protected from moisture, at temperature not exceeding 30°.

Labelling. The label states the quantity of active ingredient per pre-metered unit.

Titanium Dioxide

TiO_2 Mol. Wt. 79.9

Titanium Dioxide contains not less than 98.0 per cent and not more than 100.5 per cent of TiO_2.

Category. Pharmaceutical aid and topical protectant.

Description. A white or almost white, infusible powder; odourless.

Identification

A. When strongly heated it becomes pale yellow; the colour is discharged on cooling.

B. To 0.5 g, add 5 g of *anhydrous sodium sulphate* and 10 ml of *water* and mix. Add 10 ml of *sulphuric acid* and boil gently until clear; cool, add slowly 30 ml of a 25 per cent v/v solution of *sulphuric acid* and dilute with *water* to 100 ml (solution A). To 5 ml of solution A add 0.1 ml of *strong hydrogen peroxide solution*; an orange-red colour is produced.

C. To 5 ml of solution A add 0.5 g of *zinc*, in granules; after 45 minutes a violet-blue colour is produced.

Tests

Appearance of solution. Solution A is not more opalescent than opalescence standard OS2 (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. Shake 5.0 g with 50 ml of *carbon dioxide-free water* for 5 minutes and centrifuge until a clear solution is obtained. To 10 ml of the solution add 0.1 ml of *bromothymol blue solution*. Not more than 1.0 ml of either *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

Water-soluble substances. Not more than 0.5 per cent, determined by the following method. Boil 10.0 g for 5 minutes with 150 ml of *water* containing 0.5 g of *ammonium sulphate*. Cool, dilute to 200 ml with *water* and filter until a clear solution is obtained. Evaporate 100 ml of the filtrate to dryness ignite and weigh.

Arsenic (2.3.10). To 0.2 g in a 100 ml Kjeldahl flask, add 2 g of *anhydrous sodium sulphate*, 7 ml of *sulphuric acid* and 5 ml of *nitric acid*. Heat gently until a clear solution is obtained, cool, add 10 ml of *water*, cool again and add 5 g of *hydrazine reducing mixture* and 10 ml of *hydrochloric acid*. Immediately attach an air condenser and distil into 15 ml of cooled *water* until a total volume of 30 ml is obtained. Rinse the condenser with 5 ml of *water* and dilute the combined distillate and rinsings to 40 ml with *water*. 20 ml of the resulting solution complies with the limit test for arsenic. Use a mixture of 0.5 ml of *arsenic standard solution (1 ppm As)* and 24.5 ml of *water* to prepare the standard (5 ppm).

Barium. Shake 20.0 g with 30 ml of *hydrochloric acid*, add 100 ml of *distilled water* and boil. Filter while hot through a hardened filter paper until a clear filtrate is obtained. Wash the filter with 60 ml of *distilled water* and dilute the combined filtrate and washings to 200 ml with *distilled water*. To 10 ml of this solution add 1 ml of *1 M sulphuric acid*. After 30 minutes any opalescence is not more intense than that of a mixture of 10 ml of the test solution and 1 ml of *distilled water*.

Heavy metals (2.3.13). Dilute 10 ml of the solution prepared in the test for Barium to 20 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm).

Iron. To 8 ml of solution A, add 4 ml of *water*, mix and add 0.05 ml of *bromine water*, allow to stand for 5 minutes, remove the excess of bromine with a current of air and add 3 ml of *potassium thiocyanate solution*. Any colour in the solution is not more intense than that in a standard prepared at the same time and in the same manner using a mixture of 4 ml of *iron standard solution (2 ppm Fe)* and 8 ml of a 20 per cent w/v solution of *sulphuric acid* (200 ppm).

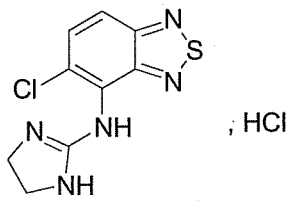
Assay. Weigh accurately about 0.5 g, transfer to a 300 ml Kjeldahl flask, add 5 g of *anhydrous sodium sulphate* and 10 ml of *water*, mix and add 10 ml of *sulphuric acid*. Boil gently until clear, cool, add slowly 40 ml of cooled *sulphuric acid* (25 per cent), cool again and dilute with *water* to 100.0 ml (solution B). To 300 g of *zinc*, in granules, add 300 ml of a 2 per cent w/v solution of *mercuric nitrate* and 2 ml of *nitric acid*, shake for 10 minutes and wash with *water*. Pack the zinc amalgam into a glass tube (400 mm x 20 mm) fitted with a tap and a filter plate. Pass through the column, at a rate of about 3 ml per minute, 100 ml of *1 M sulphuric acid* followed by 100 ml of *water*, ensuring that the amalgam is covered with liquid throughout. Pass slowly through the column, at a rate

of about 3 ml per minute, 200 ml of *0.5 M sulphuric acid* followed by 100 ml of *water*. Collect the combined eluates in a 500-ml conical flask containing 50 ml of a 15 per cent w/v solution of *ferric ammonium sulphate* in *sulphuric acid (25 per cent)* and titrate immediately with *0.1 M ceric ammonium nitrate* using *ferroin solution* as indicator until a greenish colour is obtained (n_1 ml). Pass slowly through the column 100 ml of *0.5 M sulphuric acid* followed by 20.0 ml of solution B, wash with 100 ml of *0.5 M sulphuric acid* followed by 100 ml of water. Collect the combined eluates in a 500-ml conical flask containing 50 ml of a 15 per cent w/v solution of *ferric ammonium sulphate* in *sulphuric acid (25 per cent)*, rinse the lower end of the column with *water* and titrate immediately with *0.1 M ceric ammonium nitrate* using *ferroin solution* as indicator until a greenish colour is obtained (n_2 ml). Calculate the percentage content of TiO_2 from the expression $3.99(n_2 - n_1)/w$

Where, *w* is the weight, in g, of the substance under examination used in the preparation of solution A.

Storage. Store protected from moisture. Avoid contact with aluminium.

Tizanidine Hydrochloride



$C_9H_8ClN_5S, HCl$ Mol. Wt. 290.2

Tizanidine Hydrochloride is 5-chloro-*N*-(2-imidazolin-2-yl)-2,1,3-benzothiadiazol-4-yl amine.

Tizanidine Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_9H_8ClN_5S.HCl$, calculated on the dried basis.

Category. α-adrenergic agonist.

Dose. 2 mg three times a day.

Description. A white to yellowish white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tizanidine hydrochloride RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. Gives reaction A for chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 5.3, determined on 5.0 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.1 per cent w/v solution of *tizanidine hydrochloride RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 50°,
- mobile phase: a mixture of 80 volumes of buffer solution prepared by dissolving 3.5 g of *sodium-1-pentane sulphonate* in 1000 ml of *water*, adjust the pH to 3.0 with 12 per cent *orthophosphoric acid solution* or *1 M sodium hydroxide* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0 and the column efficiency is not less than 5000 theoretical plates.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with the reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of *water*. 12 ml of this solution complies with limit test for heavy metals, Method D (20 ppm).

Total Chloride. 11.9 per cent to 12.5 per cent.

Weigh accurately about 0.5 g and dissolve in 50 ml of *water*. Titrate with *0.1 M silver nitrate*. Determine the end point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of chloride.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105° for 3 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 10.0 ml of the solution to 50.0 ml with the same solvent.

Reference solution. A 0.01 per cent w/v solution of *tizanidine hydrochloride RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of a buffer solution prepared by dissolving 6.8 g of *monobasic potassium phosphate* in 1000 ml of *water* adjusting the pH to 7.5 with *5.3 M potassium hydroxide*, and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_9H_8ClN_5S.HCl$.

Storage. Store protected from light.

Tizanidine Tablets

Tizanidine Hydrochloride Tablets

Tizanidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tizanidine, $C_9H_8ClN_5S$.

Dose. 2 mg; 4 mg.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium: 900 ml of *0.1 M hydrochloric acid*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate, suitably diluted with dissolution medium if necessary, at the maximum at about 320 nm (2.4.7).

Calculate the content of $C_9H_8ClN_5S$ in the medium from the absorbance obtained from a solution of known concentration of *tizanidine Hydrochloride RS* in the same medium.

D. Not less than 70 per cent of the stated amount of $C_9H_8ClN_5S$.

Uniformity of content. Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay.

Test solution. Crush one tablet and disperse in 50 ml *phosphate buffer pH 6.6,* dilute to 100.0 ml with *acetonitrile* and filter.

Reference solution. Weigh accurately 10 mg of *tizanidine hydrochloride RS*, dissolve in 25 ml *phosphate buffer pH 6.6* and dilute to 50.0 ml with *acetonitrile*. Dilute 5.0 ml of this solution to 50.0 ml with *acetonitrile*.

Calculate the content of $C_9H_8ClN_5S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powdered tablet containing about 20 mg of Tizanidine, disperse in 50 ml *phosphate buffer pH 6.6* and dilute to 100.0 ml with *acetonitrile* and filter.

Reference solution. Weigh accurately 10 mg of *tizanidine hydrochloride RS*, dissolve in 25 ml *phosphate buffer pH 6.6* and dilute to 50.0 ml with *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *phosphate buffer pH 6.6* and 20 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 320 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

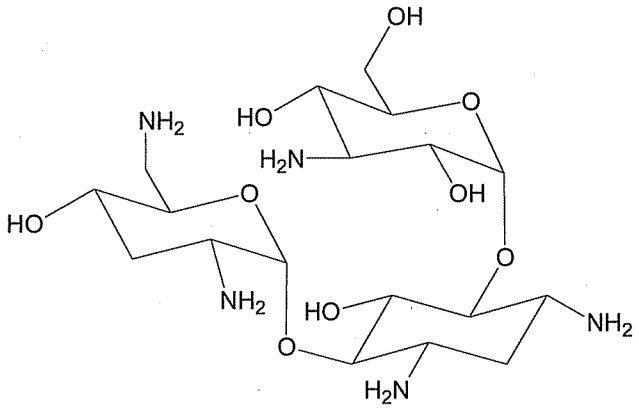
Inject the test solution and the reference solution.

Calculate the content of $C_9H_8ClN_5S$.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of Tizanidine.

Tobramycin



$C_{18}H_{37}N_5O_9$ Mol. Wt. 467.3

Tobramycin is 6-*O*-(3-amino-3-deoxy-α-D-glucopyranosyl)-2-deoxy-4-*O*-(2,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranosyl)-D-streptamine, an antimicrobial substance produced by *Streptomyces tenebrarius* or by any other means.

Tobramycin has potency not less than 930 Units per mg, calculated on the anhydrous and 2-methyl-1-propanol-free basis.

Category. Antibacterial.

Dose. By intramuscular or intravenous injection, 3 to 5 mg per kg daily, in divided doses.

Description. A white or almost white powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of 60 volumes of *methanol*, 40 volumes of *strong ammonia solution* and 20 volumes of *chloroform*.

Test solution. Dissolve 0.4 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.4 per cent w/v solution of *tobramycin RS* in *water*.

Reference solution (b). A solution containing 0.4 per cent w/v each of *kanamycin sulphate RS*, *neomycin sulphate RS* and *tobramycin RS* in *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air, spray with a mixture of equal volumes of a 46 per cent w/v solution of *sulphuric acid* and a 0.2 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated principal spots.

B. Dissolve about 5 mg in 5 ml of *water*, add 5 ml of a 0.1 per cent w/v solution of *ninhydrin* in *ethanol (95 per cent)* and heat in a water-bath for 3 minutes; a violet-blue colour develops.

Tests

pH (2.4.24). 9.0 to 11.0, determined in a 10.0 per cent w/v solution.

Specific optical rotation (2.4.22). +138° to +148°, determined in a 4.0 per cent w/v solution.

2-Methyl-1-propanol. Not more than 1.0 per cent w/w, determined by gas chromatography (2.4.13).

Test solution (a). A 10 per cent w/v solution of the substance under examination in *water*.

Test solution (b). A solution containing 10 per cent w/v of the substance under examination and 0.2 per cent v/v of *2-propanol* (internal standard).

Reference solution. A solution containing 0.1 per cent w/v of *2-methyl-1-propanol* and 0.2 per cent v/v of the internal standard.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with porous polymer beads (80 to 100 mesh) (such as Porapak Q),
- temperature. column 165°,

Calculate the percentage w/w of *2-methyl-1-propanol*.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Mobile phase. A mixture of equal volumes of *strong ammonia solution*, *2-butanone* and *ethanol (95 per cent)*.

Test solution. Dissolve 0.8 g of the substance under examination in 100 ml of *0.02 M ammonia*.

Reference solution. A 0.008 per cent w/v solution of the substance under examination in *0.02 M ammonia*.

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution (3 per cent Cl)* with *water* to contain 0.5 per cent w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue

colour with a drop of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.3 per cent.

Water (2.3.43). Not more than 8.0 per cent, determined on 0.3 g.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10).

Tobramycin intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of tobramycin.

Tobramycin intended for use in the manufacture of parenteral preparations or eye drops without a further appropriate sterilisation procedure complies with the following additional requirements.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture. If the material is sterile, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) where applicable, that it is sterile; (3) where applicable, that it is free from bacterial endotoxins and depressor substances.

Tobramycin Injection

Tobramycin Sulphate Injection

Tobramycin Injection is a sterile solution of Tobramycin in Water for Injections containing sufficient Sulphuric Acid to adjust the pH to 3.5 to 6.0.

Tobramycin Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of tobramycin, $C_{18}H_{37}N_5O_9$.

Usual strengths. 10 mg per ml; 40 mg per ml.

Description. A colourless solution.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of 60 volumes of *methanol*, 40 volumes of *strong ammonia solution* and 20 volumes of *chloroform.*

Test solution. Dilute a suitable volume of the injection with *water* to produce a solution containing 0.4 per cent w/v solution of tobramycin.

Reference solution (a). A 0.4 per cent w/v solution of *tobramycin RS* in *water.*

Reference solution (b). A solution containing 0.4 per cent w/v each of *kanamycin sulphate RS, neomycin sulphate RS* and *tobramycin RS* in *water.*

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air, spray with a mixture of equal volumes of a 46 per cent w/v solution of *sulphuric acid* and a 0.2 per cent w/v solution of *1,3-naphthalenediol* in *ethanol (95 per cent)* and heat at 105° for 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated principal spots.

B. Gives the reactions of sulphates (2.3.1).

Tests

pH (2.4.24). 3.5 to 6.0.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

Mobile phase. A mixture of equal volumes of *strong ammonia solution, 2-butanone* and *ethanol (95 per cent).*

Test solution. Dilute a suitable volume of the injection with *0.01 M ammonia* to obtain a solution containing 40 mg of Tobramycin in 4 ml. Shake with 10 ml of *ether* and use the aqueous layer.

Reference solution. A 0.008 per cent w/v solution of the substance under examination in *0.02 M ammonia.*

Apply to the plate 5 µl of each solution. After development, dry the plate in warm air, heat at 110° for 10 minutes and spray the hot plate with a solution prepared immediately before use by diluting *sodium hypochlorite solution (3 per cent Cl)* with *water* to contain 0.5 per cent w/v of available chlorine. Dry in a current of cold air until a sprayed area of the plate below the line of application gives at most a very faint blue colour with a drop of *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Bacterial endotoxins (2.2.3). Not more than 2.0 Endotoxin Units per mg of tobramycin.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

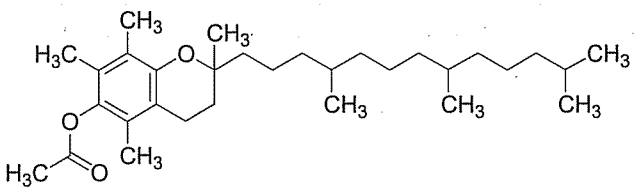
Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10).

Calculate the content of tobramycin in the injection, taking each 1000 Units found to be equivalent to 1 mg of tobramycin.

The upper fiducial limits of error is not less than 97.0 per cent and not more than 110.0 per cent of the stated potency.

Tocopheryl Acetate

α-Tocopheryl Acetate; α-Tocopherol Acetate; Vitamin E Acetate



$C_{31}H_{52}O_3$ Mol. Wt. 472.8

Tocopheryl Acetate is (2*RS*,4'*RS*,8'*RS*)-6-acetoxy-2,5,7,8- tetramethyl-2-(4', 8', 12'-trimethyltridecyl)chroman(*all-rac*-α-tocopherol acetate).

Tocopheryl Acetate contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{31}H_{52}O_3$.

Category. Vitamin E supplement.

Dose. Prophylactic, 5 to 10 mg; therapeutic, to be determined by the physician according to the needs of the patient.

Description. A clear, slightly greenish yellow, viscous, oily liquid.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *α-tocopheryl acetate RS*.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *ethanol* exhibits a maximum at about 284 nm, a shoulder at about 278 nm and a minimum at about 254 nm.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 80 volumes of *cyclohexane* and 20 volumes of *ether*.

Test solution (a). Dissolve 0.5 g of the substance under examination in 100 ml of *cyclohexane*.

Test solution (b). Dissolve 10 mg of the substance under examination in 2 ml of *5 M ethanolic sulphuric acid*, heat on a water-bath for 5 minutes, cool, add 2 ml of *water* and 2 ml of *cyclohexane* and shake for 1 minute; use the upper layer.

Reference solution (a). A 0.5 per cent w/v solution of *α-tocopheryl acetate RS* in *cyclohexane*.

Reference solution (b). Prepare in the same manner as test solution (b) but using 10 mg of *α-tocopheryl acetate RS* in place of the substance under examination.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with reference solution (a). There are two spots in each of the chromatograms obtained with test solution (b) and reference solution (b). The spots of higher R_f value are due to α-tocopheryl acetate and correspond to that in the chromatogram obtained with reference solution (a). The spots of lower R_f value are due to α-tocopheryl. Spray the plate with a mixture of 1 volume of *hydrochloric acid*, 4 volumes of a 0.25 per cent w/v solution of *ferric chloride* in *ethanol (95 per cent)* and 4 volumes of a 1.0 per cent w/v solution of *1,10-phenanthroline hydrochloride* in *ethanol (95 per cent)*. In the chromatograms obtained with test solution (b) and reference solution (b) the spot of lower R_f value α-tocopheryl is orange.

Tests

Refractive index (2.4.27). 1.494 to 1.498, determined at 20°.

Acid value (2.3.23). Not more than 2.0, determined on 2.0 g.

Free tocopherol. Not more than 2.0 per cent, determined by the following method. Weigh accurately about 0.5 g, dissolve in 100 ml of *0.25 M ethanolic sulphuric acid*, add 20 ml of *water* and 0.1 ml of a 0.25 per cent w/v solution of *diphenylamine* in *sulphuric acid* and titrate with *0.01 M ceric ammonium nitrate* until a blue colour is produced that persists for at least 5 seconds. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *ceric ammonium nitrate* required.

1 ml of *0.01 M ceric ammonium nitrate* is equivalent to 0.002154 g of tocopherol.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

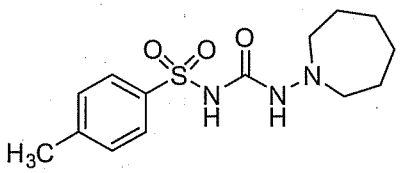
Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.3 g, dissolve in 25 ml of *ethanol (95 per cent)*, add 20 ml of *2.5 M ethanolic sulphuric acid* and heat on a water-bath under a reflux condenser for 3 hours. Cool, transfer the solution quantitatively to a 200-ml volumetric flask, rinse the apparatus with *ethanol (95 per cent)* and add the rinsings to the flask. Make up to volume with *ethanol (95 per cent)* and mix. To 25.0 ml of the resulting solution in a flask add 25 ml of *0.25 M ethanolic sulphuric acid*, 10 ml of *water* and titrate with *0.01 M ceric ammonium nitrate* using 0.1 ml of *diphenylamine* as indicator, until a blue colour persisting for at least 5 seconds is obtained. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of *ceric ammonium nitrate* required.

1 ml of *0.01 M ceric ammonium nitrate* is equivalent to 0.002364 g of $C_{31}H_{52}O_3$.

Storage. Store protected from light and moisture.

Tolazamide



$C_{14}H_{21}N_3O_3S$ Mol. Wt. 311.4

Tolazamide is 1-perhydroazepin-1-yl-3-tolyl-*p*-sulphonylurea

Tolazamide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{14}H_{21}N_3O_3S$, calculated on the dried basis.

Category. Hypoglycaemic.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolazamide RS* or with the reference spectrum of tolazamide.

B. When examined in the range 230 to 350 nm (2.4.7), a 0.04 per cent w/v solution in *ethanol (95 per cent)* exhibits maxima at 256 nm, 263 nm and 275 nm and a shoulder at 268 nm. The absorbances at the maxima are about 0.78, about 0.83 and about 0.62 respectively.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 200 volumes of *chloroform*, 100 volumes of *methanol*, 60 volumes of *cyclohexane* and 23 volumes of *13.5 M ammonia*.

Test solution. Dissolve 1.0 g of the substance under examination in 50 ml of *acetone*.

Reference solution. A 0.01 per cent w/v solution of *toluene-p-sulphonamide* in *acetone*.

Apply to the plate 10 µl of each solution. After removal of the plate, dry in a current of cold air, heat at 110° for 10 minutes, place the hot plate in a tank of chlorine gas prepared by the addition of *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry it in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch mucilage*; avoid prolonged exposure to cold air. Spray the plate with a 0.5 per cent w/v solution of *potassium iodide* in *starch mucilage*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.5 g, dissolve in 20 ml of *butan-2-one* with the aid of gentle heat. Allow to cool, add 30 ml of *ethanol (95 per cent)* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03114 g of $C_{14}H_{21}N_3O_3S$.

Tolazamide Tablets

Tolazamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tolazamide, $C_{14}H_{21}N_3O_3S$.

Usual strengths. 250 mg; 500 mg.

Identification

Triturate a quantity of the powdered tablets containing about 0.25 g of Tolazamide with 50 ml of *acetone* and filter. Evaporate the filtrate to dryness and dry the residue at 60° at a pressure of 2 kPa for 3 hours. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolazamide RS* or with the reference spectrum of tolazamide.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 23 volumes of *13.5 M ammonia*, 60 volumes of *cyclohexane*, 100 volumes of *methanol* and 200 volumes of *chloroform*.

Test solution. Shake a quantity of the powdered tablets containing about 0.2 g of Tolazamide with 10 ml of *acetone* and filter.

Reference solution. A 0.01 per cent w/v solution of *toluene-p-sulphonamide* in *acetone*.

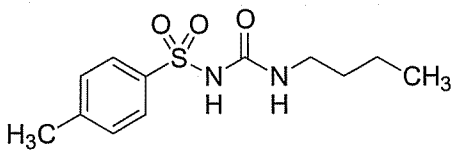
Apply to the plate 10 µl of each solution. After development, dry the plate in a current of cold air, heat at 110° for 10 minutes, place the hot plate in a tank of chlorine gas, prepared by the addition of *hydrochloric acid* to a 5 per cent w/v solution of *potassium permanganate* contained in a beaker placed in the tank and allow to stand for 2 minutes. Dry the plate in a current of cold air until an area of the plate below the line of application gives at most a very faint blue colour with a 0.5 per cent w/v solution of *potassium iodide* in *starch mucilage* avoid prolonged exposure to cold air. Spray the plate with a 0.5 per cent w/v solution of *potassium iodide* in *starch mucilage*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Shake a quantity of the powdered tablets containing about 0.5 g of Tolazamide with 50 ml of *chloroform*, filter, wash the residue with *chloroform* and evaporate the combined filtrate and washings to dryness. Dissolve the residue in 20 ml of *butan-2-one* with the aid of gentle heat. Allow to cool, add 30 ml of *ethanol (95 per cent)* and titrate the resulting solution with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.03114 g of $C_{14}H_{21}N_3O_3S$.

Tolbutamide



$C_{12}H_{18}N_2O_3S$ Mol. Wt. 270.4

Tolbutamide is 3-butyl-1-[(4-methylphenyl)sulphonyl]urea.

Tolbutamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{18}N_2O_3S$, calculated on the dried basis.

Category. Hypoglycaemic.

Dose. 500 mg to 1.5 g daily as a single dose with breakfast or in divided doses.

Description. A white, crystalline powder; almost odourless.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolbutamide RS* or with the reference spectrum of tolbutamide.

B. Dissolve 25 mg in sufficient *methanol* to produce 100.0 ml.

When examined in the range 245 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 258 nm, 263 nm and 275 nm and a shoulder at about 268 nm. Dilute the solution with *methanol* to produce a 0.001 per cent w/v solution. When examined in the range 220 nm to 235 nm, the resulting solution shows an absorption maximum at about 228 nm; absorbance at about 228 nm, about 0.50.

C. Boil 0.1 g with 8 ml of a 50 per cent v/v solution of *sulphuric acid* under a reflux condenser for 30 minutes. Make the solution strongly alkaline with *sodium hydroxide solution* and steam distil for 30 minutes, receiving the distillate in 20 ml of *0.1 M hydrochloric acid*. To 1 ml of the solution containing the distillate add 0.1 g of *sodium acetate* and 10 ml of *buffer solution pH 9.4*. Cool in an ice-bath for 10 minutes, add 1 ml of *diazotised nitroaniline solution*, set aside for 20 minutes and add dropwise 1 ml of *sodium hydroxide solution*; an orange-red colour is produced.

D. Boil 0.1 g with 8 ml of a 50 per cent v/v solution of *sulphuric acid* under a reflux condenser for 30 minutes. Cool in an ice-bath; a crystalline precipitate of *4-toluenesulphonylamide* is formed, which after recrystallisation from hot *water* and drying at 105° melts at 135° to 140° (2.4.21).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *1 M sodium hydroxide* is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.5 to 5.5, determined in a solution prepared by dissolving 2.0 g in 50 ml of *carbon dioxide-free water* by heating at 70° for 5 minutes, cooling rapidly and filtering.

Non-sulphonyl urea. Dissolve 0.5 g in 1 ml of *dilute ammonia solution* and 9 ml of *water*; not more than a faint opalescence is produced.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 8 volumes of *methanol* and 2 volumes of *anhydrous formic acid*.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of *acetone*.

Reference solution (a). A 0.015 per cent w/v solution of *4-toluenesulphonamide* in *acetone*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 5 µl of each of test solution and reference solution (a) and 10 µl of reference solution (b). After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. While still hot, place the plate in a chromatographic tank with an evaporating dish containing a 5 per cent w/v solution of *potassium permanganate*, add an equal volume of *hydrochloric acid* and close the tank. Leave the plate in the tank for 2 minutes, then place it in a current of cold air until the excess of chlorine is removed and an area of coating below the line of application gives only a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows two clearly separated spots.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Weigh accurately about 0.5 g and dissolve in a mixture of 40 ml of *ethanol (95 per cent)* and 20 ml of *water*. Titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02704 g of $C_{12}H_{18}N_2O_3S$.

Storage. Store protected from moisture.

Tolbutamide Tablets

Tolbutamide Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tolbutamide, $C_{12}H_{18}N_2O_3S$.

Usual strength. 500 mg.

Identification

Extract a quantity of the powdered tablets containing 1 g of Tolbutamide with 10 ml of *chloroform*, filter, evaporate the filtrate to dryness, scratching the sides of the vessel, if necessary, to induce crystallisation, and dry the residue at 105° for 30 minutes. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolbutamide RS* or with the reference spectrum of tolbutamide.

B. Boil 0.1 g of residue with 8 ml of a 50 per cent v/v solution of *sulphuric acid* under a reflux condenser for 30 minutes. Cool in an ice-bath; a crystalline precipitate of *4-toluenesulphonylamide* is formed, which after recrystallisation from hot *water* and drying at 105° melts at 135° to 140°.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 8 volumes of *methanol* and 2 volumes of *anhydrous formic acid*.

Test solution. Shake a quantity of the powdered tablets containing 0.5 g of Tolbutamide with 10 ml of *acetone* and filter.

Reference solution (a). A 0.015 per cent w/v solution of *4-toluenesulphonamide* in *acetone*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 5 µl of each of test solution and reference solution (a) and 10 µl of reference solution (b). After development, dry the plate in a current of warm air and heat at 110° for 10 minutes. While still hot, place the plate in a chromatographic tank with an evaporating dish containing a 5 per cent w/v solution of *potassium permanganate*, add an equal volume of *hydrochloric acid* and close the tank. Leave the plate in the tank for 2 minutes, then place it in a current of

cold air until the excess of chlorine is removed and an area of coating below the line of application gives only a very faint blue colour with *potassium iodide and starch solution*; avoid prolonged exposure to cold air. Spray the plate with *potassium iodide and starch solution* and allow to stand for 5 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows two clearly separated spots.

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of a solution containing 2.04 per cent w/v of *disodium hydrogen phosphate* and 0.135 per cent w/v of *potassium dihydrogen phosphate*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtrate suitably diluted if necessary, at the maximum at about 228 nm (2.4.7). Calculate the content of $C_{12}H_{18}N_2O_3S$ in the medium taking 417 as the specific absorbance at 228 nm.

D. Not less than 70 per cent of the stated amount of $C_{12}H_{18}N_2O_3S$.

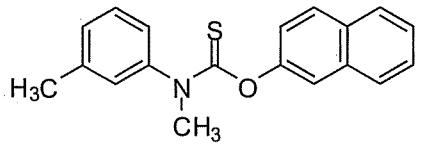
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.5 g of Tolbutamide, add 50 ml of *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution*, warm to dissolve, add 25 ml of *water* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein* solution as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02704 g of $C_{12}H_{18}N_2O_3S$.

Storage. Store protected from moisture.

Tolnaftate



$C_{19}H_{17}NOS$ Mol.Wt. 307.4

Tolnaftate is *O*-naphthalen-2-yl methyl(3-methylphenyl)-thiocarbamate.

Tolnaftate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{19}H_{17}NOS$, calculated on the dried basis.

Category. Antifungal.

Description. A white or yellowish white powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolnaftate RS* or with the reference spectrum of tolnaftate.

B. Determine by thin-layer chromatography (2.4.17), as described under Related substances.

The principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. Mix about 1 mg with 0.5 ml of *sulphuric acid*. Add 0.05 ml of *formaldehyde solution*. A greenish-blue colour develops.

D. Melting range (2.4.21). 109° to 112°.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *acetone* is clear (2.4.1) and not more intensely coloured than reference solution YS6 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Test solution (a). Dissolve 0.1 g of the substance under examination in 2 ml of *acetone*.

Test solution (b). Dilute 0.5 ml of test solution (a) to 10 ml with *acetone*.

Reference solution (a). Dissolve 25 mg of *tolnaftate RS* in 10 ml of *acetone*.

Reference solution (b). Dilute 1 ml of test solution (b) to 10 ml with *acetone*.

Reference solution (c). Dissolve 50 mg of *β-naphthol* in 1 ml of test solution (a) and dilute to 10 ml with *acetone*.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in current of air and examine under ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in vacuum at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Dissolve 50 mg in 250 ml of *methanol*. Dilute 2 ml of the solution to 50 ml with *methanol*. Measure the absorbance at the maximum at 257 nm (2.4.7).

Calculate the content of $C_{19}H_{17}NOS$ taking 720 as the specific absorbance at 257 nm.

Storage. Store protected from light.

Tolnaftate Cream

Tolnaftate Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tolnaftate, $C_{19}H_{17}NOS$.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. Toluene.

Test solution. Evaporate 10 ml of the Solution A on steam bath to dryness and dissolve the residue in 1.0 ml of *ethanol (95 per cent)*.

Reference solution. A 0.1 per cent w/v solution of *tolnaftate RS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Transfer an accurately weighed quantity of the cream containing about 10 mg of Tolnaftate to a separator containing 75 ml of *chloroform*. Wash the chloroform solution successively with two 25 ml portions of *0.1 M hydrochloric acid* and 25 ml of *water*. Filter the chloroform layer through a chloroform–washed cotton pledget into a 100-ml volumetric flask. Add *chloroform* to volume and mix (Solution A). Dilute 5.0 ml of the solution with *chloroform* to 50 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7), using 0.001 per cent w/v solution of *tolnaftate RS* in *chloroform*.

Calculate the content of $C_{19}H_{17}NOS$ in the cream.

Storage. Store protected from moisture.

Tolnaftate Gel

Tolnaftate Gel contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of tolnaftate, $C_{19}H_{17}NOS$.

Usual strength. 1 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica GF254*.

Mobile phase. Toluene.

Test solution. Evaporate 10 ml of the Solution A on steam bath to dryness and dissolve the residue in 1.0 ml of *ethanol (95 per cent)*.

Reference solution. A 0.1 per cent w/v solution of *tolnaftate RS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Gels.

Assay. Transfer an accurately weighed quantity of the gel containing about 10 mg of Tolnaftate to a separator containing 75 ml of *chloroform*. Wash the chloroform solution successively with two 25 ml portions of *0.1 M hydrochloric acid* and 25 ml of *water*. Dilute to 100 ml with *chloroform* and mix (Solution A). Dilute 5.0 ml of the solution with *chloroform* to 50 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7), using 0.001 per cent w/v solution of *tolnaftate RS* in *chloroform*.

Calculate the content of $C_{19}H_{17}NOS$ in the gel.

Storage. Store protected from moisture.

Tolnaftate Topical Powder

Tolnaftate Topical Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{19}H_{17}NOS$.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Toluene.

Test solution. Evaporate the 5.0 ml portion of the *methanol solution* reserved in the Assay, on a steam bath just to dryness and dissolve the residue in 1.0 ml of *alcohol*.

Reference solution. A 0.1 per cent w/v solution of *tolnaftate RS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Assay. Determine by liquid chromatography (2.4.14).

Internal standard solution. A 0.1 per cent w/v solution of *progesterone* in *methanol*.

Test solution. Transfer an accurately weighed quantity of the topical powder containing about 5.0 mg of tolnaftate to a screw-capped, 50 ml centrifuge tube. Add 25 ml of *methanol*, place the cap on the tube and rotate on a rotating device for 10 minutes, and centrifuge at about 2000 rpm for 5 minutes, filter the supernatant and transfer 20 ml of the filtrate to 50-ml volumetric flask, retaining the remaining portion of the filtrate for identification test. Add 5.0 ml of internal standard solution to the flask and dilute to volume with *methanol* and mix.

Reference solution. A 0.02 per cent w/v solution of *tolnaftate RS* in *methanol*. To 20 ml of this solution add 5.0 ml of internal standard solution and dilute to 50.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 30 cm x 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 2 volumes of *acetonitrile* and 1 volume of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the resolution between the tolnaftate and internal standard peak is not less than 3.0 and the relative standard deviation for replicate injections is not more than 3.0 per cent. The relative retention time of progesterone and tolnaftate are about 0.7 minute and 1.0 minute respectively.

Inject the test solution and the reference solution.

Calculate the content of $C_{19}H_{17}NOS$ in the powder.

Storage. Store protected from moisture.

Tolnaftate Topical Solution

Tolnaftate Topical Solution contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of tolnaftate, $C_{19}H_{17}NOS$.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Toluene.

Test solution. Evaporate 25 ml of solution A on steam bath to dryness and dissolve the residue in 1.0 ml of the *ethanol (95 per cent)*.

Reference solution. A 0.1 per cent w/v solution of *tolnaftate RS* in *ethanol (95 per cent)*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

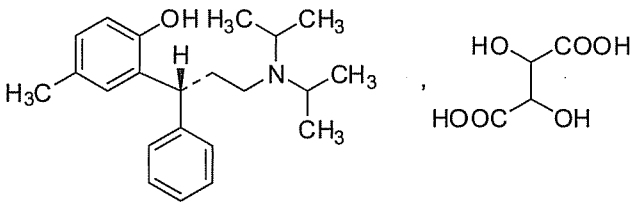
Tests

Assay. Take an accurately measured volume containing about 10 mg of Tolnaftate, add 50 ml of *chloroform* and extract with 50 ml of *0.1 M sodium hydroxide*. Filter the chloroform layer through a chloroform-washed cotton pledget into a 250- ml volumetric flask and extract the aqueous layer with two 45 ml portions of *chloroform*, filtering each into the flask. Add *chloroform* to volume and mix (Solution A). Dilute 25 ml of this solution with *chloroform* to 100 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 258 nm (2.4.7), using 0.001 per cent w/v solution of *tolnaftate RS* in *chloroform*.

Calculate the content of $C_{19}H_{17}NOS$ in topical solution.

Storage. Store protected from moisture.

Tolterodine Tartrate



$C_{22}H_{31}NO,C_4H_6O_6$ Mol Wt. 475.6

Tolterodine Tartrate is 2-[(1*R*)-3-[bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol tartrate.

Tolterodine Tartrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{31}NO,C_4H_6O_6$, calculated on the dried basis.

Description. A white to off-white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tolterodine tartrate RS* or with the reference spectrum of tolterodine tartrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +33° to +38°, determined in 1.0 per cent w/v solution in *methanol.*

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Dissolve about 50 mg of the substance under examination in 10 ml of *water* and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve about 10 mg of *tolterodine tartrate RS* in 20 ml of *water* and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 100.0 ml with the solvent mixture.

Reference solution (b). A 0.03 per cent w/v solution of tartaric acid in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil),
- mobile phase: A. a *0.05 M potassium dihydrogen orthophosphate,* adjusted to pH 3.5 with *orthophosphoric acid,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Time (in min)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0–5	65	35
5-20	65→50	35 →50
20–40	50→30	50 →70
40–41	30→65	70 →35
41–50	65	35

Inject reference solution (a). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution, reference solution (a) and (b). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore the peak due to tartaric acid corresponding to the principal peak in the chromatogram obtained with reference solution (b).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. 10 volumes of mobile phase A and 10 volumes of mobile phase B.

Test solution. Dissolve about 50 mg of the substance under examination in 20 ml of *water*, sonicate and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (a). Dissolve about 50 mg of *tolterodine tartrate RS* in 20.0 ml of *water*, sonicate and dilute to 100.0 ml with the solvent mixture. Dilute 5.0 ml of this solution to 50.0 ml with the solvent mixture.

Reference solution (b). A 0.03 per cent w/v solution of tartaric acid in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil),
- mobile phase: A. a *0.05 M potassium dihydrogen orthophosphate*, adjusted to pH 3.5 with *orthophosphoric acid,*
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Time (in min)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0–5	65	35
5-20	65 → 50	35→50
20–21	50→65	50→35
21–25	65	35

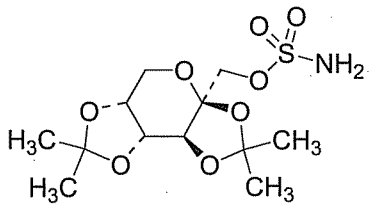
Inject reference solution (a) and (b). The test is not valid unless the theoretical plates of the principal peak is not less than 2000 and the tailing factor is not more than 2.0. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{22}H_{31}NO.C_4H_6O_6$.

Storage. Store protected from moisture.

Topiramate



$C_{12}H_{21}NO_8S$ Mol. Wt. 339.4

Topiramate is 2,3:4,5-di-*O*-1-isopropylidene-β-D-fructopyranose sulphamate.

Topiramate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{12}H_{21}NO_8S$, calculated on the anhydrous basis.

Category. Anti-convulsant.

Dose. 25 mg daily.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *topiramate RS* or with the reference spectrum of topiramate.

B. Melting range (2.4.21). 120° to 130°.

Tests

Specific optical rotation (2.4.22). –28.0° to –36.0°, determined in a 0.4 per cent w/v solution in *methanol* at 20°.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 0.4 g of the substance under examination and dissolve in 10 ml of a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*.

Reference solution. Weigh accurately about 10 mg each of *(2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose RS* (*topiramate impurity A RS*) and *2,3-O-(1-methylethylidene)-beta-D-fructopyranose sulphamate RS* (*topiramate impurity B RS*) and dissolve in sufficient mobile phase to produce 10.0 ml.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 40°,
- mobile phase: a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- refractive index detector,
- detector cell temperature 50°,
- injection volume. 50 µl.

Inject the reference solution. Run the chromatogram for 40 minutes. The retention times of topiramate impurity A and impurity B are about 2.9 and 4.5 minutes and the relative retention times with respect to topiramate are 0.54 and 0.84 respectively. The test is not valid unless the resolution between topiramate impurity A and topiramate impurity B is not less than 3.

Inject the test solution and the reference solution. For the calculation of content, multiply the peak areas of the following impurities by the corresponding correction factor: topiramate impurity A = 1.09; topiramate impurity B = 0.94

Not more than 0.15 per cent each of impurity A and impurity B, and not more than 0.1 per cent of any other impurity is found.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 0.1 g of the substance under examination and dissolve in 10 ml of the mobile phase.

Reference solution. Weigh accurately about 0.1 g of *topiramate RS and* dissolve in 10.0 ml of the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature. 50°
- mobile phase: a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate. 0.6 ml per minute,
- refractive index detector (Detector cell temperature: 50°)
- injection volume. 50 µl.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{12}H_{21}NO_8S$

Storage. Store protected from light, at a temperature not exceeding 30°.

Topiramate Tablets

Topiramate Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of topiramate, $C_{12}H_{21}NO_8S$.

Usual strengths. 25 mg; 50 mg.

Identification

A. Weigh a quantity of the powdered tablets containing 1 g of Topiramate, disperse in 30 ml of *dichloromethane*, centrifuge at 2000 rpm for 10 minutes and filter. Evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *topiramate RS* or with the reference spectrum of topiramate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. To a quantity of the powdered tablets containing 100 mg of Topiramate, add sufficient mobile phase to produce 100 ml, mix and centrifuge. Use the supernatant liquid.

Reference solution. A 0.1 per cent w/v solution of *topiramine RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS 3),
- column temperature. 50°,
- mobile phase: a mixture of 50 volumes of *acetonitrile* and 50 volumes of *water*,
- flow rate. 1 ml per minute,
- refractive index detector (Detector cell temperature 50°),
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent. The retention time of topiramate is about 5 minutes. The test is not valid unless the column efficiency is not less than 3000 theoretical plates and the tailing factor is not more than 2.0

Inject the test solution. Determine the amount of related substances by the area normalization method. The content of any individual impurity is not more than 1.0 per cent and the sum of all impurities is not more than 2.0 per cent.

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 500 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14), using the chromatographic conditions described in the test for Related substances.

Test solution. Dilute the filtrate, if necessary, with the dissolution medium.

Reference solution. Dissolve an accurately weighed quantity of *topiramate RS* in the dissolution medium and dilute with the dissolution medium to obtain a solution having a known concentration similar to the expected concentration of the test solution.

D. Not less than 70 per cent of the stated amount of $C_{12}H_{21}NO_8S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14) as described in the test for Related substances.

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 100 mg of Topiramate, disperse in 100.0 ml with the mobile phase and centrifuge. Use the supernatant liquid.

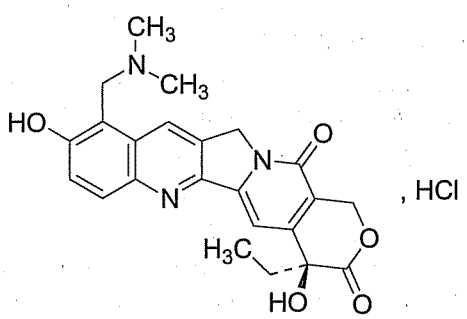
Reference solution. A 0.1 per cent w/v solution of *topiramate RS* in the mobile phase.

Inject the test solution and the reference solution.

Calculate the content of $C_{12}H_{21}NO_8S$ in the tablets.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Topotecan Hydrochloride



$C_{23}H_{23}N_3O_5.HCl$ Mol. Wt. 457.9

Topotecan Hydrochloride is (4*S*)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1*H*-pyrano[3′ ,4′ :6,7]indolizino[1,2-*b*]quinoline-3,14(4*H*,12*H*)-dione hydrochloride.

Topotecan Hydrochloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{23}H_{23}N_3O_5$,HCl, calculated on the anhydrous basis.

Category. Anticancer.

Description. A light yellow to greenish yellow powder.

CAUTION — Topotecan Hydrochloride is cytotoxic; extra care is required to prevent inhaling particles and exposing the skin to it.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *topotecan hydrochloride RS* or with the reference spectrum of topotecan hydrochloride.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 4.5, determined in a 1.0 per cent w/v solution.

Specific optical rotation (2.4.22). +30.0° to +38.0°, determined in a 1.0 per cent w/v solution in *methanol.*

Total chloride. 7.6 per cent to 8.1 per cent.

Weigh accurately about 0.5 g, dissolve in 10 ml of *methanol*, add 20 ml of *water* and 20 ml of *glacial acetic acid.* Titrate with *0.1 M silver nitrate solution* using *eosin yellow solution* as indicator. Colour changes from orange to dark pink.

1 ml of *0.1 M silver nitrate* is equivalent to 0.003545 g of Cl.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 30 volumes of *acetonitrile* and 70 volumes of a buffer solution prepared by diluting 1 ml of *trifluoroacetic acid* in 1000 ml of *water.*

Test solution. Dissolve 40 mg of the substance under examination in 100 ml of the solvent mixture.

Reference solution (a). A 0.04 per cent w/v solution of *topotecan hydrochloride RS* in the solvent mixture.

Reference solution (b). Dilute 1 ml of the reference solution (a) to 100 ml with the solvent mixture.

Reference solution (c). A solution containing 0.006 per cent w/v each of *10-hydroxy comptothecin RS* and *camptothecin RS* in *N,N'-dimethylformamide.* Add 1.0 ml of this solution to a 0.04 per cent w/v solution of *topotecan hydrochloride RS* in the solvent mixture.

Use the chromatographic system described under Assay.

Inject reference solution (c). The test is not valid unless the relative retention time for the 10-hydroxy camptothecin and camptothecin is about 1.87 and 2.62 with respect to topotecan hydrochloride and the relative response factors for 10-hydroxy camptothecin and camptothecin are about 0.79 and 0.85 with respect to topotecan hydrochloride.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.15 times the area of the peak in the chromatogram obtained with reference solution (b) (0.15 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 13.0 per cent, determined on 0.1 g.

Bacterial endotoxins (2.2.3). Not more than 16 Endotoxin Units per mg of topotecan hydrochloride.

Microbial contamination (2.2.9). Total viable aerobic count, not more than 100 cfu per g. It also meets the requirements of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella species*, and *Escherichia coli.*

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A mixture of 30 volumes of *acetonitrile* and 70 volumes of a buffer solution, prepared by diluting 1 ml of *trifluoroacetic acid* to 1000 ml with *water.*

Test solution. Dissolve 10 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution. A 0.04 per cent w/v solution of *topotecan hydrochloride RS* in the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a solution of 1 ml of *trifluoroacetic acid* in 1000 ml of *water,*
  B. *acetonitrile,*
- flow rate. 1.2 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 260 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	85	15
28	70	30
38	70	30
43	85	15
48	85	15

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{23}N_3O_5,HCl$.

Storage. Store protected from light, at a temperature between 2° to 8°.

Topotecan Injection

Topotecan Hydrochloride Injection

Topotecan Injection is a sterile, stabilised solution of Topotecan Hydrochloride in Water for Injection.

Topotecan Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of the topotecan, $C_{23}H_{23}N_3O_5$.

Usual strength. 1 mg per ml.

Description. A clear, light yellow solution.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. It gives the reaction of chlorides (2.3.1).

Tests

pH (2.4.24). 2.5 to 3.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 64.9 Endotoxin Unit per mg of topotecan.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Accurately measure the volume of injection containing 2 mg of Topotecan, dilute to 50.0 ml with mobile phase.

Reference solution. Dissolve 10 mg of *topotecan hydrochloride RS* in 5 ml of *water* and dilute to 25.0 ml with mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 78 volumes of *water*, 22 volumes of *acetonitrile* and 1 volume of *1 M hydrochloric acid,*
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

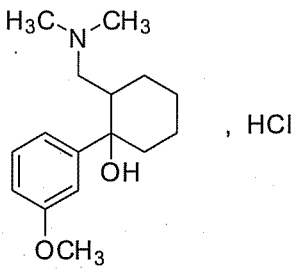
Inject the reference solution. The test is not valid unless the relative standard deviation is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{23}H_{23}N_3O_5$.

Storage. Store protected from light, at a temperature not exceeding 2° to 8°.

Tramadol Hydrochloride



$C_{16}H_{26}ClNO_2$ Mol. Wt. 299.8

Tramadol Hyrochloride is (1*RS*,2*RS*)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride.

Tramadol Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{26}ClNO_2$, calculated on the anhydrous basis.

Category. Analgesic.

Description. A white or almost white, crystalline powder.

Identification

Test B may be omitted if tests A and C are carried out. Test A may be omitted if tests B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tramadol hydrochloride RS* or with the reference spectrum of tramadol hydrochloride.

B. In the test for impurity E, the principal spot in the chromatogram obtained with test solution (b) corresponds to the principal spot in the chromatogram obtained with reference solution (a).

C. Gives reaction (a) of chlorides (2.3.1).

Tests

Appearance of solution. A 5 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Acidity. To 10 ml of 5 per cent w/v solution, add 0.2 ml of *methyl red solution* and 0.2 ml of *0.01 M hydrochloric acid*. The solution is red. Not more than 0.4 ml of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Specific optical rotation (2.4.22). −0.10° to +0.10°, determined on 5.0 per cent w/v solution.

Impurity E. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *concentrated ammonia*, 19 volumes of *2-propanol* and 80 volumes of *toluene*.

Test solution (a). Dissolve 0.1 g of the substances under examination in 2 ml of *methanol*.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

Reference solution (a). A 0.5 per cent w/v solution of *tramadol hydrochloride RS* in *methanol*.

Reference solution (b). A 0.1 per cent w/v solution of *(2RS)-2-[(dimethylamino)methyl]cyclohexanone RS* (*tramadol impurity E RS*) in *methanol*. Dilute 1 ml of this solution to 10 ml with *methanol*.

Reference solution (c). A 0.5 per cent w/v solution of *(1RS,2SR)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol RS* (*tramadol impurity A RS*) in reference solution (a).

Saturate the plate for 20 minutes with *concentrated ammonia*. Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air, expose the plate to iodine vapour for 1 hour, and examine in ultraviolet light at 254 nm. The chromatogram obtained with reference solution (c) shows 2 clearly separated spots. In the chromatogram obtained with test solution (a), any secondary spot corresponding to tramadol impurity E is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.2 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.15 g of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). Dilute 2.0 ml of the test solution to 10.0 ml with the mobile phase. Dilute 1.0 ml of this solution to 100.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *tramadol impurity A RS* in 4.0 ml of the test solution and dilute to 100.0 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm packed with endcapped octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 29.5 volumes of *acetonitrile* and 70.5 volumes of a mixture of 0.2 ml of *trifluoroacetic acid* and 100 ml of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to tramadol impurity A and tramadol is not less than 2.0. The relative retention time with reference to tramadol for tramadol impurity A is about 0.85.

Inject the test solution and reference solution (a). Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of the peak due to tramadol impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.4 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.02 per cent).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method D (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 1.0 g.

Assay. Dissolve 0.18 g in 25 ml of *anhydrous acetic acid* and add 10 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02998 g of $C_{16}H_{26}ClNO_2$.

Storage. Store protected from light.

Tramadol Capsules

Tramadol Hydrochloride Capsules

Tramadol Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tramadol hydrochloride, $C_{16}H_{25}NO_2,HCl$.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *anhydrous formic acid*, 50 volumes of *acetone* and 50 volumes of *methanol*.

Test solution. Disperse the contents of capsules containing about 50 mg of Tramadol Hydrochloride in 25 ml of *methanol* and filter through a glass fiber filter (Such as Whatmann GF/A).

Reference solution. A 0.2 per cent w/v solution of *tramadol hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and expose to iodine vapour until spots appear and examine in daylight. The principal spot in the chromatogram obtained with the test solution corresponds to spot obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse the contents of capsules containing about 0.5 g of Tramadol Hydrochloride in 80 ml of the mobile phase with the aid of ultrasound and dilute to 100 ml with the mobile phase, filter through a glass fiber filter (Such as Whatmann GF/A).

Reference solution (a). Dilute 2 ml of the test solution to 100 ml with the mobile phase. Dilute 1 ml of this solution to 10 ml with the mobile phase.

Reference solution (b). A 0.0015 per cent w/v solution of *(1RS,2SR)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol RS (tramadol impurity A RS)* in the mobile phase.

Reference solution (c). A solution containing 0.0015 per cent w/v each of *tramadol hydrochloride RS* and *tramadol impurity A RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *trifluoroacetic acid*, 30 volumes of *acetonitrile* and 69 volumes of *water*,
- flow rate. 1ml per minute,
- spectrophotometer set at 271 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to tramadol impurity A and tramadol hydrochloride is not less than 3.0.

Inject the test solution, reference solution (a) and (b). Run the chromatogram four times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of peak corresponding to tramadol impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of secondary peak other than tramadol impurity A is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all other secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent).

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the mixed contents of 20 capsules containing about 50 mg of Tramadol Hydrochloride in 150 ml of the mobile phase with the aid of ultrasound and dilute to 200 ml with the mobile phase, filter.

Reference solution (a). A 0.025 per cent w/v solution of *tramadol hydrochloride RS* in the mobile phase.

Reference solution (b). A solution containing 0.0015 per cent w/v each of *tramadol hydrochloride RS* and *tramadol impurity A RS* in the mobile phase.

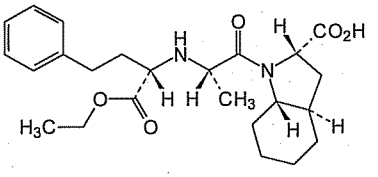
Use the chromatographic system as described under Related substances.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to tramadol impurity A and tramadol hydrochloride is not less than 3.0.

Inject the test solution and reference solution (a).

Calculate the content of $C_{16}H_{25}NO_2,HCl$ in the Capsules.

Trandolapril



$C_{24}H_{34}N_2O_5$ Mol. Wt. 430.5

Trandolapril is (2*S*,3a*R*,7a*S*)-1[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxo-propyl]octahydro-1*H*-indole-2-carboxylic acid.

Trandolapril contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{24}H_{34}N_2O_5$, calculated on the dried basis.

Category. Antihypertensive.

Description. A white to off white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trandolapril RS* or with the reference spectrum of trandolapril.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). -16.5° to -18.5°, determined on 2.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of the mobile phase.

Reference solution. A 0.0005 per cent w/v solution of *trandolapril RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 6.71 g of *monobasic potassium orthophosphate* in 1000 ml of *water*, 35 volumes of *acetonitrile* and 5 volumes of *methanol*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicates injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying at 50° for 3 hours under vacuum.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 10 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution. A 0.01 per cent w/v solution of *trandolapril RS* in the mobile phase.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil C18),
- mobile phase: a mixture of 52 volumes of buffer solution prepared by dissolving 6.71 g of *monobasic potassium orthophosphate* in 1000 ml of *water*, 43 volumes of *acetonitrile* and 5 volumes of *methanol*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{34}N_2O_5$.

Storage. Store protected from light and moisture.

Trandolapril Tablets

Trandolapril Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of trandolapril, $C_{24}H_{34}N_2O_5$.

Usual strengths. 1 mg; 2 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range of 200 nm to 400 nm (2.4.7), a 0.05 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum as obtained with *trandolapril RS* of the same concentration.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 500 ml of *water*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate, if necessary, with the dissolution medium.

Reference solution. Dissolve an accurately weighed quantity of *trandolapril RS* in the mobile phase and dilute with dissolution medium to obtain a solution having a known concentration similar to the test solution.

Use chromatographic system as described under Assay using 100 µl injection volume.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{34}N_2O_5$.

D. Not less than 80 per cent of the stated amount of $C_{24}H_{34}N_2O_5$.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution. Disperse a quantity of powdered tablets containing about 10 mg of Trandolapril in 10.0 ml of the mobile phase.

Reference solution. A 0.001 per cent w/v solution of *trandolapril RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as ACE C8),
- mobile phase: a mixture of 60 volumes of buffer solution prepared by dissolving 6.71 g of *monobasic potassium orthophosphate anhydrous* in 1000 ml of *water*, 35 volumes of *acetonitrile* and 5 volumes of *methanol*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. Run the chromatogram 5 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay with the following solutions.

Test solution. Disperse one tablet in the mobile phase, dilute to obtain a solution containing 0.005 per cent w/v of trandolapril in the mobile phase and filter.

Reference solution. Dissolve an accurately weighed quantity of *trandolapril RS* in the mobile phase and dilute to obtain a solution having a known concentration similar to the test solution.

Calculate the content of $C_{24}H_{34}N_2O_5$ in the tablet.

Water (2.3.43). Not more than 7.0 per cent, determined on 0.5 g.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and disperse 10 intact tablets in 100.0 ml with the mobile phase, filter. Dilute 5.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *trandolapril RS* in the mobile phase.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Inertsil ODS-3),
- mobile phase: a mixture of 52 volumes of buffer solution prepared by dissolving 6.71 g of *monobasic potassium orthophosphate anhydrous* in 1000 ml of *water*, 43 volumes of *acetonitrile* and 5 volumes of *methanol*, adjusted to pH 4.0 with *orthophosphoric acid*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the theoretical plates of the principal peak is not less than 2000, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

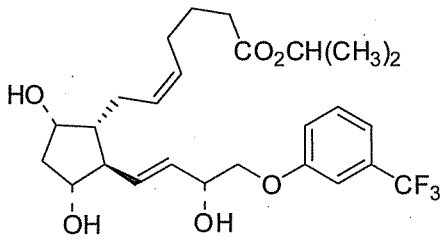
Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{34}N_2O_5$ in the tablet.

Storage. Store at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the amount of Trandolapril.

Travoprost



$C_{26}H_{35}F_3O_6$ Mol. Wt. 500.6

Travoprost is (5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-[(1*E*,3*R*)-3-hydroxy-4-[3-(trifluromethyl)phenoxy]-1-butenyl]-cyclopentyl]-5-heptenoic acid 1-methylethyl ester.

Travoprost contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{26}H_{35}F_3O_6$, calculated on the anhydrous basis.

Category. Antiglaucoma.

Description. A colourless to yellowish oil.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *travoprost RS* or with the reference spectrum of travoprost.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Appearance of solution. A 1.0 per cent w/v solution in *ethanol (95 per cent)* is not more intensely coloured than reference solution YS7 (2.4.1).

Specific optical rotation (2.4.22). +52° to +58°, determined on 2.0 per cent w/v solution in *ethanol (95 per cent)* at 20°.

Related substances. Not more than 3.5 per cent of 5,6-trans-travoprost.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in *acetonitrile* and dilute to 100.0 ml with *water*.

Reference solution. Dissolve 10 mg of *travoprost RS* in *acetonitrile* and dilute to 100.0 ml with *water*. Dilute 1.0 ml of the solution to 20.0 ml with *water*.

Use chromatographic system as described under Assay.

Inject the reference solution. The relative retention time with reference to travoprost for 5,6-trans-travoprost is about 1.06.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent). The sum of the areas of all the secondary peaks is not more than 12 times the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Water (2.3.43). Not more than 1.0 per cent, determined on 0.1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in *acetonitrile* and dilute to 100.0 ml with *water*.

Reference solution. Dissolve 10 mg of *travoprost RS* in *acetonitrile* and dilute to 10.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm), (Such as Hypersil ODS),
- column temperature. 50°,
- mobile phase: A. a mixture of 66 volumes of buffer solution prepared by diluting 1 ml of *orthophosphoric acid* to 1000 ml with *water*, adjusted to pH 5.0 with *1 M sodium hydroxide*, 30 volumes of *acetonitrile* and 4 volumes of *propanol-2-ol*,
  B. a mixture of 80 volumes of *acetonitrile*, 4 volumes of *propanol-2-ol* and 16 volumes of *water*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent V/V)
0	100	0
5-50	80	20
50-70	80→0	20→100
70-80	0	100
80-82	0→100	100→0
82-90	100	0

Inject the reference solution. The test is not valid unless the resolution between the peaks due to travoprost and 5-trans-travoprost is not less than 1.5, the theoretical plates is not less than 1500, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{26}H_{35}F_3O_6$.

Storage. Store protected from moisture, at a temperature between 2° and 8°.

Travoprost Eye drops

Travoprost Eye Drops contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of travoprost, $C_{26}H_{35}F_3O_6$.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *benzene* and 40 volumes of *dioxane*.

Test solution. Extract eye drops containing about 0.8 mg of Travoprost with 20.0 ml of *chloroform* in a separating funnel. Collect the chloroform layer in a 250 ml beaker. Give three washings with chloroform. Evaporate the chloroform layer to dryness and add 3.0 ml of *methanol.*

Reference solution. Dissolve 20 mg of *travoprost RS* in 10 ml of *acetonitrile*, sonicate and dilute to 20.0 ml with *water*. Dilute 5.0 ml of this solution to 20.0 ml with *methanol.*

Apply to the plate 20 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate and spray with 10 per cent w/v solution of *phosphomolybdic acid* in *ethanol*, heat the plate at 110° for 15 minutes and examine immediately. The principal spot in the chromatogram obtained with the test solution corresponds to the spot obtained with the reference solution.

Tests

pH (2.4.24). 5.7 to 6.3.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the eye drops to obtain a solution containing 0.004 per cent w/v of travoprost.

Reference solution. Dissolve 20 mg of *travoprost RS* in 10.0 ml of *acetonitrile,* sonicate and dilute to 20.0 ml with *water*. Dilute 1.0 ml of this solution to 25.0 ml with *water*. Further, dilute 1.0 ml of this solution to 100.0 ml with *water*.

Use chromatographic system as described under Assay.

Inject the reference solution. The relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 4 times the area of the principal peak in the chromatogram obtained with the reference solution (4.0 per cent) and the sum of the areas of all the secondary peaks is not more than 6 times the area of the principal peak in the chromatogram obtained with the reference solution (6.0 per cent).

Other tests. Complies with the tests stated under Eye Drops.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *water* and *acetonitrile.*

Test solution. Dilute a suitable volume of eye drops containing 0.004 per cent w/v of travoprost in the solvent mixture.

Reference solution. Dissolve 20 mg of *travoprost RS* with 10.0 ml of *acetonitrile,* sonicate and dilute to 20.0 ml with *water*. Dilute 1.0 ml of this solution to 25.0 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- column temperature. 50°,
- sample temperature. 5°,
- mobile phase: A. a mixture of 30 volumes of *acetonitrile*, 4 volumes of *propan-2-ol* and 66 volumes of buffer solution prepared by diluting 1.0 ml of *orthophosphoric acid* to 1000.0 ml with *water*, adjusted to pH 5.0 with *1 M sodium hydroxide* and filter,
  B. a mixture of 80 volumes of *acetonitrile*, 4 volumes of *propan-2-ol* and 16 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 100 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 5	100→80	0 →20
5 -50	80	20
50-70	80→0	20→100
70-80	0	100
80 -85	0→100	100→0

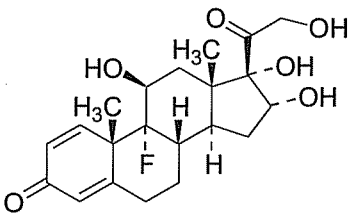
Inject the reference solution. The test is not valid unless the theoretical plates is not less than 1500, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{26}H_{35}F_3O_6$.

Storage. Store at a temperature not exceeding 30°.

Triamcinolone



$C_{21}H_{27}FO_6$ Mol. Wt. 394.4

Triamcinolone is 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione.

Triamcinolone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{21}H_{27}FO_6$, calculated on the dried basis.

Category. Corticosteroid.

Dose. 2 to 24 mg daily.

Description. A white or almost white, crystalline powder; slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triamcinolone RS* or with the reference spectrum of triamcinolone.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows an absorption maximum at about 238 nm; absorbance at about 238 nm, about 0.76.

C. Dissolve 1 mg in 6 ml of *ethanol (95 per cent)*, add 5 ml of a 1 per cent w/v solution of *butylated hydroxytoluene* in *ethanol (95 per cent)* and 5 ml of *1 M sodium hydroxide* and heat on a water-bath under a reflux condenser for 20 minutes; a pinkish lavender colour is produced.

Tests

Specific optical rotation (2.4.22). +65.0° to +72.0°, determined in a 1.0 per cent w/v solution in *dimethylformamide*.

Related substances. Determine by liquid chromatography (2.4.14).

Prepare the following solutions immediately before use and protect from light.

Test solution. Dissolve 25 mg of the substance under examination in a mixture of equal volumes of *methanol* and *water* and dilute to 10 ml with the same solvent mixture.

Reference solution. Dilute 1 ml of the test solution to 100 ml with a mixture of equal volumes of *methanol* and *water*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with base-deactivated end-capped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture prepared by mixing 525 ml of *methanol* with 400 ml of *water*, allowing to equilibrate, adjusting the volume to 1000.0 ml with *water* and mixing again,
- flow rate. 1 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject the test solution and the reference solution. Continue the chromatography for 4.5 times the retention time of triamcinolone (about 11 minutes).

In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1 per cent); not more than two such peaks have an area greater than half the area of the principal peak in the chromatogram obtained with the reference solution (0.5 per cent); the sum of the areas of all the peaks other than the principal peak is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Heavy metals (2.3.13). 0.8 g complies with the limit test for heavy metals, Method B (25 ppm).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1.0 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Assay. Weigh accurately about 25 mg, dissolve in sufficient *ethanol (95 per cent)* to produce 100.0 ml and mix. Dilute 2.0 ml to 50.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7).

Calculate the content of $C_{21}H_{27}FO_6$ taking 380 as the specific absorbance at 238 nm.

Storage. Store protected from light and moisture.

Triamcinolone Tablets

Triamcinolone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of triamcinolone, $C_{21}H_{27}FO_6$.

Usual strengths. 2 mg; 4 mg; 8 mg.

Identification

A. Dissolve 1 mg of the residue obtained in the test for Related substances in 6 ml of *ethanol (95 per cent)*, add 5 ml of a 1 per

cent w/v solution of *butylated hydroxytoluene* in *ethanol (95 per cent)* and 5 ml of *1 M sodium hydroxide* and heat on a water-bath under a reflux condenser for 20 minutes; a pinkish lavender colour is produced.

B. In the Assay, the chromatogram obtained with the principal peak obtained with the test solution corresponds to the peak due to triamcinolone in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 15 mg of Triamcinolone with 15 ml of *ethanol* for 15 minutes, filter under reduced pressure through a fine filter paper (such as Whatman No 42) and evaporate the filtrate to dryness using a rotary evaporator. Reserve 1 mg of the residue for Identification test A. Dissolve the remainder of the residue in 15 ml of *methanol*.

Reference solution (a). Dilute 4 ml of the test solution to 100 ml with *methanol*.

Reference solution (b). Dilute 5 ml of the test solution to 50 ml with *methanol*. Dilute 5 ml of the resulting solution to 50 ml with the same solvent.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of *methanol* and *water*, adjusted so that the retention time of triamcinolone is about 5 minutes (approximately equal volumes of *methanol* and *water*),
- flow rate. 2 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Inject reference solution (b) and record the chromatogram for 4 times the retention time of the triamcinolone peak.

The test is not valid unless the column efficiency determined from the principal peak in the chromatogram obtained with reference solution (a) is at least 10,000 theoretical plates per metre.

In the chromatogram obtained with the test solution the area of any secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent) and not more than one such peak has an area more than that of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent). The sum of the areas of any such peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (4 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Crush one tablet to a fine powder, add 100 ml of *ethanol (95 per cent)* and shake for 10 minutes. Filter, dilute 2.0 ml of the filtrate with sufficient *ethanol (95 per cent)* to produce a solution containing 0.002 per cent w/v of Triamcinolone and measure the absorbance of the resulting solution at the maximum at about 238 nm (2.4.7).

Calculate the content of $C_{21}H_{27}FO_6$ in the tablet taking 380 as the specific absorbance at 238 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 2.5 mg of Triamcinolone, shake with 5 ml of *methanol* and 20 ml of a mixture of 5 volumes of *methanol* and 3 volumes of *water*. Shake for 15 minutes, mix with the aid of ultrasound for 10 minutes, centrifuge and use the supernatant liquid.

Reference solution (a). Prepare in the same manner as the test solution but add 5 ml of a 0.06 per cent w/v solution of *testosterone* (internal standard) in *methanol* (solution A) in place of the 5 ml of *methanol*.

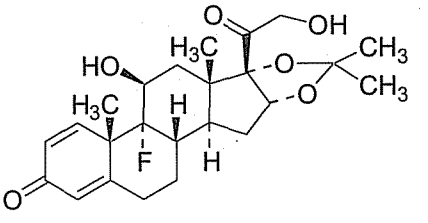
Reference solution (b). Add 5 ml of solution A to 5 ml of a 0.08 per cent w/v solution of *triamcinolone RS* in *methanol* and add 15 ml of *methanol (50 per cent)*.

Chromatographic system
- a stainless steel column 20 cm × 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 70 volumes of *methanol*, 30 volumes of *water* and 0.1 volume of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 238 nm,
- injection volume. 20 µl.

Calculate the content of $C_{21}H_{27}FO_6$ in the tablets.

Storage. Store protected from moisture.

Triamcinolone Acetonide



$C_{24}H_{31}FO_6$ Mol. Wt. 434.5

Triamcinolone Acetonide is 9α-fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione.

Triamcinolone Acetonide contains not less than 96.0 per cent and not more than 104.0 per cent of $C_{24}H_{31}FO_6$, calculated on the anhydrous basis.

Category. Corticosteroid.

Dose. By deep intramuscular injection, 40 mg to 100 mg.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triamcinolone acetonide RS.*

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide.*

Mobile phase. A mixture of 115 volumes of *cyclohexane,* 56 volumes of *chloroform* and 29 volumes of *toluene.*

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *triamcinolone acetonide RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm × about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add about 2 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

Light absorption (2.4.7). When examined in the range 230 nm to 360 nm, a 0.001 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 239 nm; absorbance at about 239 nm, 0.34 to 0.37.

Specific optical rotation (2,4,22). +100° to +107°, determined in a 1.0 per cent w/v solution in *dioxan.*

Related substances. Determine by liquid chromatography (2.4.14).

NOTE – Carry out the test protected from light.

Test solution. Dissolve 25 mg of the substance under examination in 7 ml of *methanol* and dilute to 10 ml with *water.*

Reference solution (a). Dissolve 2 mg of *triamcinolone acetonide RS* and 2 mg of *triamcinolone* in the mobile phase and dilute to 100 ml with the mobile phase.

Reference solution (b). Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture prepared by mixing 525 ml of *methanol* with 400 ml of *water,* allowing to equilibrate, adjusting the volume to 1000.0 ml with *water* and mixing again,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Equilibrate the column with the mobile phase for about 10 minutes.

Inject reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 per cent of the full scale of the recorder.

Inject reference solution (a). The retention times are; triamcinolone, about 5 minutes and triamcinolone acetonide about 17 minutes. The test is not valid unless the resolution between the peaks corresponding to triamcinolone and triamcinolone acetonide is at least 15; if necessary, adjust the concentration of *methanol* in the mobile phase.

Inject the test solution and reference solution (b). Continue the chromatography for 3.5 times the retention time of the principal peak in the chromatogram obtained with the test solution. In the chromatogram obtained with the test solution the area of any peak other than the principal peak is not more than 0.25 times the area of the principal peak in the

chromatogram obtained with the reference solution (b) (0.25 per cent); the sum of the areas of all the peaks other than the principal peak is not greater than half the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent). Ignore any peak due to the solvent and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.2 g.

Assay. Weigh accurately about 25 mg and dissolve in sufficient *ethanol* to produce 100.0 ml and mix. Dilute 5.0 ml to 100.0 ml with *ethanol (95 per cent)* and measure the absorbance of the resulting solution at the maximum at about 239 nm (2.4.7).

Calculate the content of $C_{24}H_{31}FO_6$ taking 354 as the specific absorbance at 239 nm.

Storage. Store protected from light and moisture.

Triamcinolone Acetonide Injection

Triamcinolone Acetonide Injection is a sterile suspension of Triamcinolone Acetonide in very fine particles in Water for Injections containing suitable dispersing agents.

Triamcinolone Acetonide Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of triamcinolone acetonide, $C_{24}H_{31}FO_6$.

Usual strength. 40 mg per ml.

Identification

Extract a volume containing about 50 mg of Triamcinolone Acetonide with two quantities, each of 10 ml, of *peroxide-free ether* and discard the ether extracts. Filter the aqueous layer through a sintered-glass filter, wash the residue with four quantities, each of 5 ml *of water* and dry at 105° for 1 hour. The residue complies with the following tests.

Test A may be omitted if tests B and C are carried out. Test C may be omitted if tests A and B are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triamcinolone acetonide RS* or with the reference spectrum of triamcinolone acetonide.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *formamide*.

Mobile phase. A mixture of 115 volumes of *cyclohexane*, 56 volumes of *chloroform* and 29 volumes of *toluene*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). Dissolve 25 mg of *triamcinolone acetonide RS* in 10 ml of the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Heat 0.5 ml of *chromic-sulphuric acid* in a test-tube (5 cm × about 6 mm) in a naked flame until white fumes are evolved; the solution wets the sides of the tube readily and there is no greasiness. Add about 2 mg of the substance under examination and again heat in a naked flame until white fumes appear; the solution does not wet the sides of the tube and does not pour easily from the tube.

Tests

pH (2.4.24). 5.0 to 7.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Mix an accurately measured volume of the injection, diluted with *methanol*, with sufficient of a solution of *prednisolone RS* (internal standard) with *methanol* to obtain a final concentration of 0.02 per cent w/v of triamcinolone acetonide and 0.01 per cent w/v of prednisolone, centrifuge and use the clear supernatant liquid.

Reference solution (a). A solution containing 0.02 per cent w/v of *triamcinolone acetonide RS* and 0.01 per cent w/v of *prednisolone RS* in *methanol*.

Reference solution (b). Prepare in the same manner as test solution but omitting the internal standard.

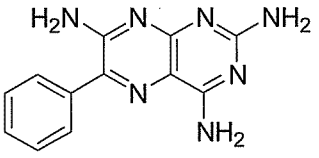
Chromatographic system
- a stainless steel column 25 cm × 4 mm, packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 56 volumes of *methanol* and 44 volumes of *water*,
- flow rate. 1.4 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Adjust the flow rate of the mobile phase such that the separation of the triamcinolone acetonide and internal standard is optimised with a retention time of about 15 minutes for triamcinolone acetonide.

Calculate the content of $C_{24}H_{31}FO_6$ in the injection.

Storage. Store protected from light.

Triamterene



$C_{12}H_{11}N_7$ Mol. Wt. 253.3

Triamterene is 2,4,7-triamino-6-phenylpteridine.

Triamterene contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{12}H_{11}N_7$, calculated on the dried basis.

Category. Potassium sparing diuretic.

Dose. 150 to 250 mg daily, in divided doses.

Description. A yellow, crystalline powder; odourless.

Identification

A. When examined in the range 250 nm to 380 nm (2.4.6), a 0.001 per cent w/v solution in a mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *1 M hydrochloric acid* shows absorption maxima at about 262 nm and 360 nm, and a shoulder at about 285 nm.

B. A 0.1 per cent w/v solution in *anhydrous formic acid*, when examined in ultraviolet light at 365 nm shows an intense blue fluorescence. Solutions in other acids also exhibit a blue fluorescence.

Tests

Acidity. Boil 1.0 g with 20 ml of *water* for 5 minutes, cool, filter and wash the filter with three quantities, each of 10 ml, of *water*. Combine the filtrate and washings and add 0.3 ml of *phenolphthalein solution*. Not more than 1.5 ml of *0.01 M sodium hydroxide* is required to change the colour of the solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *ethyl acetate*, 10 volumes of *18 M ammonia* and 10 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 20 ml of *dimethyl sulphoxide* and dilute 2 ml of the resulting solution to 50 ml with *methanol*.

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in 5 ml of *anhydrous formic acid*, add 100 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02533 g of $C_{12}H_{11}N_7$.

Storage. Store protected from light and moisture.

Triamterene Capsules

Triamterene Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of triamterene, $C_{12}H_{11}N_7$.

Usual strength. 50 mg.

Identification

The final solution obtained in the Assay has a bluish fluorescence and when examined in the range 250 nm to 380 nm (2.4.7), shows an absorption maximum at about 360 nm.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 90 volumes of *ethyl acetate*, 10 volumes of *18 M ammonia* and 10 volumes of *methanol*.

Test solution. Dissolve a quantity of the contents of the capsules containing 0.1 g of Triamterene in sufficient *dimethyl sulphoxide* to produce 20 ml and dilute 2 ml to 50 ml with *methanol.*

Reference solution. Dilute 1 volume of the test solution to 200 volumes with *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvent is no longer detectable and examine in ultraviolet light at 365 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

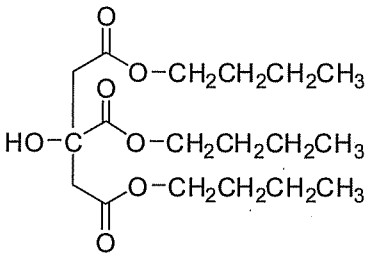
Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the contents of 20 capsules containing about 0.1 g of Triamterene, dissolve in 50 ml of a mixture of equal volumes of *glacial acetic acid* and *water* with the aid of gentle heat, cool and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with *1 M acetic acid* and measure the absorbance of the resulting solution at the maximum at about 360 nm (2.4.7).

Calculate the content of $C_{12}H_{11}N_7$ from the absorbance obtained by repeating the operation using *triamterene RS* in place of the contents of the capsules.

Storage. Store protected from moisture.

Tributyl Citrate



$C_{18}H_{32}O_7$ Mol. Wt. 360.5

Tributyl Citrate contains not less than 99.0 per cent of $C_{18}H_{32}O_7$, calculated on the anhydrous basis.

Category. Excipient.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tributyl citrate RS* or with the reference spectrum of tributyl citrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Weight per ml (2.4.29). 1.037 to 1.045.

Refractive index (2.4.27). 1.443 to 1.445.

Acidity. Dissolve 32.0 g in 30 ml of *ethanol (95 per cent)*, previously neutralised to *bromothymol blue*, add *bromothymol blue solution* and titrate with *0.1 M sodium hydroxide* to a faint blue endpoint; not more than 1.0 ml is required.

Water (2.3.43). Not more than 0.2 per cent.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve about 300 mg of substance under examination in 10 ml of *toluene.*

Reference solution. A solution containing 3.0 per cent w/v of *tributyl citrate RS* and *acetyltributyl citrate RS* in *toluene.*

Chromatographic system
- a glass or stainless steel column 30 m x 0.32 mm, packed with bonded with a 0.5-µm layer of phase G42,
- temperature:
  column. 80° to 220° from 0.5 to 10 minutes,
  inlet port. 85° to 250° from 0.5 to 10 minutes (increased @ 20° per minute) and detector 275°,
- a flame ionisation detector,
- flow rate. 2.3 ml per minute of the nitrogen as carrier gas.

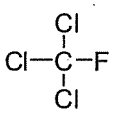
Inject 1 µl of the reference solution. The test is not valid unless the relative retention times with reference to acetyltributyl citrate for tributyl citrate is about 0.9, the resolution between the peaks corresponding to tributyl citrate and acetyltributyl citrate is not less than 1.5 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject 1 µl of the test solution and the reference solution.

Calculate the content $C_{18}H_{32}O_7$.

Storage. Store protected from moisture.

Trichloromonofluoromethane



CCl_3F Mol. Wt. 137.4

Trichloromonofluoromethane contains not less than 99.6 per cent and not more than 100.0 per cent of CCl_3F, calculated on the anhydrous basis.

Category. Pharmaceutical aid (Excipient).

Identification

Determine by infrared absorption spectrophotometry (2.4.6), compare the spectrum with the obtained with *trichloromonoflurometharne RS.*

Tests

Boiling temperature. Transfer a 100 ml sample at about 24° to a tared, pear-shaped, 100-ml centrifuge tube containing a few boiling stones, and weigh. Suspend a thermometer in the liquid, and place the tube in a medium maintained at a temperature of 32° above the expected boiling temperature. When the thermometer reading becomes constant, record as the boiling temperature the thermometer reading after at least 5per cent of the substance under examination has distilled. Retain the remainder of the substance for the determination of High-Boiling Residues.

Water (2.3.43). Not more than 0.001 per cent (Method 3) with the following modifications (a) provide the closed-system titrating vessel with an opening through which passes a coarse-porosity gas dispersion tube connected to a sample cylinder; (b) dilute the reagent with anhydrous methanol to give a water equivalence factor of between 0.2 and 1.0 mg per ml, age this diluted solution for not less than 16 hours before sanitation; (c) obtain a 100 g sample as directed under inhalation preparation, and introduce the same into the titration vessel through the gas dispersion tube at a rate of about 100 ml of gas per minute.

High-boiling residues. Not more than 0.01 per cent, Allow 85 ml of the sample to distill as directed in the test for Approximate Boiling Temperature, and transfer the centrifuge tube containing the remaining 15 ml of substance to a medium maintained at a temperature 10° above the boiling temperature. After 30 minutes, remove the tube from the water-bath, blot dry, and weigh. Calculate the weight of the residue.

Inorganic chlorides. Place 5 ml of anhydrous *methanol* in a test tube, add 3 drops of a saturated solution of silver nitrate in anhydrous *methanol*, shake, and add 7 g of Trichloromonofluoromethane; no opalescence or turbidity is produced.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Introduce the liquid phase of Trichloromono-fluoromethane into an evacuated headspace vial

Reference solution. Introduce a liquid-phase mixture of dichlorodifluoromethane, dichlorotetrafluoroethane, and trichloromonofluoromethane into an evacuated headspace vial.

Chromatographic system

- a steel column 1.8 m × 2 mm, packed with support S12, containing 1 per cent phase G25,
- temperature:
  column 70° to 170° @10° per minute and 170° for 5 minutes,
  inlet port at 110° and detector at 200°,
- a flame ionisation detector,
- flow rate. 20 ml per minute of the nitrogen as carrier gas

Head-space conditions which may be used:

- bath temperature 100°,
- valve/loop temperature 105°,
- sampling time is 3 seconds.

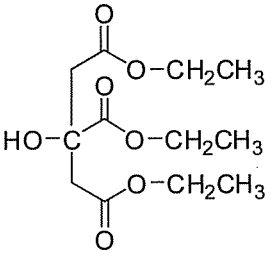
The relative retention time with reference to trichloromonofluoromethane for dichlorodifluoromethane is about 0.5 and for dichlorotetrafluoroethane is about 0.8. The sum of these two peak areas is not more than 0.2 per cent of the total of all peak areas and the sum of the areas of all peaks other than that for trichloromonofluoromethane is not more than 0.4 per cent of the total of all peak areas.

Inject the test solution and reference solution. The test is not valid unless the resolution between the peaks due dichlorotetrafluoroethane and trichloromonofluoromethane is not less than 2.0.

Calculate the content of CCl_3F.

Storage. Store protected from moisture and prevent exposure to excessive heat.

Triethyl Citrate



$C_{12}H_{20}O_7$ Mol. Wt. 276.3

Triethyl Citrate is triethyl 2-hydroxypropane-1, 2, 3-tricarboxylate.

Triethyl Citrate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{12}H_{20}O_7$, calculated on the anhydrous basis.

Category. Excipient.

Description. A clear, viscous, colourless or almost colourless, hygroscopic liquid.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triethyl citrate RS* or with the reference spectrum of triethyl citrate.

B. To 0.5 ml add 5 ml of *ethanol (95 per cent)* and 4 ml of *dilute sodium hydroxide solution*. Boil under reflux for about 10 minutes, 2 ml of the solution gives the reactions of citrates (2.3.1).

C. It gives the reactions of esters (2.3.1).

Tests

Appearance of solution. The substance under examination is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Acidity. Dilute 10 g with 10 ml of previously neutralized *ethanol (95 per cent)*. Add 0.5 ml of *bromothymol blue solution*. Not more than 0.3 ml of *0.1 M sodium hydroxide* is required to change the color of the indicator to blue.

Refractive index (2.4.27). 1.440 to 1.446.

Related substances. Determine by gas chromatography (2.4.13).

Test solution. Disperse 1.0 ml of the substance under examination in 50.0 ml of *dichloromethane*.

Reference solution. Disperse 1.0 ml of the substance under examination and 0.5 ml of *methyl tridecanoate* in 50.0 ml of *dichloromethane*.

Chromatographic system
- a fused silica column 30 m x 0.32 mm, packed with poly (dimethyl) siloxane (5 µm),
- temperature:
  column.200°,
  injection port and detector at 220°,
- a flame ionization detector,
- linear velocity about 26 cm per second using nitrogen as carrier gas

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to triethyl citrate and methyl tridecanoate is not less than 1.5.

Inject 1 µl each of the test solution and the reference solution. Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 per cent the area of the principal peak in the chromatogram obtained with the reference solution. The sum of the areas of all the secondary peaks is not more than 0.5 per cent the area of the principal peak in the chromatogram obtained with the reference solution. Ignore any peak with an area less than 0.04 per cent the area of the principal peak in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). Dissolve 4.0 g in 8 ml of *ethanol (95 per cent)* and dilute to 20 ml with *water*. 12 ml of the resulting solution complies with the limit test for the heavy metals, method D (5 ppm). Prepare the standard solution using *lead standard solution (1 ppm Pb)* obtained by diluting *lead standard solution (100 ppm Pb)* with a mixture of equal volumes of *ethanol (95 per cent)* and *water*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

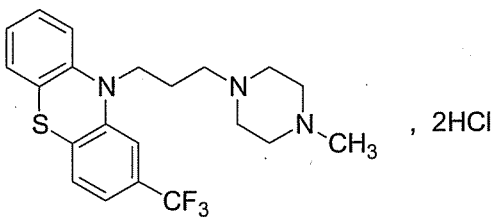
Water (2.4.19). Not more than 0.25 per cent, determined on 1.0 g.

Assay. To 1.5 g, add 25 ml of *2-propanol*, 50 ml of *water*, 25.0 ml of *1 M sodium hydroxide* and a few glass beads into a 250-ml borosilicate-glass flask fitted with a reflux condenser. Heat under a reflux condenser for 1 hour and allow to cool. Titrate with *1 M hydrochloric acid*, using 1 ml of *phenolphthalein solution* as indicator. Carry out a blank titration.

1 ml of *1 M sodium hydroxide* is equivalent to 0.0921 g of $C_{12}H_{20}O_7$.

Storage. Store protected from moisture.

Trifluoperazine Hydrochloride



$C_{21}H_{24}F_3N_3S,2HCl$ Mol. Wt. 480.4

Trifluoperazine hydrochloride is 10-[3-(4-methylpiperazin-1-yl)propyl]-2-trifluoromethylphenothiazine dihydrochloride.

Trifluoperazine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{21}H_{24}F_3N_3S$, 2HCl, calculated on the dried basis.

Category. Antipsychotic; antiemetic.

Dose. As antipsychotic, orally, the equivalent of 2 to 30 mg of trifluoperazine daily, in divided doses; by intramuscular injection, the equivalent of 1 to 3 mg of trifluoperazine daily, in divided doses. As antiemetic, orally, the equivalent of 2 to 4 mg of trifluoperazine daily; by intramuscular injection, the equivalent of 1 to 3 mg of trifluoperazine daily, in divided doses.

Description. A white to pale yellow, crystalline powder; odourless or almost odourless; slightly hygroscopic.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

A. Dissolve 20 mg in 10 ml of *water*, make the solution alkaline to *litmus paper* with *5 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *light petroleum (60° to 80°)*. Combine the extracts, wash with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate carefully to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trifluoperazine hydrochloride RS* or with the reference spectrum of trifluoperazine hydrochloride.

B. Complies with the test for identification of phenothiazines (2.3.3), using as reference solution a 0.2 per cent w/v solution of *trifluoperazine hydrochloride RS* in *chloroform*.

C. When examined in the range 280 nm to 360 nm (2.4.7), a 0.01 per cent w/v solution in *0.1 M hydrochloric acid* measured immediately after preparation shows an absorption maximum at about 305 nm. Dilute 10 ml of the solution to 100 ml with *0.1 M hydrochloric acid*. When examined in the range 230 nm to 280 nm, the resulting solution shows an absorption maximum at about 256 nm; absorbance at about 256 nm, about 0.65.

D. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced.

E. A 10 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 1.7 to 2.6, determined in a 10.0 per cent w/v solution.

Related substances. Complies with the test for related substances in phenothiazines (2.3.5), using mobile phase A.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.4 g, dissolve in 50 ml of *anhydrous glacial acetic acid* add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02402 g of $C_{21}H_{24}F_3N_3S, 2HCl$.

Storage. Store protected from light and moisture.

Trifluoperazine Injection

Trifluoperazine Hydrochloride Injection

Trifluoperazine Injection is a sterile solution of Trifluoperazine Hydrochloride in Water for Injections.

Trifluoperazine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of trifluoperazine, $C_{21}H_{24}F_3N_3S$.

Usual strength. The equivalent of 1 mg of trifluoperazine per ml.

Description. A clear, colourless solution.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in Assay shows an absorption maximum at about 256 nm.

Tests

pH (2.4.24). 4.0 to 5.0.

Other tests. Complies with the tests stated under Parenteral preparations (Injections).

Assay. To an accurately measured volume of the injection containing about 50 mg of trifluoperazine add 10 ml of *2 M sulphuric acid* and extract with three quantities, each of 25 ml, of *carbon tetrachloride*. Discard the carbon tetrachloride extract after each extraction. Add 10 ml of *strong ammonia solution* and extract with five quantities, each of 50 ml, of *cyclohexane*. Extract the combined cyclohexane extracts with five quantities, each of 50 ml, of *0.1 M hydrochloric acid*. Dilute the combined acid extracts to 500.0 ml and mix. Measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7).

Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the specific absorbance at 256 nm.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of trifluoperazine per ml.

Trifluoperazine Tablets

Trifluoperazine Hydrochloride Tablets

Trifluoperazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of trifluoperazine, $C_{21}H_{24}F_3N_3S$. The tablets are coated.

Usual strengths. The equivalent of 1 mg and 5 mg of trifluoperazine.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of trifluoperazine with 30 ml of *1 M hydrochloric acid* for 10 minutes, filter, make the filtrate alkaline to litmus paper with *5 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *light petroleum (60° to 80°)*. Combine the extracts, wash with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate carefully to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trifluoperazine hydrochloride RS* or with the reference spectrum of trifluoperazine hydrochloride.

B. Extract a quantity of the powdered tablets containing 5 mg of trifluoperazine with 5 ml of *acetone*, filter and evaporate the filtrate to dryness. Add 2 ml of *sulphuric acid* to the residue and allow to stand for 5 minutes; an orange colour is produced.

Tests

Uniformity of content. Comply with the test stated under Tablets.

Place one tablet in a 100-ml volumetric flask, add 50 ml of a mixture of 19 volumes of *water* and 1 volume of *hydrochloric acid*, shake until the tablet has completely disintegrated, dilute to volume with the same mixture, mix and filter, rejecting the first few ml of the filtrate. Dilute suitably, if necessary with the same solvent mixture and measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7). Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the specific absorbance at 256 nm.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 5 mg of trifluoperazine, shake for 15 minutes with 400 ml of a mixture of 19 volumes of *water* and 1 volume of *hydrochloric acid*, dilute to 500.0 ml with the same solvent mixture, mix and filter. Measure the absorbance of the filtrate at the maximum at about 256 nm (2.4.7).

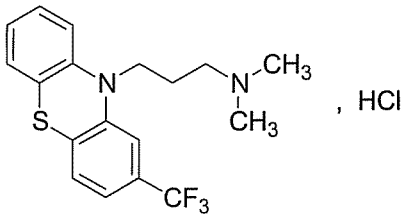
Calculate the content of $C_{21}H_{24}F_3N_3S$ taking 743 as the specific absorbance at 256 nm.

Storage. Store protected from light and moisture.

Labelling. The label states the strength in terms of the equivalent amount of trifluoperazine.

Triflupromazine Hydrochloride

Flupromazine Hydrochloride



$C_{18}H_{19}F_3N_2S, HCl$ Mol. Wt. 388.9

Triflupromazine Hydrochloride is 10-[3-(dimethylamino)-propyl)]-2-trifluoromethylphenothiazine hydrochloride.

Triflupromazine Hydrochloride contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{18}H_{19}F_3N_2S$, HCl, calculated on the dried basis.

Category. Antipsychotic; antiemetic.

Dose. As antipsychotic, orally, 30 to 150 mg of triflupromazine daily, in divided doses; by intramuscular injection, 10 mg repeated 4-hourly, if necessary; by intravenous injection, 1 to 3 mg repeated 4-hourly, if necessary. As antiemetic, 20 to 30 mg of triflupromazine daily; by intramuscular injection 1 to 3 mg.

Description. A white to pale yellowish brown, crystalline powder; odour slight and characteristic.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

A. Dissolve 20 mg in 10 ml of *water*, make the solution alkaline to *litmus paper* with *5 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *light petroleum (60° to 80°)*. Combine the extracts, wash with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate carefully to dryness.

The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triflupromazine hydrochloride RS* or with the reference spectrum of triflupromazine hydrochloride.

B. Complies with the test for identification of phenothiazines (2.3.3), using as reference solution a 0.2 per cent w/v solution of *triflupromazine hydrochloride RS* in *chloroform*.

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows

an absorption maximum at about 256 nm and a less well-defined maximum at about 305 nm.

D. A 10 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Related substances. Complies with the test for Related substances in phenothiazines (2.3.5), using *mobile phase A*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 0.8 g, dissolve in 50 ml of *anhydrous glacial acetic acid* add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03889 g of $C_{18}H_{19}F_3N_2S$, HCl.

Storage. Store protected from light and moisture.

Trifluprομazine Injection

Trifluprομazine Hydrochloride Injection; Flupromazine Hydrochloride Injection; Flupromazine Injection

Trifluprομazine Injection is a sterile solution of Trifluprομazine Hydrochloride in Water for Injections.

Trifluprομazine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of trifluprομazine hydrochloride, $C_{18}H_{19}F_3N_2S$, HCl.

Usual strength. 10 mg per ml.

Description. A clear, almost colourless solution.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

A. To an appropriate quantity of the injection add sufficient *0.1 M hydrochloric acid* to produce a solution containing 0.001 per cent w/v solution of Trifluprομazine Hydrochloride.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 256 nm.

B. To a volume containing about 100 mg of Trifluprομazine Hydrochloride add 5 ml of *8 M nitric acid* and mix; a pink to amber colour develops which quickly turns dark brown and then changes to a clear solution having a yellow tint.

Tests

pH (2.4.24). 3.5 to 5.2.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume of the injection containing about 20 mg of Trifluprομazine Hydrochloride add sufficient of a mixture of 19 volumes of *water* and 1 volume of *hydrochloric acid* to produce 100.0 ml and mix. Dilute 5.0 ml of the solution to 100.0 ml with the same mixture and mix. Measure the absorbance of the resulting solution at the maximum at about 256 nm (2.4.7).

Calculate the content of $C_{18}H_{19}F_3N_2S$, HCl taking 700 as the specific absorbance at 256 nm.

Storage. Store protected from light.

Trifluprομazine Tablets

Trifluprομazine Hydrochloride Tablets; Flupromazine Hydrochloride Tablets; Flupromazine Tablets

Trifluprομazine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of trifluprομazine hydrochloride, $C_{18}H_{19}F_3N_2S$, HCl.

Usual strengths. 10 mg; 25 mg.

NOTE — In the following procedures, the test and standard specimens and the solutions containing them should be protected by carrying out the tests without delay and in subdued light.

Identification

A. Shake a quantity of the powdered tablets containing 20 mg of Trifluprομazine Hydrochloride with 30 ml of *1 M hydrochloric acid* for 10 minutes, filter, make the filtrate alkaline to *litmus paper* with *5 M sodium hydroxide* and extract with two quantities, each of 20 ml, of *light petroleum (boiling range 60° to 80°)*. Combine the extracts, wash with 10 ml of water, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate carefully to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trifluprομazine hydrochloride RS* or with the reference spectrum of trifluprομazine hydrochloride.

B. Extract a quantity of the powdered tablets containing 5 mg of Trifluprομazine Hydrochloride with 5 ml of *acetone*, filter and evaporate the filtrate to dryness.

When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 256 nm.

Tests

Uniformity of content. *(For tablets containing 10 mg or less.*

Comply with the test stated under Tablets.

Crush one tablet and transfer to a 100-ml volumetric flask with the aid of a mixture of 19 volumes of *water* and 1 volume of *hydrochloric acid.* Shake well, dilute to volume with the same mixture and complete the procedure described under the Assay beginning at the words "mix and filter,...".

Calculate the content of $C_{18}H_{19}F_3N_2S$, HCl in the tablet.

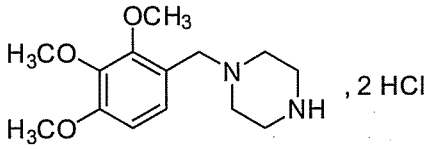
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 50 mg of Triflupromazine Hydrochloride and shake for 15 minutes with 200 ml of a mixture of 19 volumes of *water* and 1 volume of *hydrochloric acid.* Dilute to 500.0 ml with the same solvent mixture, mix and filter, rejecting the first few ml of the filtrate. Dilute 10.0 ml to 100.0 ml with the same solvent mixture. Measure the absorbance of the filtrate at the maximum at about 256 nm (2.4.7).

Calculate the content of $C_{18}H_{19}F_3N_2S$, HCl taking 700 as the specific absorbance at 256 nm.

Storage. Store protected from light and moisture.

Trimetazidine Hydrochloride



$C_{14}H_{22}N_2O_3$, 2HCl Mol. Wt. 339.3

Trimetazidine Hydrochloride is 1-[(2,3,4-trimethoxyphenyl)-methyl]piperazine dihydrochloride.

Trimetazidine Hydrochloride contains not less than 98.5 per cent and not more than 101.5 per cent, calculated on the dried basis.

Category. Antiischemic metabolic agent.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trimetazidine dihydrochloride RS* or with the reference spectrum of trimetazidine dihydrochloride.

B. Gives reaction (a) for chlorides (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution is clear (2.4.1) and not more intensely coloured than reference solution BYS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.2 g of the substance under examination in 50 ml of *water.*

Reference solution. Dilute 2.0 ml of the test solution to 100.0 ml with *water.* Dilute 5.0 ml of this solution to 100.0 ml with *water.*

Chromatographic system

- a stainless steel column15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 35.7 volumes of *methanol* and 64.3 volumes of a 0.29 per cent w/v solution of *sodium heptanesulphonate*, adjusted to pH 3.0 with *orthophosphoric acid,*
  B. *methanol,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 50	95 → 75	5 → 25
50 - 52	75 → 95	25 → 5

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to trimetazidine for (2,3,4-trimethoxyphenyl)methanol (trimetazidine impurity D) is about 0.2, for 2,3,4-trimethoxybenzaldehyde (trimetazidine impurity C) is about 0.4, for ethyl 4-(2,3,4-trimethoxybenzyl)piperazine-1-carboxylate (trimetazidine impurity H) is about 0.6, for 1-(3,4,5-trimethoxybenzyl)piperazine (trimetazidine impurity A) and *N*-methyltrimetazidine (trimetazidine impurity I) is about 0.9, for 1-(2,4,5-trimethoxybenzyl)piperazine (trimetazidine impurity E) is about 0.95, for 1-(2,4,6-trimethoxybenzyl) piperazine (trimetazidine impurity F) is about 1.4 and for 1,4-bis(2,3,4-trimethoxybenzyl) piperazine (trimetazidine impurity B) is about 1.8.

Inject the test solution and the reference solution. In the chromatogram obtained with the test solution, the area of each peak due to trimetazidine impurity A, B, C, D, E, F, H, I is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent), the area of any other secondary peak is not more than

the area of the principal peak in the chromatogram obtained with the reference solution (0.1 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

Impurity G. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 20 volumes of *concentrated ammonia* and 80 volumes of *ethanol (95 per cent).*

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of *methanol.*

Reference solution. Dissolve 22.6 mg of *piperazine hydrate* (impurity G) in 100 ml of *methanol.* Dilute 10 ml of this solution to 100 ml with *methanol.*

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 8 cm. Dry the plate at 105° for 30 minutes and spray with *iodoplatinate reagent.* Any secondary spot corresponding to trimetazidine impurity G is not more intense than the spot in the chromatogram obtained with the reference solution (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

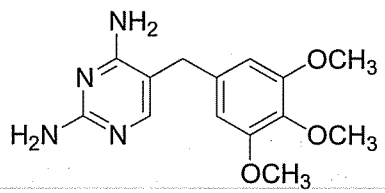
Loss on drying (2.4.19). Not more than 2.5 per cent, determined on 1.0 g by drying in an oven at 105 ° over *diphosphorus pentoxide* at a pressure not exceeding 15 kPa.

Assay. Dissolve 0.12 g in 50.0 ml of *water.* Add 1 ml of *nitric acid* and titrate with *0.1 M silver nitrate,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M silver nitrate* is equivalent to 0.01696 g of $C_{14}H_{24}Cl_2N_2O_3$.

Storage. Store protected from moisture.

Trimethoprim



$C_{14}H_{18}N_4O_3$ Mol. Wt. 290.3

Trimethoprim is 5-(3,4,5-trimethoxybenzyl)pyrimidine-2,4-diamine.

Trimethoprim contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{14}H_{18}N_4O_3$, calculated on the dried basis.

Category. Antibacterial.

Dose. 200 mg twice daily.

Description. A white or yellowish white powder; odourless or almost odourless.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trimethoprim RS* or with the reference spectrum of trimethoprim.

B. Dissolve 25 mg in 25 ml of *ethanol (95 per cent)* and dilute 2.0 ml to 100 ml with *0.1 M sodium hydroxide.*

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 287 nm; absorbance at about 287 nm, about 0.49.

C. Dissolve about 25 mg in 5 ml of *0.005 M sulphuric acid,* with heating if necessary, add 2 ml of a 1.6 per cent w/v solution of *potassium permanganate* in *0.1 M sodium hydroxide.* Heat to boiling and to the hot solution add 0.4 ml of *formaldehyde solution.* Mix, add 1 ml of *0.5 M sulphuric acid,* mix and heat to boiling. Cool and filter. Add 2 ml of *chloroform* to the filtrate and shake vigorously. The chloroform layer exhibits a green fluorescence when examined in ultraviolet light at 365 nm.

Tests

Appearance of solution. A 5.0 per cent w/v solution in a mixture of 10 volumes of *chloroform,* 9 volumes of *methanol* and 2 volumes of *water* is not more intensely coloured than reference solution BYS7 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 85 volumes of *ethyl acetate,* 10 volumes of *methanol,* 5 volumes of *water* and 2 volumes of *anhydrous formic acid.*

Test solution. Dissolve 0.4 g of the substance under examination in 10 ml of a mixture of 10 volumes of *chloroform,* 9 volumes of *methanol* and 2 volumes of *water.*

Reference solution. A 0.008 per cent w/v solution of the substance under examination in a mixture of 10 volumes of *chloroform,* 9 volumes of *methanol* and 2 volumes of *water.*

Apply to the plate 5 µl of each solution. Allow the mobile phase to rise 17 cm. Dry the plate in a current of cold air for 5 minutes and examine in ultraviolet light at 254 nm. Place an

evaporating dish containing a mixture of 2 volumes of a 1.5 per cent w/v solution of *potassium permanganate*, 1 volume of *7 M hydrochloric acid* and 1 volume of *water* at the bottom of a closed tank and allow to stand for 15 minutes. Place the dried plate in the closed tank and expose to the chlorine vapour for 5 minutes. Remove the plate from the tank and remove the chlorine in a current of cold air until an area below the line of application does not give a blue colour on the addition of 0.05 ml of *potassium iodide* and *starch solution*. Spray the plate with *potassium iodide* and *starch solution* and examine in daylight. By both methods of visualisation any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.25 g, dissolve in 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02903 g of $C_{14}H_{18}N_4O_3$.

Storage. Store protected from light.

Trimethoprim and Sulphamethoxazole Oral Suspension

Sulphamethoxazole and Trimethoprim Oral Suspension; Co-trimoxazole Oral Suspension; Co-trimoxazole Mixture

Trimethoprim and Sulphamethoxazole Oral Suspension is a suspension of Trimethoprim and Sulphamethoxazole in a suitable flavoured vehicle. It contains 5 parts of Sulphamethoxazole for 1 part, by weight, of Trimethoprim.

Trimethoprim and Sulphamethoxazole Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of trimethoprim, $C_{14}H_{18}N_4O_3$, and sulphamethoxazole, $C_{10}H_{11}N_3O_3S$.

Category. Antibacterial.

Dose. For children upto 1 year, Trimethoprim, 40 mg and Sulphamethoxazole, 200 mg daily, in divided doses. For children 1 to 5 years of age, Trimethoprim, 80 mg and Sulphamethoxazole, 400 mg daily, in divided doses. For adults, Trimethoprim, 160 to 480 mg and Sulphamethoxazole, 800 mg to 2.4 g daily, in divided doses.

Usual strengths. Trimethoprim 40 mg and Sulpha- methoxazole 200 mg in 5 ml; Trimethoprim 80 mg and Sulphamethoxazole 400 mg in 5 ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *chloroform*, 2 volumes of *methanol* and 1 volume of *dimethylformamide*.

Test solution. Add 20 ml of *methanol* (or a suitable volume of *methanol* to yield 0.4 per cent w/v solution of Trimethoprim) to 5 ml of the suspension, mix, shake with 10 g of *anhydrous sodium sulphate*, centrifuge and use the supernatant liquid.

Reference solution (a). A 2.0 per cent w/v solution of *sulphamethoxazole RS* in *methanol*.

Reference solution (b). A 0.4 per cent w/v solution of *trimethoprim RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a) and the other corresponds to that in the chromatogram obtained with reference solution (b)

Tests

pH (2.4.24). 5.0 to 6.5.

Other tests. Complies with the tests stated under Oral liquids.

Assay. *For trimethoprim* — Weigh accurately about 4 g, add 30 ml of *0.1 M sodium hydroxide*, shake and extract with four quantities, each of 50 ml, of *chloroform*, washing each extract with the same two quantities, each of 10 ml, of *0.1 M sodium hydroxide*. Reserve the combined aqueous solution and washings for the Assay for sulphamethoxazole. Extract the combined chloroform extracts with four quantities, each of 50 ml, of *1 M acetic acid*. Wash the combined extracts with 5 ml of *chloroform* and dilute the aqueous extracts to 250.0 ml with *1 M acetic acid*. To 10.0 ml of this solution add 10 ml of *1 M acetic acid* and sufficient *water* to produce 100.0 ml, mix and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7).

Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm. Determine the weight per ml of the suspension (2.4.29), and calculate the content of $C_{14}H_{18}N_4O_3$, weight in volume.

For sulphamethoxazole — Carry out the following procedure protected from light.

Dilute the combined aqueous solution reserved in the Assay for trimethoprim to 250.0 ml with *water*, filter and dilute 5.0 ml of the filtrate to 200.0 ml with water (solution A). To 2.0 ml of solution A add 0.5 ml of *4 M hydrochloric acid* and 1 ml of a 0.1 per cent w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 ml of a 0.5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 3 minutes. Add 1 ml of a 0.1 per cent w/v solution of *N- (1-napthyl) ethylenediamine dihydrochloride* and allow to stand for 10 minutes. Dilute the solution to 25.0 ml with *water* and measure the absorbance of the resulting solution at about 538 nm (2.4.7), using as the blank a solution prepared in the same manner but using 2 ml of *water* in place of solution A. Weigh accurately about 0.25 g of *sulphamethoxazole RS*, dissolve in 50 ml of *0.1 M sodium hydroxide* and dilute to 250.0 ml with *water*. Dilute 5.0 ml of the resulting solution to 200.0 ml with *water* (solution B). Repeat the procedure using 2.0 ml of solution B and beginning at the words "add 0.5 ml of *4 M hydrochloric acid.....*".

Calculate the content of $C_{10}H_{11}N_3O_3S$ from the values of the absorbances obtained. Calculate the content of $C_{10}H_{11}N_3O_3S$, weight in volume from the weight per ml determined in the Assay for trimethoprim.

Storage. Store protected from light and moisture. The suspension should not be allowed to freeze.

Labelling. The label states (1) the content of Trimethoprim and of Sulphamethoxazole in each 5 ml of the suspension; (2) that the contents should be shaken before use; (3) that a suspension containing 40 mg of Trimethoprim and 200 mg of Sulphamethoxazole in 5 ml is meant for paediatric use.

Trimethoprim and Sulphamethoxazole Tablets

Sulphamethoxazole and Trimethoprim Tablets; Co-trimoxazole Tablets

Trimethoprim and Sulphamethoxazole Tablets contain 5 parts of Sulphamethoxazole for 1 part, by weight, of Trimethoprim.

Trimethoprim and Sulphamethoxazole Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amounts of trimethoprim, $C_{14}H_{18}N_4O_3$, and sulphamethoxazole, $C_{10}H_{11}N_3O_3S$.

Category. Antibacterial.

Dose. For children upto 1 year, Trimethoprim, 40 mg and Sulphamethoxazole, 200 mg daily, in divided doses. For children 1 to 5 years of age, Trimethoprim, 80 mg and Sulphamethoxazole, 400 mg daily, in divided doses. For adults, Trimethoprim, 160 to 480 mg and Sulphamethoxazole, 800 mg to 2.4 g daily, in divided doses.

Usual strengths. Trimethoprim 20 mg and Sulpha- methoxazole 100 mg; Trimethoprim 80 mg and Sulphamethoxazole 400 mg; Trimethoprim 160 mg and Sulphamethoxazole 800 mg.

Identification

A. To a quantity of the powdered tablets containing 50 mg of Trimethoprim add 30 ml of *0.1 M sodium hydroxide* and extract with two quantities, each of 50 ml, of *chloroform*. Wash the combined chloroform extracts with two quantities, each of 10 ml, of *0.1 M sodium hydroxide* and then with 10 ml of *water*. Combine the aqueous extract and washings (solution A) and reserve for test B. Shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate to dryness using a rotary evaporator.

The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trimethoprim RS* or with the reference spectrum of trimethoprim.

B. Filter solution A, add, dropwise, sufficient *2 M hydrochloric acid* to the filtrate to make it just acidic and extract with 50 ml of *ether*. Wash the ether layer with 10 ml of *water*, shake with 5 g of *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness using a rotary evaporator. Dissolve the residue in the minimum volume of a 5 per cent w/v solution of *sodium carbonate*, add *1 M hydrochloric acid* dropwise until precipitation is complete and filter. Wash the residue sparingly with water and dry at 105°.

The residue complies with the following test. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphamethoxazole RS* or with the reference spectrum of sulphamethoxazole.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 20 volumes of *chloroform*, 2 volumes of *methanol* and 1 volume of *dimethylformamide*.

Test solution. Shake a quantity of the powdered tablets containing 0.4 g of Sulphamethoxazole with 20 ml of *methanol* and filter.

Reference solution (a). A 2.0 per cent w/v solution of *sulphamethoxazole RS* in *methanol*.

Reference solution (b). A 0.4 per cent w/v solution of *trimethoprim RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with *dilute potassium iodobismuthate solution*. One of the principal spots in the chromatogram obtained with the test solution corresponds to

that in the chromatogram obtained with reference solution (a) and the other corresponds to that in the chromatogram obtained with reference solution (b)

Tests

Other tests. Comply with the tests stated under Tablets.

Assay. *For trimethoprim* — Weigh accurately a quantity of the powdered tablets containing about 50 mg of Trimethoprim, add 30 ml of *0.1 M sodium hydroxide* and extract with four quantities, each of 50 ml, of *chloroform*, washing each extract with the same two quantities, each of 10 ml, of *0.1 M sodium hydroxide*. Combine the chloroform extracts and extract with four quantities, each of 50 ml, of *1 M acetic acid*. Wash the combined extracts with 5 ml of *chloroform* and dilute the aqueous extracts to 250.0 ml with *1 M acetic acid*. To 10.0 ml of the solution add 10 ml of *1 M acetic acid* and sufficient *water* to produce 100.0 ml, mix and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7).

Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm.

For sulphamethoxazole — Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.2 g of Sulphamethoxazole, dissolve as completely as possible in 60 ml of *water* and 10 ml of *hydrochloric acid*. Add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02533 g of $C_{10}H_{11}N_3O_3S$.

Storage. Store protected from light and moisture.

Labelling. The label states the quantities of Trimethoprim and of Sulphamethoxazole in each tablet.

Trimethoprim Tablets

Trimethoprim Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of trimethoprim, $C_{14}H_{18}N_4O_3$.

Usual strengths. 100 mg; 200 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 10 ml of *chloroform*, filter and evaporate the filtrate to dryness.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *trimethoprim RS* or with the reference spectrum of trimethoprim.

B. Shake a quantity of the powdered tablets containing 0.1 g of Trimethoprim with 60 ml of *0.1 M hydrochloric acid* for 20 minutes, add sufficient *0.1 M hydrochloric acid* to produce 100 ml, filter and dilute 5 ml of the filtrate to 250 ml with *0.1 M sodium hydroxide*.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum at about 287 nm.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Trimethoprim with 50 ml of the mobile phase, filter and use the filtrate.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 5 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 0.14 per cent w/v solution of *sodium perchlorate* in *methanol (60 per cent)* adjusted to pH 3.1 with *0.1 M hydrochloric acid*,
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

The column efficiency, determined using the peak due to trimethoprim in the chromatogram obtained with reference solution, should be at least 8,000 theoretical plates per metre.

In the chromatogram obtained with the test solution the sum of the areas of any secondary peaks is not greater than the area of the principal peak in the chromatogram obtained with reference solution.

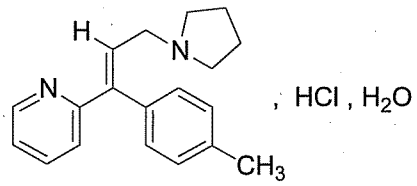
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 0.1 g of Trimethoprim, add 100 ml of *glacial acetic acid*, shake for 20 minutes, dilute to 200.0 ml with *glacial acetic acid* and filter. To 5.0 ml of the filtrate add 15 ml of *glacial acetic acid* and dilute to 100.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7).

Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm.

Storage. Store protected from moisture.

Triprolidine Hydrochloride



$C_{19}H_{22}N_2,HCl,H_2O$ Mol. Wt. 332.9

Triprolidine Hydrochloride is (*E*)-2-(3-pyrrolidin-1-yl-1-(*p*-tolyl)prop-1-enyl)pyridine hydrochloride monohydrate.

Triprolidine Hydrochloride contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{22}N_2$, HCl, calculated on the anhydrous basis.

Category. Histamine H_1-receptor antagonist.

Dose. 10 to 20 mg daily, in divided doses.

Description. A white, crystalline powder; almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triprolidine hydrochloride RS* or with the reference spectrum of triprolidine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution shows absorption maxima at about 230 nm and 276 nm; absorbance at about 230 nm, about 0.50 and at about 276 nm, about 0.25.

C. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.05 M sulphuric acid* shows an absorption maximum at about 290 nm; absorbance at about 290 nm, about 0.6.

D. Dissolve 0.1 g in 2 ml of *2 M hydrochloric acid* and add 0.5 ml of *potassium mercuri-iodide solution*; a pale yellow precipitate is produced.

E. A 5 per cent w/v solution gives the reactions of chlorides (2.3.1).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of equal volumes of *2-butanone* and *dimethylformamide*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *methanol*.

Reference solution (a). Dissolve 10 mg of the substance under examination in 100 ml of *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *Z-triprolidine RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to Z-triprolidine is not more intense than the spot in the chromatogram obtained with reference solution (b) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). 4.0 to 6.0 per cent, determined on 0.4 g.

Assay. Weigh accurately about 0.25 g and dissolve in a mixture of 50 ml of *anhydrous glacial acetic acid* and 0.5 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01574 g of $C_{19}H_{22}N_2$, HCl.

Storage. Store protected from light and moisture.

Triprolidine Tablets

Triprolidine Hydrochloride Tablets

Triprolidine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of triprolidine hydrochloride, $C_{19}H_{22}N_2,HCl,H_2O$.

Usual strength. 10 mg.

Identification

A. Extract a quantity of the powdered tablets containing 10 mg of Triprolidine Hydrochloride with *ether*, filter, discard the ether extract and dry the residue. Extract the residue with *chloroform*, filter and evaporate the filtrate to dryness. Add 0.1 ml of *ether*, stir and allow to evaporate.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *triprolidine hydrochloride RS* or with the reference spectrum of triprolidine hydrochloride.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of equal volumes of *2-butanone* and *dimethylformamide.*

Test solution. Extract a quantity of powdered tablets containing 10 mg of Triprolidine Hydrochloride with *methanol*, filter, evaporate to dryness and dissolve the residue in 1 ml of *methanol.*

Reference solution (a). A 0.02 per cent w/v solution of *Z-triprolidine RS* in *methanol.*

Reference solution (b). A 1.0 per cent w/v solution of *triprolidine hydrochloride RS* in *methanol.*

Reference solution (c). A 0.01 per cent w/v solution of *triprolidine hydrochloride RS* in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot corresponding to Z-triprolidine is not more intense than the spot in the chromatogram obtained with reference solution (a) and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (c).

Uniformity of content. *(For tablets containing 10 mg or less).* Comply with the test stated under Tablets.

Powder one tablet, weigh accurately a quantity of the powder containing 7.5 mg of Triprolidine Hydrochloride and carry out the Assay beginning at the words "add 15 ml of *water*....".

Calculate the content of $C_{19}H_{22}N_2$,HCl,H_2O in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 7.5 mg of Triprolidine Hydrochloride, add 15 ml of *water* and 1 g of *sodium chloride*, shake for 2 to 3 minutes and add sufficient *5 M sodium hydroxide* to make it alkaline. Extract with four quantities, each of 20 ml, of *ether* and wash the combined extracts with two quantities, each of 5 ml, of a mixture of equal volumes of a saturated solution of *sodium chloride* and *water*. Extract the ether solution with 20 ml of *0.1 M hydrochloric acid*, wash the ether with two quantities, each of 5 ml, of *water* and add the washings to the acid extract. Heat on a water-bath for 30 minutes, cool and add sufficient *water* to produce 50.0 ml. Dilute 10.0 ml to 100.0 ml with *0.1 M hydrochloric acid.* Measure the absorbance of the resulting solution at the maximum at about 290 nm (2.4.7).

Calculate the content of $C_{19}H_{22}N_2$, HCl, H_2O taking 290 as the specific absorbance at 290 nm.

Storage. Store protected from light and moisture.

Trisodium Edetate Concentrate for Injection

Trisodium Edetate Concentrate for Injection is a sterile solution in Water for Injections containing 20 per cent w/v solution of trisodium edetate prepared by the interaction of Disodium Edetate and Sodium Hydroxide. It should be diluted with a suitable diluent in accordance with the manufacturer's instructions.

Trisodium Edetate Concentrate for Injection contains not less than 19.0 per cent w/v and not more than 21.0 per cent w/v solution of trisodium edetate, $C_{10}H_{13}N_2Na_3O_8$.

Category. Used in the treatment of hypercalcaemia.

Dose. By slow intravenous infusion, upto 70 mg per kg daily over 2 to 3 hours. For topical use in the eye, dilute 1 ml to 50 ml with Water for Injection.

Description. A colourless solution.

Identification

A. Dissolve 2 g in 25 ml of *water,* add 6 ml of *lead nitrate solution*, shake and add 3 ml of *potassium iodide solution*; no yellow precipitate is produced. Make alkaline to red litmus paper with *2 M ammonia* and add 5 ml of *ammonium oxalate solution*; no precipitate is produced.

B. Dissolve 0.5 g in 10 ml of *water*, add 0.5 ml of a 10 per cent w/v solution of *calcium chloride*, make alkaline to red litmus paper with *2 M ammonia* and add 3 ml of *ammonium oxalate solution*; no precipitate is produced.

C. Evaporate to dryness and ignite. The residue gives the reactions of sodium salts (2.3.1).

Tests

pH (2.4.24). 7.0 to 8.0.

Pyrogens (2.2.8). Complies with the test, using per kg of the rabbit's weight 5 ml of a solution prepared in the following manner. To 1 volume of the injection add 2.5 volumes of *calcium gluconate solution.* Dilute the resulting solution with sufficient water for injection to give a final concentration of 5.0 per cent w/v solution of trisodium edetate.

Other tests. Complies with the tests stated under Parenteral Preparations (Concentrated Solutions for Injection).

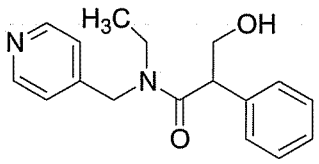
Assay. Dilute 10.0 ml to 100.0 ml with *water* and use this solution to titrate a mixture of 25.0 ml of *0.05 M magnesium sulphate* and 10 ml of *ammonia buffer pH 10.9* using *mordant black II mixed triturate* as indicator.

1 ml of *0.05 M magnesium sulphate* is equivalent to 0.01791 g of $C_{10}H_{13}N_2Na_3O_8$.

Storage. Store in hermetically-sealed, lead-free glass containers.

Labelling. The label states (1) the strength in terms of anhydrous trisodium edetate contained in a suitable dose-volume; (2) 'Trisodium Edetate Concentrate for Injection'; (3) that the solution must be diluted with either Sodium Chloride Intravenous Infusion or Dextrose Intravenous Infusion before administration.

Tropicamide



$C_{17}H_{20}N_2O_2$ Mol. Wt. 284.4

Tropicamide is (*RS*)-*N*-ethyl-3-hydroxy-2-phenyl-*N*-(pyrid-4-ylmethyl)propionamide

Tropicamide contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2O_2$, calculated on the dried basis.

Category. Mydriatic; cycloplegic.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tropicamide RS* or with the reference spectrum of tropicamide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum only at about 254 nm; absorbance at about 254 nm, about 0.54.

C. Dissolve 5 mg in 3 ml of a mixture of 9 ml of *acetic anhydride*, 1 ml of *6 M acetic acid* and 0.1 g of *citric acid* and heat on a water-bath for 5 to 10 minutes; a reddish yellow colour is produced.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 95 volumes of *chloroform*, 5 volumes of *methanol* and 0.5 volume of *strong ammonia solution*.

Test solution. Dissolve 0.1 g of the substance under examination in 10 ml of *chloroform*.

Reference solution (a). A 0.005 per cent w/v solution of the substance under examination in *chloroform*.

Reference solution (b). A 0.002 per cent w/v solution of the substance under examination in *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 0.5 g, dissolve in 80 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein* solution as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02844 g of $C_{17}H_{20}N_2O_2$.

Storage. Store protected from light and moisture.

Tropicamide Eye Drops

Tropicamide Eye Drops are a sterile solution of Tropicamide in Purified Water. They may contain stabilisers, suitable antimicrobial agents and suitable substances to increase the viscosity of the solution.

Tropicamide Eye Drops contain not less than 95.0 per cent and not more than 110.0 per cent of the stated amount of tropicamide, $C_{17}H_{20}N_2O_2$.

Usual strengths. 0.5 per cent w/v; 1.0 per cent w/v.

Identification

A. Shake a volume containing 20 mg of Tropicamide with 10 ml of *chloroform*, dry the chloroform layer over *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dissolve the residue in minimum quantity of *chloroform*, add dropwise to finely powdered *potassium bromide IR*, mix and dry at 60°.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tropicamide RS* or with the reference spectrum of tropicamide.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum at about 254 nm.

Tests

pH (2.4.24). 4.0 to 5.8.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 95 volumes of *chloroform*, 5 volumes of *methanol* and 0.5 volume of *strong ammonia solution*.

Test solution. A volume of the eye drops containing 0.2 mg of Tropicamide.

Reference solution (a). Dilute 1 volume of the eye drops to 200 volumes with *chloroform*.

Reference solution (b). Dilute 1 volume of the eye drops to 500 volumes with *chloroform*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Comply with the tests stated under Eye Drops.

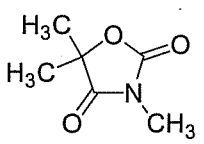
Assay. To a volume containing about 30 mg of Tropicamide add sufficient *water* to produce 100.0 ml. To 10.0 ml of the resulting solution add 2 ml of a 10 per cent w/v solution of *anhydrous sodium carbonate* and extract with four quantities, each of 20 ml, of *chloroform*. Wash the combined *chloroform* extracts with 25 ml of *phosphate buffer pH 6.5*. Wash the aqueous layer with 10 ml of *chloroform*, combine the chloroform extracts and shake with four quantities, each of 20 ml, of *0.5 M sulphuric acid*. Combine the acid extracts, dilute to 100.0 ml with *0.5 M sulphuric acid* and measure the absorbance of the resulting solution at the maximum at about 254 nm (2.4.7).

Calculate the content of $C_{17}H_{20}N_2O_2$ taking 172 as the specific absorbance at 254 nm.

Storage. Store in a refrigerator (8° to 15°). It should not be allowed to freeze.

Troxidone

Trimethadione



$C_6H_9NO_3$ Mol. Wt. 143.1

Troxidone is 3,5,5-trimethyloxazolidine-2,4-dione.

Troxidone contains not less than 98.0 per cent and not more than 102.0 per cent of $C_6H_9NO_3$, calculated on the dried basis.

Category. Anticonvulsant.

Dose. For a child, 250 mg to 500 mg daily, in divided doses; for an adult, 1 to 2 g daily, in divided doses.

Description. Colourless or almost colourless crystals; odour, slightly camphoraceous.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *troxidone RS*.

B. To 2 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) add 1 ml of *barium hydroxide solution*; a white precipitate is produced which dissolves on the addition of 1 ml of *2 M hydrochloric acid*.

C. Dissolve 0.3 g in a mixture of 5 ml of *ethanolic potassium hydroxide solution* and 5 ml of *ethanol (95 per cent)* and allow to stand for 10 minutes. Add 0.05 ml of *phenolphthalein solution* and carefully add *hydrochloric acid* until the solution is neutral. Evaporate to dryness on a water-bath, shake the residue with four quantities, each of 5 ml, of *ether*, filter the combined ether extracts and evaporate the filtrate to dryness. The residue, after recrystallisation from 5 ml of *toluene* and drying, melts at about 80° (2.4.21).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Acidity or alkalinity. To 10 ml of solution A add 0.1 ml of *methyl red solution*. Not more than 0.1 ml of *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide* is required to change the colour of the solution.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *anhydrous silica gel* for 6 hours.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh accurately 0.1 g of the substance under examination and dissolve in sufficient solutions prepared by dissolving 0.125 g of *1-decanol* (internal standard) in sufficient *ethanol* to produce 25.0 ml (solution B).

Reference solution. Dissolve 0.1 g of *troxidone RS* in sufficient solution B to produce 10.0 ml.

Chromatographic system
- a stainless steel column 0.75 m x 3 mm, packed with porous polymer beads (120 to 150 µm),
- temperature:
  column 210°,
  inlet port at 240° and detector at 270°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the test solution and the reference solution.

Calculate the content of $C_6H_9NO_3$.

Storage. Store protected from light and moisture.

Troxidone Capsules

Trimethadione Capsules

Troxidone Capsules contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of troxidone, $C_6H_9NO_3$.

Usual strength. 250 mg.

Identification

To a quantity of the contents of the capsules containing 1 g of Troxidone add 25 ml of *ether*, set aside in a stoppered flask for 20 minutes, decant the ether through a filter, and if an insoluble residue remains, digest it with another 10-ml portion of *ether* as before, and filter into the first ether filtrate. Evaporate the ether extracts to dryness with the aid of a current of air and dry the residue at a pressure of 2 kPa for 2 hours. The residue complies with the following tests.

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *troxidone RS* or with the reference spectrum of troxidone.

B. To 2 ml of a 5.0 per cent w/v solution in *carbon dioxide-free water* (solution A) add 1 ml of *barium hydroxide solution*; a white precipitate is produced which dissolves on the addition of 1 ml of *2 M hydrochloric acid*.

C. Dissolve 0.3 g in a mixture of 5 ml of *ethanolic potassium hydroxide solution* and 5 ml of *ethanol (95 per cent)* and allow to stand for 10 minutes. Add 0.05 ml of *phenolphthalein solution* and carefully add *hydrochloric acid* until the solution is neutral. Evaporate to dryness on a water-bath, shake the residue with four quantities, each of 5 ml, of *ether*, filter the combined ether extracts and evaporate the filtrate to dryness. The residue, after recrystallisation from 5 ml of *toluene* and drying, melts at about 80° (2.4.21).

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by gas chromatography (2.4.13).

Test solution. To a quantity of the contents of the capsules containing about 1.0 g of Troxidone add 25 ml of a 0.5 per cent w/v solution of *1-decanol* (internal standard) in *ethanol*, shake for 30 minutes, add sufficient of internal standard solution to produce 100.0 ml, mix and centrifuge. Use the supernatant liquid.

Reference solution. Dissolve 0.1 g of *troxidone RS* in sufficient solution B to produce 10.0 ml.

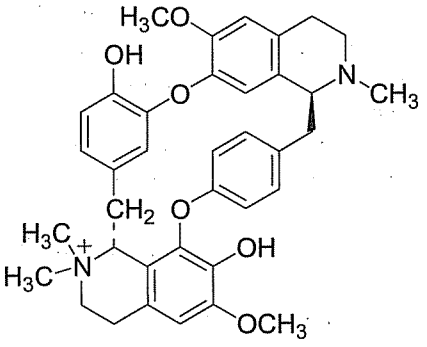
Chromatographic system
- a stainless steel column 0.75 m × 3 mm, packed with porous polymer beads (120 to 150 µm),
- temperature:
  column 210°,
  inlet port at 240° and detector at 270°,
- flow rate. 30 ml per minute of the carrier gas.

Inject 1 µl of the test solution and the reference solution.

Calculate the content of $C_6H_9NO_3$ in the capsules.

Storage. Store protected from moisture.

Tubocurarine Chloride



Cl, HCl, $5H_2O$

$C_{37}H_{41}ClN_2O_6,HCl,5H_2O$ Mol. Wt. 771.7

Tubocurarine Chloride is 7′ ,12′ -dihydroxy-6,6′ -dimethoxy-2,2′ ,2′ -trimethyltubocuraranium chloride hydrochloride pentahydrate.

Tubocurarine Chloride contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{37}H_{41}ClN_2O_6,HCl$, calculated on the anhydrous basis.

Category. General anaesthetic.

Dose. By intramuscular or intravenous injection, 100 to 300 µg per kg body weight, total dose not exceeding 40 mg.

Description. A white or yellowish white, crystalline powder.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tubocurarine chloride RS* or with the reference spectrum of tubocurarine chloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution shows an absorption maximum at about 280 nm and a minimum at about 255 nm, 0.56 to 0.62, calculated on the anhydrous basis.

C. To 1 ml of a 2.5 per cent w/v solution add 0.2 ml of *ferric chloride solution* and heat in a water-bath for 1 minute; a green colour is produced; 1 ml of *water* treated in the same manner gives a brown colour.

D. To 20 ml of a 0.05 per cent w/v solution add 0.2 ml of *sulphuric acid* and 2 ml of a 1 per cent w/v solution of *potassium iodate*, mix and warm on a water-bath for 30 minutes; a yellow colour is produced.

E. Gives reaction A of chlorides and the reactions of alkaloids (2.3.1).

Tests

Appearance of solution. Dissolve 0.5 g in sufficient *carbon dioxide-free water* to produce 50.0 ml (solution A). Solution A is clear (2.4.1), and not more intensely coloured than reference solution YS6 (2.4.1).

pH (2.4.24). 4.0 to 6.0, determined in Solution A.

Specific optical rotation (2.4.22). +210° to +222°, determined in solution A 3 hours after preparation.

Chloroform-soluble substances. Not more than 2 per cent, determined by the following method. Dissolve 0.25 g in 150 ml of *water* contained in a separating funnel with a grease-free stopcock. Add 5 ml of a saturated solution of *sodium bicarbonate* and extract with three quantities, each of 20 ml, of *chloroform*. Wash the combined chloroform extracts with 10 ml of *water*, filter the *chloroform* solution into a tared beaker and wash the filter with two successive quantities, each of 5 ml, of *chloroform*. Add the washings to the filtrate. Remove the chloroform on a water-bath, dry the residue at 105° for 1 hour, cool and weigh. The residue does not dissolve in 10 ml of *water* but dissolves on the addition of 1 ml of *2 M hydrochloric acid.*

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. The lower layer of a mixture of equal volumes of *chloroform*, *methanol* and a 12.5 per cent w/v solution of *trichloroacetic acid.*

Test solution. Dissolve 0.25 g of the substance under examination in 10 ml of *water.*

Reference solution (a). A 0.0375 per cent w/v solution of the substance under examination in *water.*

Reference solution (b). A 0.01875 per cent w/v solution of the substance under examination in *water.*

Apply to the plate 5 µl of each solution. After development, dry the plate in a current of cold air and spray with a mixture of 1 volume of *potassium ferricyanide solution*, 1 volume of *water* and 2 volumes of *ferric chloride solution*, prepared immediately before use. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Sulphated ash (2.3.18). Not more than 0.25 per cent, determined on 0.2 g.

Water (2.3.43). 9.0 to 12.0 per cent, determined on 0.3 g.

Assay. Weigh accurately about 25 mg, dissolve in sufficient *water* to produce 500.0 ml and measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7).

Calculate the content of $C_{37}H_{41}ClN_2O_6,HCl$ from the absorbance obtained by repeating the operation using 25 mg, accurately weighed, of *tubocurarine chloride RS* in place of the substance under examination.

Storage. Store protected from moisture.

Tubocurarine Injection

Tubocurarine Chloride Injection

Tubocurarine Injection is a sterile solution of Tubocurarine Chloride in Water for Injections. It may contain suitable buffering agents.

Tubocurarine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tubocurarine chloride, $C_{37}H_{41}ClN_2O_6,HCl,5H_2O$.

Usual strength. 10 mg per ml.

Description. A colourless or faintly coloured solution.

Identification

A. Mix a volume containing 15 mg of Tubocurarine Chloride with 5 ml of *acetone*, evaporate the liquid at a pressure of

2 kPa and add successive quantities of 2 ml of *acetone*, evaporating each quantity at a pressure of 2 kPa until a dry residue is obtained.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tubocurarine chloride RS* or with the reference spectrum of tubocurarine chloride.

B. Dilute 1 ml to 30 ml with *water*. To 1 ml of the resulting solution add 0.5 ml of *mercuric nitrate solution*; a cherry-red colour slowly develops.

Tests

pH (2.4.24). 4.0 to 6.0.

Optical rotation (2.4.22).+0.172° to +0.206° for each mg of tubocurarine chloride, $C_{37}H_{41}ClN_2O_6,HCl,5H_2O$ per ml stated on the label.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. The lower layer of a mixture of equal volumes of *chloroform*, *methanol* and a 12.5 per cent w/v solution of *trichloroacetic acid*.

Test solution. A volume of the injection containing 10 mg of Tubocurarine Chloride diluted to 1 ml.

Reference solution (a). Dilute 3 volumes of test solution to 200 volumes with *water*.

Reference solution (b). Dilute 1 volume of reference solution (a) to 2 volumes with *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in a current of cold air and spray with a mixture of 1 volume of *potassium ferricyanide solution*, 1 volume of *water* and 2 volumes of *ferric chloride solution*, prepared immediately before use. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a volume containing about 50 mg of Tubocurarine Chloride to 1000.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 280 nm (2.4.7).

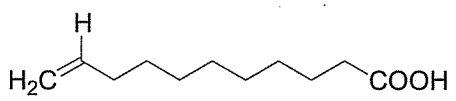
Calculate the content of $C_{37}H_{41}ClN_2O_6,HCl,5H_2O$ taking 105 as the specific absorbance at 280 nm.

Storage. Store protected from moisture.

U

Undecenoic Acid

Undecylenic Acid



$C_{11}H_{20}O_2$ Mol. Wt. 184.3

Undecenoic Acid is 10-undecenoic acid.

Undecenoic Acid contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{11}H_{20}O_2$.

Category. Antifungal (topical).

Description. A white or very pale yellow, crystalline mass or colourless or pale yellow liquid; odour, characteristic.

Identification

A. Dissolve 0.1 g in a mixture of 2 ml of *1 M sulphuric acid* and 5 ml of *glacial acetic acid* and add dropwise 0.25 ml of *potassium permanganate solution*; the colour of the permanganate solution is discharged.

B. Boil 2 g under a reflux condenser with 2 ml of freshly distilled *aniline* for 10 minutes, allow to cool, add 30 ml of *ether* and extract with three quantities, each of 20 ml, of *2 M hydrochloric acid* and then with 20 ml of *water*. Evaporate the organic layer to dryness on a water-bath. The residue, after recrystallising twice from *ethanol (70 per cent)* and drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa for 3 hours melts at 66° to 68°.

Tests

Congealing range (2.4.10). 21° to 24°.

Refractive index (2.4.27). 1.447 to 1.450.

Peroxide value (2.3.35). Not more than 10.

Iodine value (2.3.28). 131 to 140.

Fixed and mineral oils. Boil 1.0 g with 25 ml of *water* and 5 ml of *sodium carbonate solution* for 3 minutes. The hot solution is not more opalescent than opalescence standard OS2 (2.4.1).

Water-soluble acids. Shake 2.0 g with 20 ml of warm *water*, allow to separate and filter the aqueous layer through a moistened filter paper. To 5 ml of the filtrate add 0.01 ml of *dilute phenolphthalein solution*. Not more than 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

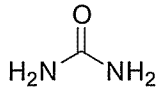
Sulphated ash (2.3.18). Not more than 0.15 per cent.

Assay. Weigh accurately about 0.75 g, dissolve in 10 ml of *ethanol (95 per cent)* and titrate with *0.5 M sodium hydroxide* using 0.1 ml of *dilute phenolphthalein solution* as indicator.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.09214 g of $C_{11}H_{20}O_2$.

Storage. Store protected from light and moisture.

Urea



CH_4N_2O Mol. Wt. 60.1

Urea is the diamide of carbonic acid.

Urea contains not less than 99.0 per cent and not more than 101.0 per cent of CH_4N_2O, calculated on the dried basis.

Category. Keratolytic.

Dose. 5 to 15 g.

Description. A white, crystalline powder or transparent crystals; odourless or almost odourless, but may gradually develop a slight odour of ammonia upon long standing; slightly hygroscopic.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *urea RS* or with the reference spectrum of urea.

B. Heat 0.5 g in a test-tube; it liquefies, and ammonia is evolved which is recognised by its characteristic odour. Heat further until the liquid is turbid, cool and dissolve in 10 ml of *water*. Add 1 ml of a 10 per cent w/v solution of *sodium hydroxide* and 0.05 ml of *copper sulphate solution*; a reddish violet colour is produced.

C. Dissolve 0.1 g in 1 ml of *water* and add 1 ml of *nitric acid*; a white, crystalline precipitate is produced.

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

Alkalinity. To 10 ml of a 5.0 per cent w/v solution (solution A) add 0.1 ml of *methyl red solution* and 0.4 ml of *0.01 M hydrochloric acid*; the resulting solution is red to orange.

Biuret. Not more than 0.1 per cent, determined by the following method. To 10 ml of a 20 per cent w/v solution add 5 ml of *water*, 0.5 ml of a 0.5 per cent w/v solution of *copper sulphate* and 0.5 ml of *10 M sodium hydroxide* and allow to stand for 5 minutes. Any reddish violet colour obtained is not more intense than that in a standard prepared at the same time and in the same manner using 10 ml of a 0.02 per cent w/v solution of *biuret* in place of the substance under examination.

Ethanol-insoluble matter. Not more than 0.04 per cent, determined by the following method. Dissolve 5.0 g in 50 ml of warm *ethanol (95 per cent)*, filter through a tared filter, wash the filter with 20 ml of warm *ethanol (95 per cent)* and dry at 105° for 1 hour.

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of *water* and 5 ml of *0.1 M hydrochloric acid*. The solution complies with the limit test for heavy metals, Method A (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Assay. Weigh accurately about 0.5 g, dissolve in sufficient of a 10 per cent v/v solution of *sulphuric acid* to produce 100.0 ml and mix. Place 5.0 ml of the resulting solution in a long-necked flask, add 10 ml of *sulphuric acid* and heat gently until evolution of gas ceases. Boil gently for 10 minutes, cool, cautiously add 40 ml of *water*, cool again and place in a steam-distillation apparatus. Add 50 ml of *10 M sodium hydroxide* and distil immediately by passing steam through the mixture. Continue the distillation for 1 hour, collecting the distillate in 40 ml of a 4 per cent w/v solution of *boric acid*. Titrate with *0.1 M hydrochloric acid*, using 0.25 ml of *methyl red-methylene blue solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M hydrochloric acid* is equivalent to 0.003003 g of CH_4N_2O.

Storage. Store protected from moisture.

Urea Cream

Urea Cream contains Urea in a suitable basis.

Urea Cream contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of urea, CH_4N_2O.

Usual strength. 10 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. For the first development use *2,2,4-trimethylpentane*. Dry the plate in air. For the second development use a mixture of 99 volumes of *ethanol* and 1 volume of *strong ammonia solution*.

Test solution. Disperse with heating a quantity of the cream containing 50 mg of Urea in 1 ml of *water*, cool, add 4 ml of *acetone*, mix and filter.

Reference solution (a). Dissolve 50 mg of *urea RS* in 1 ml of *water* and add 4 ml of *acetone*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 10 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.5 per cent w/v solution of *4-dimethylaminobenzaldehyde* and 0.5 per cent v/v of *sulphuric acid* in *ethanol*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The chromatogram obtained with reference solution (b) shows a single, compact spot.

B. To a quantity containing 0.1 g of Urea add 50 ml of *water* and heat until dispersed, cool in ice and filter through glass wool. Adjust the pH to 6.0 to 7.0 using *0.1 M hydrochloric acid* or *0.1 M sodium hydroxide* as necessary. To 5 ml add 5 ml of a 0.1 per cent w/v suspension of urease-active meal and allow to stand for 30 minutes in a stoppered flask at 37°. When the resulting solution is heated in a water-bath a vapour is produced that turns moist red *litmus paper* blue.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Weigh accurately a quantity containing about 42 mg of Urea, shake with 150 ml of hot *water* for 20 minutes, allow to cool and dilute to 500.0 ml with *water*. Filter through a fine glass microfibre filter paper or by any other means, transfer 1.0 ml of the filtrate to a 100-ml volumetric flask, add 2 ml of a 0.1 per cent w/v suspension of urease-active meal, stopper the flask and allow to stand for 15 minutes at 37°. Immediately add 25 ml of a solution containing 12 g of *sodium salicylate* and 0.24 g of *sodium nitroprusside* in 200 ml and 25 ml of a solution prepared by diluting a volume of *sodium hypochlorite solution* containing 0.66 g of available chlorine with *0.2 M sodium hydroxide* to 1000 ml. Mix well, allow to stand at 37° for 5 minutes and dilute to 100.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 665 nm (2.4.17), using as the blank a solution prepared in the same manner but using 1.0 ml of *water* in place of 1.0 ml of the filtrate.

Calculate the content of CH_4N_2O from the absorbance obtained by using 42 mg of *urea RS* in place of the substance under examination.

Storage. Store in accordance with the instructions of the manufacturer.

Urokinase

Urokinase is an enzyme, obtained from human urine that activates plasminogen. It consists of a mixture of low molecular weight and high molecular weight forms, the high molecular weight form being predominant. The molecular weights of the low and high molecular weight forms are 33,000 and 54,000 respectively.

It is prepared in conditions designed to minimise microbial and viral contamination. In particular, adequate measures, such as heating the substance in solution at 60° for 10 hours, are taken to inactivate viruses.

Urokinase contains not less than 70,000 Units of urokinase activity per mg of protein.

Category. Fibrinolytic enzyme.

Dose. By instillation into arteriovenous shunt and for intraocular administration, 5000 to 37,500 Units in 2 to 3 ml of Sodium Chloride Injection.

Description. A white or almost white, amorphous powder.

Identification

A. Place 0.5 ml of citrated human plasma and 0.5 ml of citrated bovine plasma in two separate haemolysis tubes maintained in a water-bath at 37°. To each tube add 0.1 ml of a solution of the substance under examination containing 1000 Units per ml in *phosphate buffer pH 7.4* and 0.1 ml of a solution of thrombin containing 20 Units per ml in *phosphate buffer pH 7.4* and shake immediately; in both tubes, a clot forms and lyses within 30 minutes.

B. Carry out a suitable immunodiffusion test.

Tests

Appearance of solution. A 0.1 per cent w/v solution in *water* is clear (2.4.1), and colourless (2.4.1).

Molecular fractions. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *0.02 M phosphate buffer pH 8.0*.

Chromatographic system
- a column 90 cm × 16 mm, packed with a cross-linked dextran suitable for fractionation of proteins in the range of molecular weight from 4000 to 150,000 (such as Sephadex G-100), at 5°,
- mobile phase: 1.75 per cent w/v solution of *sodium chloride* in *0.02 M phosphate buffer pH 8.0*,
- flow rate. 6 ml per hour,
- spectrophotometer set at 280 nm.

Apply 1 ml of the test solution to the column, rinse twice with 0.5-ml portions of the buffer and then carry out the elution. The eluate may be collected in 1-ml fractions. Plot the individual absorbances on a graph. Draw perpendicular lines towards the axis of the abscissae from the minima before the high molecular weight peak, between the high and the low molecular weight peaks, and after the low molecular weight peak. Combine separately the high and low molecular weight fractions and for each combined fraction determine the activity by the method described under Assay. The ratio of the activity in the combined high molecular weight fraction to that in the combined low molecular weight fraction is not less than 2.0.

Total protein. Determine by Method C for the determination of nitrogen (2.3.30), using 10 mg of the substance under examination and multiplying the result by 6.25 to obtain the content of protein.

Hepatitis-B surface antigen. Examine by a suitably sensitive method such as radio-immunoassay; hepatitis-B surface antigen is not detected.

Abnormal toxicity (2.2.1). Complies with the test, using a solution containing 2,500 Units in 0.5 ml of *saline solution*.

Thromboplastic contaminants. Dissolve suitable quantities of the substance under examination in *barbitone buffer pH 7.4* to obtain solutions containing 5000, 2500, 1250, 625 and 312 Units per ml. Into each of six haemolysis tubes, 1 cm in internal diameter, place 0.1 ml of citrated rabbit plasma. Add 0.1 ml of one of each of the solutions of the substance under examination to each of five of the tubes and 0.1 ml of *barbitone buffer pH 7.4* to the sixth (control). Incubate the six tubes at 25° ± 0.5° for 5 minutes and then add 0.1 ml of a 0.3675 per cent w/v solution of *calcium chloride*. Using a stop-watch, measure the clotting time for each solution and the control. Plot the shortening of the recalcification time (control clotting time minus clotting time for each solution) against log concentration. Extrapolate the best-fitting straight line through the five points until it reaches the log concentration axis. The urokinase activity at the intersection point represents the limit concentration for clotting activity (zero clotting activity). The zero clotting activity is not less than 150 Units per ml.

Vasoactive substances. Anesthetise a rabbit by intraperitoneal injection of 0.15 g of *phenobarbitone sodium* per kg of body weight. Dissolve in *normal saline solution* a sufficient quantity of the substance under examination to give a solution containing 40,000 Units per ml. Administer by intravenous infusion at a rate of 1 ml per minute a sufficient volume of the solution of the substance under examination such that the dose is 20,000 Units per kg of body weight. Measure the arterial pressure and heart rate at intervals of 15 minutes for 5 hours after the infusion. No significant and lasting alterations in arterial pressure or heart rate are produced, except those arising from the effects of the anaesthetic.

Assay. The potency of urokinase is determined by comparing its ability to activate human plasminogen to form plasmin with that of the Standard Preparation. The plasmin generated is determined by measurement of the time taken to lyse a fibrin clot under the conditions of a suitable method of Assay.

Standard Preparation

The Standard Preparation is the 1st International Reference Preparation for Urokinase, human, established in 1968, consisting of partially purified freeze-dried urokinase from human urine with lactose (supplied in ampoules containing 4800 Units of urokinase activity) or another suitable preparation the activity of which has been determined in relation to the International Reference Preparation.

Method

Unless otherwise prescribed, use *phosphate buffer pH 7.4* containing 3 per cent w/v solution of *bovine albumin* for the preparation of solutions and dilutions.

Prepare a solution of the Standard Preparation containing 1000 Units of urokinase activity per ml and prepare a solution of the preparation under examination expected to have the same concentration; keep the solutions in ice and use within 6 hours. Prepare three 1.5-fold serial dilutions of the solution of the Standard Preparation so that the longest clot-lysis time is less than 20 minutes and the shortest clot-lysis time is greater than 3 minutes. Prepare three similar dilutions of the solution of the preparation under examination. Keep the solutions in ice and use within 1 hour. Using 24 tubes 8 mm in diameter, label the tubes S_1, S_2, S_3 for the dilutions of the Standard Preparation and T_1, T_2, T_3 for the dilutions of the preparation under examination, allocating four tubes to each dilution. Place the tubes in ice. Into each tube introduce 0.2 ml of the appropriate dilution, 0.2 ml of *phosphate buffer pH 7.4* containing 3 per cent w/v solution of *bovine albumin* and 0.1 ml of a solution containing 20 Units of *thrombin* per ml. Place the tubes in a water-bath at 37° and allow to stand for 2 minutes to attain temperature equilibrium. Using an automatic pipette, introduce into the bottom of the first tube 0.5 ml of a 1.0 per cent w/v solution of *bovine euglobulin fraction* ensuring mixing. At 5-second intervals introduce successively into the remaining tubes 0.5 ml of a 1.0 per cent w/v solution of *bovine euglobulin fraction*. Using a stop-watch, measure for each tube the time in seconds that elapses between the addition of the euglobulin and the lysis of the clot.

Using the logarithms of the lysis times, calculate the result of the assay by standard statistical methods.

The estimated potency is not less than 90.0 per cent and not more than 111.0 per cent of the stated potency.

The fiducial limits of error are not less than 80 per cent and not more than 125 per cent of the stated potency.

Urokinase intended for use in the manufacture of parenteral preparations or ophthalmic preparations complies with the following additional requirements.

Bacterial endotoxins (2.2.3). Not more than 0.002 Endotoxin Unit per Unit of urokinase activity.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light in a refrigerator (2° to 8°). The containers should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units of urokinase activity in the container; (2) the number of Units of urokinase activity per mg of protein; (3) the storage conditions; (4) whether or not it is intended for use in the manufacture of parenteral preparations or ophthalmic preparations.

V

Valproate Injection

Valproate Sodium Injection

Valproate Injection is a sterile solution of sodium valproate in Water for Injections with one or more suitable buffering or sequestering agents.

Valproate Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$.

Usual strength. 20 mg per ml.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Gives the reaction of sodium salts (2.3.1).

Tests

pH (2.4.24). 7.0 to 9.0.

Bacterial endotoxins (2.2.3). Not more than 23.0 Endotoxin Units per ml.

Sterility (2.2.11). Complies with the test for sterility.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.5 per cent w/v solution of biphenyl in *dichloromethane*.

Test solution. Dilute a volume of the injection containing about 400 mg of valproic acid with 20 ml of 5 per cent v/v solution of *hydrochloric acid* and add 50 ml of internal standard solution. Shake for 1 hour, if emulsion forms, stir with a glass rod. Take 5 ml of the organic layer and dilute to 50 ml with *dichloromethane*.

Reference solution (a). A 0.8 per cent w/v solution of *valproic acid RS* in internal standard solution.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 50 ml with *dichloromethane*.

Chromatographic system
- a glass column 1.8 m x 2 mm, packed with 10 per cent phase G34 on 80 to 100 mesh support S1A,
- temperature:
  column. 155°,
  injection port at 275° and detector at 300°,
- a flame ionization detector,
- flow rate. 20 ml per minute using nitrogen as the carrier gas.

Inject 2 µl of reference solution (b). The test in not valid unless the resolution between the peaks due to valproic acid and biphenyl peaks is not less than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

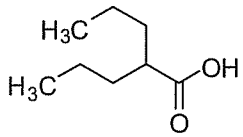
Inject 2 µl of the test solution and reference solution (a).

Calculate the content of $C_8H_{16}O_2$ in the injection.

Storage. Store at a temperature not exceeding 30° and in single dose containers, preferably of Type 1 glass.

Labelling. The label states the amount of any buffering or sequestering agents used.

Valproic Acid



$C_8H_{16}O_2$ Mol. Wt. 144.2

Valproic acid is 2-propylpentanoic acid.

Valproic acid contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_{16}O_2$.

Category. Anticonvulsant.

Description. A colourless or very slightly yellow liquid, slightly viscous.

Identification

Test A may be omitted if tests B and C are carried out. Tests B and C may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *valproic acid RS* or with the reference spectrum of valproic acid.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *ether* and 50 volumes of *dichloromethane*.

Test solution. A 1.0 per cent w/v solution of the substance under examination in *methanol*.

Reference solution. A 1.0 per cent w/v solution of *valproic acid RS* in *methanol*.

Apply 2 µl of each solution. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with *bromocresol green solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. To 1 ml add 3.0 ml of dilute *sodium hydroxide solution*. Add 3.0 ml of *water* and 1.0 ml of a 10 per cent w/v solution of *cobalt nitrate*. A violet precipitate is formed, filter it. The precipitate dissolves in *dichloromethane*.

Tests

Appearance of solution. A 20.0 per cent w/v solution in *dilute sodium hydroxide* is clear (2.4.1) and not more intensely coloured than reference solution YS5 (2.4.1).

Refractive index (2.4.27). 1.422 to 1.425.

Related substances. Determine by gas chromatography (2.4.13).

Internal standard solution. A 0.005 per cent w/v solution of *butyric acid* in *heptane*.

Test solution. A 5.0 per cent w/v solution of the substance under examination in the internal standard solution. Dilute 1.0 ml of this solution to 10 ml with *heptane*.

Reference solution. Dissolve 20 mg of the substance under examination and 20 mg of *2-(1-methylethyl)pentanoic acid RS (valproic acid impurity C RS)* in 10 ml of *heptane*. Dilute 1.0 ml of this solution to 10 ml with *heptane*.

Chromatographic system
- a fused-silica capillary column 30 m x 0.53 mm, coated with wide-bore macrogol 20000 2-nitroterephthalate (0.5 µm),
- temperature.
  column 130° from 0-10 minutes and 130° - 190° from 10-30 minutes
- inlet port and detector 220°,
- a flame ionisation detector,
- flow rate. 8 ml per minute of the carrier gas (helium).

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to valproic acid impurity C and valproic acid is not less than 3.0.

Inject 1 µl of the test solution and the reference solution. In the chromatogram obtained with the test solution the area of peak due to any impurity, for each impurity is not more than 0.1 times the area of the peak due to the internal standard (0.1 per cent). The sum of areas of all the secondary peaks is not more than 0.3 times the area of the peak due to the internal standard (0.3 per cent). Ignore any peak with an area less than 0.01 times the area of the peak due to the internal standard (0.01 per cent).

Heavy metals (2.3.13). Dissolve 2.0 g in 20 ml *ethanol (80 per cent)* and use 12 ml of the solution. The resulting solution complies with the limit test for heavy metals, Method D (20 ppm). Prepare the standard using *lead standard solution (2 ppm Pb)* obtained by diluting *lead standard solution (100 ppm Pb)* with *ethanol (80 per cent)*.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.1g and dissolve in 25 ml of *ethanol (95 per cent)*. Add 2 ml of *water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01442 g of $C_8H_{16}O_2$.

Storage. Store protected from moisture.

Valproic Acid Capsules

Valproic Acid Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$.

Usual strength. 250 mg.

Identification

A. In the Assay, the retention time ratio of the principal peak to the internal standard peak in the chromatogram obtained with the test solution and reference solution, are not more than 2.0 per cent.

B. Disperse a quantity of the content of capsules containing about 250 mg of valproic acid, add 20 ml of *1 M sodium hydroxide*, shake and allow the layers to separate. Filter the aqueous layer, add 4 ml of *hydrochloric acid*, mix and extract with 40 ml of *n-heptane*. Filter the *n-heptane* layer through glass wool and evaporate the solvent completely on a steam bath with the aid of a current of air. Transfer 2 drops of the residue to a test tube containing 0.5 ml each of 2 per cent v/v of *potassiunm iodide solution* and 4 per cent v/v of *potassium iodate solution* and mix, a yellow colour is produced.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a 0.5 per cent w/v solution of *sodium lauryl sulphate* in simulated intestinal fluid prepared by dissolving 6.8 g of *monobasic sodium phosphate* in 250 ml *water*, mix and 77 ml of *0.2 M sodium hydroxide*, add 500 ml of *water*. Adjust the pH to 6.8 with *0.2 M sodium hydroxide* or *0.2 M hydrochloric acid* dilute to 1000 ml with *water* adjust to pH 7.5 with *5 M sodium hydroxide*,
Speed and time. 50 rpm and 60 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by gas chromatography (2.4.13) as described under Assay, using the following solutions.

Test solution. To 10 ml of the filtrate, add about 3.0 g of *sodium chloride*, and mix on a vortex mixer for 5 minutes. Add about

1.0 ml of *6 M hydrochloric acid* and 5.0 ml of internal standard solution, shake and allow to separate, remove the *n-heptane* layer and filter. Discard the aqueous layer.

Reference solution. Weigh accurately a quantity of *valproic acid RS* and dilute with the dissolution medium to obtain a solution having a concentration similar to that of the test solution. Take 10 ml of this solution and proceed as in test solution beginning with the words "add about 3.0 g.....".

Calculate the content of $C_8H_{16}O_2$ in the capsule.

D. Not less than 85 per cent of the stated amount of $C_8H_{16}O_2$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Take 20 capsules in a container and add 150 ml of *dichloromethane*, cool in a *solid carbon dioxide-acetone mixture* until the contents have solidified. If necessary, transfer the mixture of capsules and *dichloromethane* to a blender jar and blend until all the solids are reduced to fine particles. Transfer the mixture to a 500-ml flask, add *n-heptane* to volume, mix and allow the solids to settle. Transfer an accurately measured volume of this solution, containing about 250 mg of valproic acid, to a 100 ml flask, dilute with *n-heptane* to volume and mix. Transfer 5.0 ml to a container equipped with a closure. Add 2.0 ml of internal standard solution, close the container and mix.

Reference solution. A 0.25 per cent w/v solution of *valproic acid RS* in *n-heptane*. Transfer 5.0 ml to a container equipped with a closure. Add 2.0 ml of internal standard solution, close the container and mix.

Internal standard solution. A 0.5 per cent w/v solution of *biphenyl* in *n-heptane.*

Chromatographic system
- a glass column 1.8 m x 2mm, packed with 10 per cent phase G34 on 80 to 100 mesh support S1A,
- temperature:
  column. 150°,
  injection port and detector. 250°,
- a flame ionization detector,
- flow rate. 40 ml per minute using nitrogen as carrier gas.

Inject 2 µl of the reference solution. The test is not valid unless the resolution between the peak due to valproic acid and biphenyl is not less than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to valproic acid for biphenyl is about 2.0.

Inject 2 µl of the test solution and the reference solution.

Calculate the content of $C_8H_{16}O_2$ in the capsules.

Storage. Store protect from moisture, at a temperature not exceeding 30°.

Valproic Acid Oral Solution

Valproic Acid Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valproic acid, $C_8H_{16}O_2$.

Usual strengths. 40 mg per ml; 50 mg per ml.

Identification

A. In the Assay, the retention time ratio of the principal peak to the internal standard peak in the chromatogram obtained with the test solution and reference solution is not more than 2.0 per cent.

B. Dissolve a volume of solution containing about 250 mg of valproic acid in 40 ml of *water* and 2.0 ml of *hydrochloric acid.* Mix and extract with 40 ml of *n-heptane*. Filter the *n-heptane* layer through glass wool into a beaker, and evaporate the solvent completely on a steam bath with the aid of a current of air. Transfer 2 drops of the residue to a test tube containing 0.5 ml each 2 per cent v/v of *potassium iodide solution* and 4 per cent v/v solution of *potassium iodate solution* and mix; a yellow colour is produced.

Tests

pH (2.4.24). 7.0 to 8.0.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Dissolve a volume of solution containing about 250 mg of valproic acid in 40 ml of *water,* add 2.0 ml of *hydrochloric acid* and extract with 80 ml of *n-heptane* until the aqueous layer is clear. Filter the *n-heptane* layer through glass wool with small portions of *n-heptane*, add the rinsings to the flask, dilute to 100.0 ml with *n-heptane* and mix. Transfer 5.0 ml to a container equipped with a closure. Add 2.0 ml of the internal standard solution, close the container and mix.

Reference solution. A 0.25 per cent w/v solution of *valproic acid RS* in *n-heptane.* Transfer 5.0 ml to a container equipped with a closure. Add 2.0 ml of internal standard solution and mix.

Internal standard solution. A 0.5 per cent w/v solution of *biphenyl* in *n-heptane.*

Chromatographic system
- glass column 1.8 m x 2 mm, packed with 10 per cent phase G34 on 80 to 1010 mesh support S1A,
- temperature:
  column. 150°,
  injection port and detector at 250°,
- a flame ionization detector,
- flow rate. 40 ml per minute using nitrogen as carrier gas.

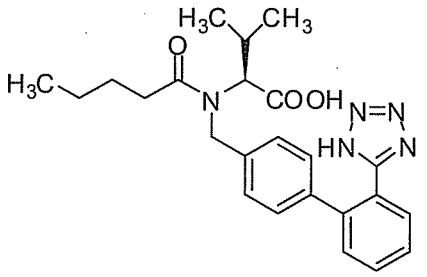
Inject 2 µl of the reference solution. The test is not valid unless the resolution between valproic acid and biphenyl is not less than 3.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to valproic acid for biphenyl is about 2.0.

Inject 2 µl each of the test solution and the reference solution.

Calculate the content of $C_8H_{16}O_2$ in oral solution.

Storage. Store protected from moisture.

Valsartan



$C_{24}H_{29}N_5O_3$ Mol. Wt. 435.5

Valsartan is *N*-pentanonyl-*N*-[2′ -(1*H*-tetrazol-5-yl)biphenyl-4-ylmethyl]-L-valine.

Valsartan contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{24}H_{29}N_5O_3$, calculated on the anhydrous basis.

Category. Antihypertensive.

Dose. 80-160 mg daily.

Description. A white to almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *valsartan RS* or with the reference spectrum of valsartan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

C. When examined in the range 200 nm to 300 nm (2.4.7), a 0.001 per cent w/v solution in *0.1 M hydrochloric acid* exhibits a maximum at about 248 nm.

Tests

Specific optical rotation (2.4.22). – 60° to – 67°, determined in 1.0 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 100.0 ml of the mobile phase.

Reference solution (a). A 0.05 per cent w/v solution of *valsartan RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of reference solution (a) to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil),
- mobile phase: a mixture of 50 volumes of *water*, 50 volumes of *acetonitrile* and 0.1 volume of *glacial acetic acid*,
- flow rate. 0.4 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume. 10 µl.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatogram three times the retention time of the principal peak. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (b) (0.5 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 1 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution *of valsartan RS* in the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Purospher 18e),
- mobile phase: a mixture of 50 volumes of *water*, 50 volumes of *acetonitrile* and 0.1 volume of *glacial acetic acid*,
- flow rate. 0.4 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{29}N_5O_3$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Valsartan Tablets

Valsartan Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valsartan, $C_{24}H_{29}N_5O_3$.

Usual strengths. 40 mg; 80 mg; 160 mg; 320 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Valsartan with 40 ml of *methanol*, filter and evaporate the filtrate to dryness. On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained from *valsartan RS* or with the reference spectrum of valsartan.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *phosphate buffer pH 6.8*,
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm (2.4.7). Calculate the content of $C_{24}H_{29}N_5O_3$ in the medium from the absorbance obtained from a solution of known concentration of *valsartan RS* prepared by dissolving in minimum amount of *methanol* and diluted with the dissolution medium.

D. Not less than 70 per cent of the stated amount of $C_{24}H_{29}N_5O_3$.

Related Substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 25 mg of Valsartan with 15 ml of mobile phase and dilute to 25.0 ml with the mobile phase, filter.

Reference solution (a). A 0.1 per cent w/v solution of *valsartan RS* in the mobile phase.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent) and the sum of areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing 50 mg of Valsartan, disperse in 25 ml of the mobile phase and dilute to 100.0 ml with the mobile phase and filter. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *valsartan RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 50 volumes of *water*, 50 volumes of *acetonitrile* and 0.1 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 273 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{29}N_5O_3$ in the tablets.

Storage. Store protected from light and moisture.

Valsartan and Hydrochlorothiazide Tablets

Valsartan and Hydrochlorothiazide Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of valsartan, $C_{24}H_{29}N_5O_3$ and hydrochlorothiazide, $C_7H_8ClN_3O_4S_2$.

Usual Strength . Valsartan 80 mg and Hydrochlorothiazide 12.5 mg; Valsartan 60 mg and Hydrochlorothiazide 12.5 mg.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *ethyl acetate*, 20 volumes of *dehydrated alcohol* and 10 volumes of a 25 per cent w/v solution of *ammonium hydroxide.*

Test solution. Disperse a quantity of powdered tablets containing about 20 mg of valsartan in 100 ml of *acetone*, sonicate for 15 minutes and filter.

Reference solution. A solution containing 0.02 per cent w/v each of *valsartan RS* and *hydrochlorothiazide RS* in *acetone.*

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 7 cm. Dry the plate in a current of warm air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 1000 ml of *phosphate buffer pH 6.8,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of the filtered solution, suitably diluted with the medium if necessary, at the maximum at about 250 nm for valsartan and 272 nm for hydrochlorothiazide (2.4.7). Calculate the content of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ in the medium from the absorbance obtained from a solution of known concentration of *valsartan RS* and *hydrochlorothiazide RS* in the same medium.

Calculate the content of valsartan dissolved by the formula:

$$\frac{(AT2 \times A1\ per\ cent\ H_{272nm}) - (AT1 \times A1\ per\ cent\ H_{250nm})}{(A1\ per\ cent\ V_{250nm} \times A1\ per\ cent\ H_{272nm}) - (A1\ per\ cent\ V_{272nm} \times A1\ per\ cent\ H_{250nm})} \times 12{,}500$$

Calculate the content of hydrochlorothiazide dissolved by the formula:

$$\frac{(AT1 \times A1\ per\ cent\ V_{250nm}) - (AT2 \times A1\ per\ cent\ V_{272nm})}{(A1\ per\ cent\ H_{272nm} \times A1\ per\ cent\ V_{250nm}) - (A1\ per\ cent\ H_{250nm} \times A1\ per\ cent\ V_{272nm})} \times 80{,}000$$

where, *AT1* = absorbance of the test solution at 272 nm,

AT2 = absorbance of the test solution at 250 nm,

A1 per cent $V_{272\ nm}$ = absorptivity (1 per cent, 0.2 cm, 272 nm) of valsartan in medium,

A1 per cent $V_{250\ nm}$ = absorptivity (1 per cent, 0.2 cm, 250 nm) of valsartan in medium,

A1 per cent $H_{272\ nm}$ = absorptivity (1 per cent, 0.2 cm, 272 nm) of hydrochlorothiazide in medium,

A1 per cent $H_{250\ nm}$ = absorptivity (1 per cent, 0.2 cm, 250 nm) of hydrochlorothiazide in medium,

D. Not less than 80 per cent of the stated amount of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$.

Related substances. Determine by liquid chromatography (2.4.14), as described under Assay using the following solutions.

Solvent mixture. Equal volumes of *acetonitrile* and *water.*

Test solution. Disperse a quantity of powdered tablets containing about 62.5 mg of Hydrochlorothiazide with 5.0 ml of *water* and 100 ml of solvent mixture, sonicate for 15 minutes and shake for 30 minutes. Dilute to 250 ml with the solvent mixture, centrifuge and dilute 25.0 ml of the supernatant to 200 ml with the solvent mixture.

Reference solution (a). A solution containing 0.003 per cent w/v of *benzothiadiazine impurity A RS*, 0.006 per cent w/v of *hydrochlorothiazide RS*, 0.008 per cent w/v of *valsartan RS* and 0.02 per cent w/v of *valsartan impurity B RS* in the solvent mixture.

Reference solution (b). Dilute 5.0 ml of reference solution (a) to 100 ml with the solvent mixture.

Reference solution (c). Dilute 10 ml of reference solution (b) to 100 ml with the solvent mixture.

Inject reference solution (b) and (c). The test is not valid unless in the chromatogram obtained with reference solution (b), the resolution between valsartan impurity B and valsartan and between benzothiadiazine impurity A and hydrochlorothiazide is not less than 1.4. In the chromatogram obtained with reference solution (c) the relative standard deviation for replicate injections is not more than 10 per cent.

Inject the test solution, reference solution (a), (b) and (c). The area of the peak due to benzothiadiazine impurity A is not more than 1.0 per cent; the area of any secondary peak other than valsartan impurity A is not more than 0.2 per cent; and the sum of areas of all the secondary peaks other than valsartan impurity A is not more than 1.3 per cent. (Valsartan impurity A is the enantiomer of valsartan and coelutes with valsartan in this test.)

Uniformity of content. Comply with the tests stated under Tablets.

Determine by liquid chromatography (2.4.14), as described under Assay using the following solution as the test solution.

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse 1 tablet with 5.0 ml of *water* and about 100 ml of solvent mixture, sonicate for 15 minutes and dilute to 200 ml with the solvent mixture. Centrifuge a portion of this solution and dilute a volume of the clear supernatant with the solvent mixture to obtain a 0.02 per cent w/v solution of valsartan.

Calculate the content of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *acetonitrile* and *water*.

Test solution. Disperse a quantity of powdered tablets containing about 62.5 mg of Hydrochlorothiazide with 5.0 ml of *water* and 100 ml of solvent mixture, sonicate for 15 minutes and shake for 30 minutes. Dilute to 250 ml with the solvent mixture, centrifuge and dilute 25.0 ml of the supernatant to 200 ml with the solvent mixture. Dilute a suitable volume of this solution with the solvent mixture to obtain a 0.02 per cent w/v solution of Valsartan.

Reference solution. Transfer about 12.5 mg of *hydrochlorothiazide RS* to a 200 ml volumetric flask and add about 12.5 J mg of *valsartan RS*, J being the ratio of the stated amount, in mg, of valsartan to the stated amount, in mg, of hydrochlorothiazide per tablet. Add about 100 ml of solvent mixture, sonicate for 15 minutes, dilute to volume and mix. Transfer 25 ml of this solution to a 50 ml flask, dilute with solvent mixture to volume and mix. Dilute a suitable volume of this solution with the solvent mixture to obtain a 0.02 per cent w/v solution of *valsartan RS*.

Chromatographic system

- a stainless steel column 12.5 cm x 3.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of *water*, 10 volumes of *acetonitrile* and 0.1 volume of *trifluroacetic acid*,
  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and 0.1 volume of *trifluroacetic acid*,
- a linear gradient programme using the conditions given below,
- flow rate. 0.4 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0–25	90 → 10	10 → 90
25–27	10 → 90	90 → 10
27–40	90	10

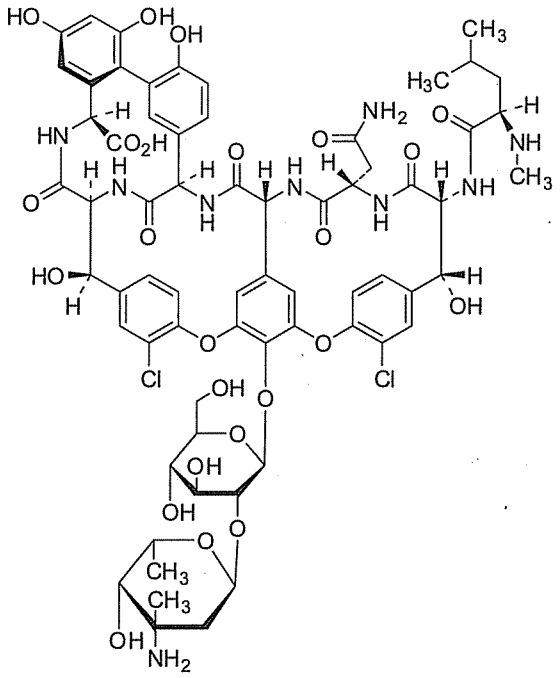
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{24}H_{29}N_5O_3$ and $C_7H_8ClN_3O_4S_2$ in the tablet.

Storage. Store protected from moisture.

Vancomycin Hydrochloride



, HCl

$C_{66}H_{75}Cl_2N_9O_{24}$, HCl Mol.Wt. 1485.7

Vancomycin Hydrochloride is a mixture of related glycopeptides, consisting principally of the monohydrochloride of (3*S*,6*R*,7*R*,22*R*,23*S*,26*S*,30a*S*,36*R*,38a*R*)-3-(2-amino-2-oxoethyl)-44-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-β-L-*lyxo*-hexopyranosyl)-β-D-glucopyranosyl]oxy]-10,19-dichloro-7,22,28,30,32-pentahydroxy-6-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,5,24,38,39-pentaoxo-2,3,4,5,6,7,23,24,25,26,36,37,38,38a-tetradecahydro-22*H*-8,11:18,21-dietheno-23,36-(iminomethano)-13,16:31,35-dimetheno-1*H*,13*H*-[1,6,9]oxadiazacyclohexadecinol[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-26-carboxylic acid (vancomycin B).

It is produced by certain strains of *Amycolatopsis orientalis* or obtained by any other means.

Vancomycin Hydrochloride contains not less than 1050 IU per mg of $C_{66}H_{75}Cl_2N_9O_{24}$, HCl, calculated on the anhydrous basis and not less than 93.0 per cent of vancomycin B.

Category. Antibacterial.

Description. A white or almost white hygroscopic powder.

Identification

A. In the test for Vancomycin B, the principal peak in the chromatogram obtained with test solution (a) corresponds to that in the chromatogram obtained with the reference solution.

B. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10.0 per cent w/v solution in *water* is clear (2.4.1), absorbance of the solution at 450 nm (2.4.7) is not more than 0.1.

pH (2.4.24). 2.5 to 4.5, determined in a 5.0 per cent w/v solution in *carbon-dioxide free water*.

Vancomycin B. Determine by liquid chromatography (2.4.14).

NOTE—Use freshly prepared solutions.

Test solution (a). Dissolve 0.2 g of the substance under examination in 100 ml of the mobile phase A.

Test solution (b). Dilute 2.0 ml of test solution (a) to 50 ml with mobile phase A.

Test solution (c). Dilute 0.5 ml of test solution (b) to 20 ml with mobile phase A.

Reference solution. A 0.5 per cent w/v solution of *vancomycin hydrochloride RS* in *water*. Heat at 65° for 24 hours, allow to cool.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 1 volume of *tetrahydrofuran*, 7 volumes of *acetonitrile* and 92 volumes of buffer solution prepared by diluting 1 ml of *triethylamine* to 499 ml with *water*, adjust the pH to 3.2 with *orthophosphoric acid*,

  B. a mixture of 1 volume of *tetrahydrofuran*, 29 volumes of *acetonitrile* and 70 volumes of buffer solution,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-13	100	0
13-22	100→0	0→100
22-26	0	100
26-27	100	0

Inject test solution (b), (c) and the reference solution. The test is not valid unless the resolution between the 2 principal peaks in the chromatogram obtained with the reference solution is not less than 5.0, signal-to-noise ratio for the principal peak in the chromatogram obtained with test solution (c) is not less than 5.0 and tailing factor for the peak due to vancomycin in the chromatogram obtained with the test solution (b) is not more than 1.6.

Inject test solution (b) and the reference solution.

Calculate the content of vancomycin B hydrochloride.

Related substances. Determine by liquid chromatography (2.4.14) as described under Vancomycin B with the following modifications.

Inject test solution (a), (b) and (c). In the chromatogram obtained with the test solution the area of any secondary peak due to N-demethylvancomycin B (Vancomycin Hydrochloride impurity A), (4*S*,7*R*,8*R*,23*R*,24*S*,27*S*,31a*S*a,37*R*,39a*R*)-45-[[2-*O*-(3-amino-2,3,6-trideoxy-3-*C*-methyl-α-L-lyxo-hexopyranosyl)-β -D-glucopyranosyl]oxy]-11,20-dichloro-8,23,29,31,33-pentahydroxy-7-[[(2*R*)-4-methyl-2-(methylamino)pentanoyl]amino]-2,6,25,39,40-pentaoxo-1,2,3,4,5,6,7,8,24,25,26,27,37,38,39,39a-hexadecahydro-23*H*-9,12:19,22-dietheno-24,37-(iminomethano)-14,17:32,36-dimetheno-14*H*-[1,6,10]oxadiazacycloheptadecino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-4,27-dicarboxylic acid ([β Asp^3] vancomycin B) (Vancomycin Hydrochloride impurity B), aglucovancomycin B (Vancomycin Hydrochloride impurity C) and desvancosaminylvancomycin B (Vancomycin Hydrochloride impurity D) is not more than 4.0 per cent. The sum of area of all the secondary peaks is not more than 7.0 per cent. Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (30 ppm).

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per mg.

Vancomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine by the microbiological assay of antibiotics, Method B (2.2.10) using *vancomycin hydrochloride RS*.

Storage. Store protected from light and moisture.

If it is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-proof and sealed so as to exclude micro-organisms.

Vancomycin Capsules

Vancomycin Hydrochloride Capsules

Vancomycin Capsules contain not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of vancomycin, $C_{66}H_{75}Cl_2N_9O_{24}$.

Usual strength. 125 mg.

Identification

Determine by paper chromatography (2.4.15), using a suitable sheet of chromatographic filter paper.

Mobile phase. A mixture of 6 volumes of *butyl alcohol*, 4 volumes of *water* and 3 volumes of *pyridine*.

Test solution. Disperse the content of capsules containing about 100 mg of vancomycin in 100.0 ml of water.

Reference solution. A 0.1 per cent w/v solution of *vancomycin hydrochloride RS* in water.

Apply to the plate 5 µl of each solution. Develop by descending chromatography for about 7 hours. After development, dry the paper and place it on an inoculated agar surface of sufficient area to accommodate the paper and prepared for vancomycin assay described in microbiological assay of antibiotics (2.2.10) except to use Medium B. Remove the paper from the agar surface after 30 minutes and incubate the agar medium at 37° for 18 hours; clear zones of inhibition are produced at corresponding positions on the two chromatograms.

Tests

Dissolution (2.5.2).

Apparatus No. 2,
Medium. 900 ml of *water*,
Speed and time. 100 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter.

Carry out the method as described under Assay.

D. Not less than 85 per cent of the stated amount of $C_{66}H_{75}Cl_2N_9O_{24}$.

Water (2.3.43). Not more than 8.0 per cent.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by the microbiological assay of antibiotics, Method A (2.2.10) on a solution prepared in the following manner.

Place not less than 5 capsules in a glass blender jar and blend at high speed for 3 to 5 minutes with a sufficient volume of Buffer No. 3 to yield a stock solution having a convenient concentration of vancomycin. Dilute an accurately measured volume of this stock solution with Buffer No. 3 to obtain a test dilution having a concentration assumed to be equal to the median dose level of the standard.

Storage. Store protected from moisture.

Vancomycin Intravenous Infusion

Vancomycin Intravenous Infusion is a sterile solution of Vancomycin Hydrochloride in Water for Injections. It is prepared by dissolving Vancomycin Hydrochloride for Intravenous Infusion in Water for Injections and then diluting with the requisite volume of a suitable diluent in accordance with the manufacturer's instructions.

The intravenous infusion complies with the tests stated under Parenteral Preparations.

Storage. Vancomycin Intravenous Infusion should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Usual strengths. 500 mg; 1 g.

Vancomycin Hydrochloride for Intravenous Infusion

Vancomycin Hydrochloride for Intravenous Infusion is a sterile material consisting of Vancomycin Hydrochloride with or without excipients. It is filled in a sealed container.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Vancomycin Hydrochloride for Intravenous Infusion contains not less than 88.0 per cent of Vancomycin B.

Identification

A. In the test for vancomycin B, the retention time of the principal peak in the chromatogram obtained with the test

solution corresponds to that in the chromatogram obtained with the reference solution.

B. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 2.5 to 4.5, determined on a 5.0 per cent w/v solution of Vancomycin Hydrochloride.

Appearance of solution. A 10.0 per cent w/v solution of Vancomycin Hydrochloride is clear (2.4.1) and the absorbance of the solution at 450 nm (2.4.7) is not more than 0.1.

Vancomycin B. Determine by liquid chromatography (2.4.14).

NOTE—Use the solutions within 4 hours of preparation.

Test solution (a). Dissolve a quantity of the substance under examination in sufficient mobile phase A to produce a solution containing 2,000 IU of vancomycin per ml.

Test solution (b). Dilute 1 ml of test solution (a) to 25 ml with mobile phase A.

Test solution (c). Dilute 1 ml of test solution (b) to 40 ml with mobile phase A.

Reference solution. A 0.05 per cent w/v solution of *vancomycin hydrochloride RS* in *water*. Heat at 65° for 24 hours and allow to cool.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: A. a mixture of 1 volumes of *tetrahydrofuran*, 7 volumes of *acetonitrile* and 92 volumes of buffer solution prepared by diluting 1 ml of *triethylamine* to 500 ml with *water*, adjust the pH to 3.2 with *orthophosphoric acid*,

  B. a mixture of 1 volume of *tetrahydrofuran*, 29 volumes of *acetonitrile* and 70 volumes of buffer solution,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-13	100	0
13-21	100→0	0→100
21-25	0	100
25-35	100	0

Inject the reference solution, test solution (b) and (c). The test is not valid unless in the chromatogram obtained with test solution (c), signal-to- noise ratio of the principal peak is not less than 5; the tailing factor of the vancomycin peak in the chromatogram obtained with test solution (b) is not more than 1.6 and the resolution between the two principal peaks in the chromatogram obtained with the reference solution is not less than 5.0.

Related substances. Determine by liquid chromatography (2.4.14), using test solutions (a), (b) and (c) as described under Vancomycin B.

Chromatographic system as described under Vancomycin B.

In the chromatogram obtained with test solution (a) calculate the content of each impurity.

The content of any impurity is not more than 4.0 per cent and the sum of the contents of any such impurities is not more than 12.0 per cent. Ignore any peak with an area less than that of the principal peak in the chromatogram obtained with test solution (c) (0.1 per cent).

Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Bacterial endotoxins (2.2.3). Dissolve the contents of the sealed container in *tris-chloride buffer pH 7.4* prepared using *water BET* to give a solution containing 9000 Units of vancomycin per ml. Carry out the test on the resulting solution; the maximum allowable endotoxin concentration of the solution is 2.5 Units of endotoxins per ml. Carry out the test using the maximum valid dilution of the prepared solution calculated from the declared sensitivity of the lysate used in the test.

Assay. Determine the weight of the contents of ten containers as described in the test for Uniformity of weight under Parenteral Preparations (Powders for Injection).

Determine on the mixed contents of ten containers by the microbiological assay of antibiotics, Method B (2.2.10).

The upper fiducial limit of error is not less than 95.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units.

Labelling. The label states (1) the total number of Units of vancomycin in the container and (2) the number of Units of vancomycin per mg.

Vancomycin Oral Solution

Vancomycin Oral Solution is a solution of Vancomycin Hydrochloride in a suitable flavoured vehicle. It is prepared by dissolving Vancomycin Hydrochloride for Oral Solution in the requisite amount of a suitable diluent.

The oral solution complies with the tests stated under Oral Liquids.

Storage. Vancomycin Oral Solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer when prepared and stored strictly in accordance with the manufacturer's instructions.

Usual strength. 50 mg per ml.

Vancomycin Hydrochloride for Oral Solution

Vancomycin Hydrochloride for Oral Solution is a dry powder consisting of Vancomycin Hydrochloride with or without excipients. It is filled in a sealed container.

The contents of the sealed container comply with the requirements stated under Powders and Granules for Oral Solutions and Oral Suspensions stated under Oral Liquids and with the following requirements.

Vancomycin Hydrochloride for Oral Solution contains not less than 88.0 per cent of Vancomycin B.

Identification

A. In the test for Vancomycin B, the retention time of the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 2.5 to 4.5, determined on a 5.0 per cent w/v solution of Vancomycin Hydrochloride.

Appearance of solution. A 10.0 per cent w/v solution is clear (2.4.1) and the absorbance at 450 nm (2.4.7) is not more than 0.1.

Vancomycin B. Determine by liquid chromatography (2.4.14).

NOTE—Use the solutions within 4 hours of preparation.

Test solution (a). Dissolve a quantity of the powder in mobile phase A to produce a solution containing 2,000 IU of vancomycin per ml.

Test solution (b). Dilute 1 ml of test solution (a) to 25 ml with mobile phase A.

Test solution (c). Dilute 1 ml of test solution (b) to 40 ml with mobile phase A.

Reference solution. A 0.05 per cent w/v solution of *vancomycin hydrochloride RS* in *water*. Heat at 65° for 24 hours and allow to cool.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with end-capped octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil ODS),
- mobile phase: A. a mixture of 4 volumes of *triethylamine* diluted with 1996 volumes of *water*, adjusted to pH 3.2 with *orthophosphoric acid* (solution A), 10 volumes of *tetrahydrofuran* and 70 volumes of *acetonitrile* to 920 volumes of solution A,

  B. a mixture of 10 volumes of *tetrahydrofuran*, 290 volumes of *acetonitrile* and 700 volumes of solution A,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 13	100	0
13 – 21	100 → 0	0 → 100
21 – 25	0	100
25 – 35	100	0

Inject each solution. The test is not valid unless in the chromatogram obtained with test solution (c) signal-to- noise ratio of the principal peak is not less than 5.0. The tailing factor of the principal peak in the chromatogram obtained with test solution (b) is not more than 1.6; and the resolution between the two principal peaks in the chromatogram obtained with the reference solution is not less than 5.0.

Related substances. Determine by liquid chromatography (2.4.14), using test solution (a), (b) and (c) as described under Vancomycin B.

Use chromatographic system as described under Vancomycin B.

Inject test solution (c) and the reference solution. The area of any secondary peak is not more than 4.0 per cent and the sum of the areas of all the secondary peaks is not more than 12.0 per cent. Ignore any peak with an area less than the area of the principal peak in the chromatogram obtained with the test solution (c) (0.1 per cent).

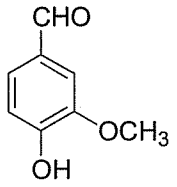
Water (2.3.43). Not more than 5.0 per cent, determined on 0.5 g.

Assay. Determine on the mixed contents of ten containers by the microbiological assay of antibiotics, Method A or Method B (2.2.10).

The upper fiducial limit of error is not less than 95.0 per cent and the lower fiducial limit of error is not more than 115.0 per cent of the stated number of Units.

Labelling. The label states (1) the total number of Units of vancomycin in the container and (2) the number of Units of vancomycin per mg.

Vanillin



$C_8H_8O_3$ Mol. Wt. 152.2

Vanillin is 4-hydroxy-3-methoxybenzaldehyde.

Vanillin contains not less than 99.0 per cent and not more than 101.0 per cent of $C_8H_8O_3$, calculated on the dried basis.

Category. Pharmaceutical aid (flavouring agent).

Description. A white or slightly yellow powder or crystalline needles; odour, characteristic of vanilla.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B, C and D may be omitted if test A is carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *vanillin RS*.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (b). Examine the chromatograms in daylight after spraying.

C. To 5 ml of a saturated solution add 0.2 ml of *ferric chloride solution*; a blue colour is produced. Heat to 80°; the solution becomes brown and a white precipitate is produced on cooling.

D. Melting range (2.4.21). 81° to 84°.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *ethanol (95 per cent)* is clear (2.4.1) and not more intensely coloured than reference solution BS6 (2.4.1).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 98.5 volumes of *dichloromethane*, 1 volume of *methanol* and 0.5 volume of *anhydrous acetic acid*.

Test solution (a). Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Test solution (b). Dissolve 0.2 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). Dissolve 10 mg of the substance under examination in 100 ml of *methanol*.

Reference solution (b). A 0.2 per cent w/v solution of *vanillin RS* in *methanol*.

Use an unsaturated tank and allow the mobile phase to rise 10 cm. Apply to the plate 5 µl of each solution. After development, dry the plate in cold air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Spray with *dinitrophenylhydrazine-aceto-hydrochloric solution* and examine in daylight. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* for 4 hours.

Assay. Weigh accurately about 0.12 g, dissolve in 20 ml of *ethanol (95 per cent)*, add 60 ml of *carbon dioxide-free water*. Titrate with *0.1 M sodium hydroxide*, determining the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01521 g of $C_8H_8O_3$.

Storage. Store protected from light and moisture.

Vasopressin Injection

Vasopressin Injection is a sterile aqueous solution containing the water soluble pressor principle obtained from the posterior lobe of the pituitary of healthy oxen or other mammals or by synthesis.

Vasopressin Injection contains a pressor activity equivalent to not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units.

Usual strength. 20 Units per ml.

Description. A clear, colourless or practically colourless liquid; odour, faint and characteristic.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Inject into the vein of a mammal anaesthetised by a general anaesthetic or by destruction of the brain; it causes a rise of blood pressure.

Tests

pH (2.4.24). 2.5 to 4.5.

Bacterial endotoxins (2.2.3). Less than 17.0 Endotoxin Units per Unit of vasopressin.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Vasopressin injection containing Vasopressin of natural origin obtained by extraction and purification complies with the following additional requirement.

Oxytocin impurity. Not more than 1.2 Units per ml.

Determine by liquid chromatography (2.4.14).

Test solution. Use the injection under examination.

Reference solution. Dissolve the contents of one vial of *oxytocin RS* in a 1.65 per cent w/v solution of *sodium dihydrogen orthophosphate* to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the injection.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (Such as Nucleosil C18),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a 0.2 per cent v/v solution of *orthophosphoric acid* and 15 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 50,000.

Inject the test solution and the reference solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$ in the injection.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Dissolve 5.0 g of chlorobutanol in 5.0 ml of *glacial acetic acid*, add 1.1 g of *sodium acetate*, 5.0 g of *ethanol*, and dilute to 1000 ml with *water* and mix.

Test solution. Dilute 2.0 ml of injection under examination to 25 ml with 0.25 per cent w/v of *glacial acetic acid* and mix.

Reference solution. Dissolve the contents of one vial of *vasopressin RS* in a known volume of solvent mixture. If necessary dilute the prepared solution to a working concentration range.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- column temperature. 30°,
- mobile phase: a mixture of 87 volumes of 0.66 per cent w/v solution of *dibasic ammonium phosphate*, adjusted to pH 3.0 with *orthophosphoric acid* and 13 volumes of *acetonitrile*, filter through 0.45 µm nylon membrane,
- flow rate. 1 ml per minute,
- spectrophometer set at 220 nm,
- injection volume. 20 µl.

Equilibrate the column at least for one hour. Run the chromatogram minimum of 60 minutes.

Inject the reference solution. The test is not valid unless the resolution between vasopressin and the adjacent peak is not less than 1.5 and relative standard deviation for replicate injections is not more than 2.0 per cent.

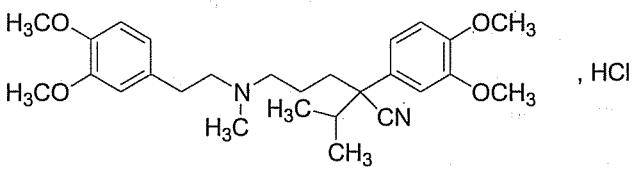
Inject the reference solution and the test solution.

Storage. Store at the temperature between 2° to 8°.

Labelling. The label states (1) the number of Units of the vasopressor activity per ml; (2) either the animal source of the vasopressin or that it is synthetic.

Verapamil Hydrochloride

Verapamil Chloride; Iproveratril Hydrochloride



$C_{27}H_{38}N_2O_4, HCl$, Mol. Wt. 491.1

Verapamil Hydrochloride is (*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-(1-methylethyl)pentanenitrile hydrochloride.

Verapamil Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{27}H_{38}N_2O_4$, HCl, calculated on the dried basis.

Category. Calcium channel blocker.

Dose. Orally, as antiarrhythmic, 40 to 120 mg thrice daily; as antianginal, 80 to 120 mg thrice daily; as antihypertensive, 240 to 480 mg daily, in 2 to 3 divided doses; by slow intravenous injection, 5 to 10 mg.

Description. A white, crystalline powder.

Identification

Test A may be omitted if tests B, C and D are carried out. Tests B and C may be omitted if tests A and D are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *verapamil hydrochloride RS* or with the reference spectrum of verapamil hydrochloride.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.01 M hydrochloric acid* shows absorption maxima at about 229 nm and 278 nm and there may be a shoulder at about 282 nm. The ratio of the absorbance at the maximum at about 278 nm to that at the maximum at about 229 nm is 0.35 to 0.39.

C. In test A for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

D. Gives reaction B of chlorides (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution in *carbon dioxide-free water* prepared with the aid of gentle heat is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.5 to 6.0, determined in a 5.0 per cent w/v solution prepared with the aid of gentle heat.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 63 volumes of mobile phase A and 37 volumes of mobile phase B.

Test solution. Dissolve 25 mg of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution (a). Dissolve 5 mg each of of *verapamil hydrochloride RS* and *(2RS)-2-(3,4-dimethoxyphenyl)-2-[2-[[2-(3,4-dimethoxy-phenyl)ethyl](methyl)amino]ethyl]-3-methylbutanenitrile RS (verapamil impurity A RS)* in 20.0 ml of the solvent mixture. Dilute 1 ml of this solution to 10 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with palmitamidopropylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.7 per cent w/v solution of *dipotassium hydrogen phosphate* adjusted to pH 7.2 with *orthophosphoric acid,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 - 22	63	37
22 – 27	63 → 35	37 → 65
27 - 35	35	65
35 – 36	35 → 63	65 → 37
36 - 50	63	37

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to verapamil and verapamil impurity A is not less than 5.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.01 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g, dissolve in 40 ml of *anhydrous glacial acetic acid*, add 6 ml of *mercuric acetate solution.* Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04911 g of $C_{27}H_{38}N_2O_4$, HCl.

Storage. Store protected from light and moisture.

Verapamil Injection

Verapamil Hydrochloride Injection; Verapamil Chloride Injection; Iproveratril Hydrochloride Injection

Verapamil Injection is a sterile solution of Verapamil Hydrochloride in Water for Injections.

Verapamil Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$, HCl.

Usual strength. 2.5 mg per ml.

Identification

A. Dilute a volume containing 10 mg of Verapamil Hydrochloride to 5 ml with *0.1 M hydrochloric acid*, extract with 5 ml of *ether*, discard the ether extract and make the aqueous layer just alkaline with *2 M potassium carbonate*. Extract with 5 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *verapamil hydrochloride RS* treated in the same manner or with the reference spectrum of verapamil.

B. To a volume containing 5 mg of Verapamil Hydrochloride add 0.2 ml of a 5 per cent w/v solution of *mercuric chloride*; a white precipitate is produced.

C. To a volume containing 5 mg of Verapamil Hydrochloride add 0.5 ml of *3 M sulphuric acid* and 0.2 ml of *dilute potassium permanganate solution*; a violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

Tests

pH (2.4.24). 4.5 to 6.0.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *toluene*, 20 volumes of *methanol*, 5 volumes of *glacial acetic acid* and 5 volumes of *acetone*.

Test solution. Evaporate a volume containing 5 mg of Verapamil Hydrochloride carefully to dryness on a water-bath in a current of *nitrogen* and dissolve the residue as completely as possible in 0.25 ml of *chloroform*.

Reference solution. Dilute 1 volume of the test solution to 100 volumes with *chloroform* and dilute 1 volume of the resulting solution to 10 volumes with *chloroform*.

Apply to the plate 30 µl of each solution. After development, dry the plate in air for 10 minutes and repeat the development. Dry the plate at 110° for 30 minutes and allow to stand until the odour of solvent is no longer detectable. Spray with a solution prepared by dissolving 5 g of *ferric chloride hexahydrate* and 2 g of *iodine* in sufficient of a mixture of equal volumes of *acetone* and a 20 per cent w/v solution of (+)-*tartaric acid* to produce 100 ml, applying a total of 15 to 20 ml of the reagent for a plate (20 cm x 20 cm), and examine immediately. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the principal spot in the chromatogram obtained with the reference solution. Ignore any spot remaining on the line of application.

B. Carry out test A but using a mixture of 85 volumes of *cyclohexane* and 15 volumes of *diethylamine* as the mobile phase and applying separately to the plate 30 µl of each of the test solution and the reference solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute an accurately measured volume containing 5 mg of Verapamil Hydrochloride to 100.0 ml with *0.01 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{27}H_{38}N_2O_4$, HCl taking 118 as the specific absorbance at 278 nm.

Storage. Store protected from light.

Verapamil Tablets

Verapamil Hydrochloride Tablets; Verapamil Chloride Tablets; Iproveratril Hydrochloride Tablets

Verapamil Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of verapamil hydrochloride, $C_{27}H_{38}N_2O_4$, HCl. The tablets may be coated.

Usual strengths. 40 mg; 80 mg; 120 mg; 160 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 25 ml of *0.1 M hydrochloric acid*, filter, extract the filtrate with 25 ml of *ether*, discard the ether extract and make the aqueous layer just alkaline with *2 M potassium carbonate*. Extract with 25 ml of *ether*, filter the ether layer through *anhydrous sodium sulphate* and evaporate to dryness. The residue complies with the following tests.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *verapamil hydrochloride RS* treated in the same manner or with the reference spectrum of verapamil.

B. Shake a quantity of the powdered tablets containing 0.1 g of Verapamil Hydrochloride with 10 ml of *dichloromethane*, filter, evaporate the filtrate to dryness and dissolve the residue in 10 ml of *water* (solution A). To 2 ml of the resulting solution add 0.2 ml of a 5 per cent w/v solution of *mercuric chloride*; a white precipitate is produced.

C. To 2 ml of solution A obtained in test B add 0.5 ml of *3 M sulphuric acid* and 0.2 ml of *dilute potassium permanganate solution*; a violet precipitate is produced which quickly dissolves to produce a very pale yellow solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing about 0.24 g of Verapamil Hydrochloride with 100.0 ml of the mobile phase.

Reference solution (a). Dilute 1 ml of the test solution to 100.0 ml with the mobile phase. Further dilute 1.0 ml of this solution to 10.0 ml with the mobile phase.

Reference solution (b). A solution containing 0.005 per cent w/v each of *verapamil hydrochloride RS* and *(2RS)-2-(3,4-dimethoxyphenyl)-2-[2-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]ethyl]-3-methylbutanenitrile RS (verapamil impurity A RS)* in the mobile phase.

Chromatographic system

- a stainless steel column 12.5 cm x 4 mm, packed with octadecylsilane bonded to porous silica (3 µm) (Such as Hypersil ODS),
- mobile phase: a mixture of 1 volume of n-*heptylamine*, 4.7 volumes of *glacial acetic acid* , 58 volumes of *acetonitrile* and 137 volumes of *0.01 M sodium acetate,*
- flow rate. 0.85 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 10 µl.

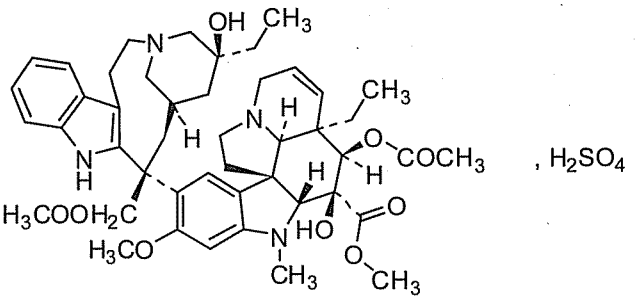
Inject reference solution (b). The test is not valid unless the resolution between the peaks due to verapamil and verapamil impurity A is not less than 2.0.

Inject the test solution and reference solution (a). Run the chromatogram 4 times the retention time of the principal peak. In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than three times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.1 g of Verapamil Hydrochloride, shake with 150 ml of *0.1 M hydrochloric acid* for 10 minutes, add sufficient *0.1 M hydrochloric acid* to produce 200.0 ml and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{27}H_{38}N_2O_4$, HCl taking 118 as the specific absorbance at 278 nm.

Vinblastine Sulphate



$C_{46}H_{58}N_4O_9,H_2SO_4$ Mol. Wt. 909.1

Vinblastine Sulphate is methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*)-4-acetoxy-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7-methanoazacycloundecino[(5,4-*b*)indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3a,4,5,5a,6,11,12,13a-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate sulphate.

Vinblastine Sulphate contains not less than 95.0 per cent and not more than 104.0 per cent of $C_{46}H_{58}N_4O_9$, H_2SO_4, calculated on the dried basis.

Category. Anticancer.

Dose. By intravenous injection, according to the needs of the patient.

Description. A white or yellowish, amorphous or crystalline powder; very hygroscopic.

CAUTION— Handle Vinblastine Sulphate with great care since it is a potent cytotoxic agent. Avoid contact with eyes; irritant to tissues.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *vinblastine sulphate RS.*

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to vinblastine sulphate in the chromatogram obtained with reference solution (b).

C. A 10 per cent w/v solution gives the reaction of sulphates (2.3.1).

Tests

Appearance of solution. A 0.5 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in a 0.15 per cent w/v solution.

Related substances. In the Assay, the area of any secondary peak in the chromatogram obtained with the test solution is not greater than that of the principal peak in the chromatogram obtained with reference solution (c) and the sum of the areas of any such peaks is not greater than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c). Ignore any peak with an area less than that of the peak in the chromatogram obtained with reference solution (d).

Loss on drying (2.4.19). Not more than 15.0 per cent, determined by Method B, on an appropriately calibrated instrument using about 3.0 mg, accurately weighed. Heat the substance under examination at the rate of 5° per minute between ambient temperature and 200° in a current of nitrogen for chromatography with a flow rate of 40 ml per minute. From the thermogram, determine the accumulated loss in weight between ambient temperature and a point on the plateau before decomposition is indicated (at about 160°).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *water*.

Reference solution (a). A solution containing 0.1 per cent w/v each of *vinblastine sulphate RS* and *vincristine sulphate RS* in *water*.

Reference solution (b). A 0.1 per cent w/v solution of *vinblastine sulphate RS* in *water*.

Reference solution (c). A 0.002 per cent w/v solution of *vinblastine sulphate RS* in *water*.

Reference solution (d). A 0.0001 per cent w/v solution of *vinblastine sulphate RS* in *water*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm), (b) a guard column packed with a suitable silica gel placed between the pump and the injection device,
- mobile phase: a mixture of 50 volumes of *methanol*, 38 volumes of a 1.5 per cent v/v solution of *diethylamine* adjusted to pH 7.5 with *phosphoric acid* and 12 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 262 nm,
- injection volume. 10 µl.

NOTE — Store all solutions in ice before use.

Inject each solution and record the chromatograms for 3 times the retention time of the peak due to vinblastine.

The assay is not valid unless the resolution between the peaks due to vincristine and vinblastine in the chromatogram obtained with reference solution (a) is at least 4 and the signal-to-noise ratio of the peak in the chromatogram obtained with reference solution (d) is at least 5.

Calculate the percentage content of $C_{46}H_{58}N_4O_9$, H_2SO_4.

Vinblastine Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per mg.

Vinblastine Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture in a deep freezer (Below -18°). If the material is intended for use in the manufacture of parenteral preparations, it should be stored in sterile, tamper-evident glass containers and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are suitable for use in the manufacture of parenteral preparations.

Vinblastine Injection

Vinblastine Sulphate Injection

Vinblastine Injection is a sterile material consisting of Vinblastine Sulphate with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile 0.9 per cent w/v solution of Sodium Chloride, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Vinblastine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of vinblastine sulphate, $C_{46}H_{58}N_4O_9,H_2SO_4$.

Usual strength. 10 mg.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

CAUTION — Handle Vinblastine Injection with great care since it is a potent cytotoxic agent. Avoid contact with eyes; irritant to tissues.

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay shows an absorption maximum at about 267 nm.

B. In the test for Related substances, the principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To 1 ml add 0.2 ml of a freshly prepared 1 per cent w/v solution of *vanillin* in *hydrochloric acid*; a pink colour is produced in about 1 minute (distinction from Vincristine Sulphate).

Tests

pH (2.4.24). 3.5 to 5.0, determined in a 0.15 per cent w/v solution of the dried contents.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 80 volumes of *toluene*, 40 volumes of *chloroform* and 6 volumes of *diethylamine*.

Test solution. Dissolve a quantity of the contents of a container in sufficient *methanol* to produce a solution containing the equivalent of 1 per cent w/v of dried vinblastine sulphate.

Reference solution (a). A 1 per cent w/v solution of *vinblastine sulphate RS* in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *vincristine sulphate RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

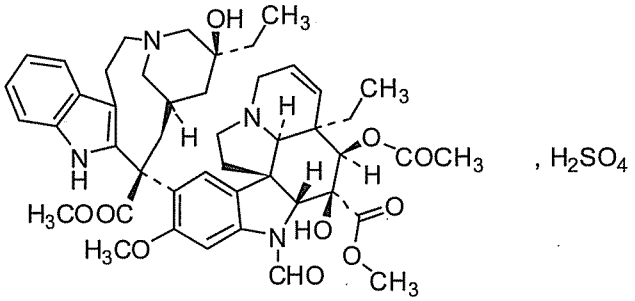
Bacterial endotoxins (2.2.3). Not more than 10.0 Endotoxin Units per mg of the dried contents.

Assay. Weigh the contents of 20 sealed containers. Weigh accurately a quantity of the mixed contents containing about 20 mg of dried vinblastine sulphate and dissolve it in 100.0 ml of *methanol*. Dilute 10.0 ml to 100.0 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 267 nm (2.4.7). Calculate the content of $C_{46}H_{58}N_4O_9,H_2SO_4$ taking 185 as the specific absorbance at 267 nm.

Storage. Store in sealed containers in a deep freezer (Below −18°).

Labelling. The label states (1) the strength in terms of the weight of dried vinblastine sulphate contained in it; (2) the names of auxiliary substances, if any; (3) that the contents are to be used by intravenous injection only; (4) the storage conditions.

Vincristine Sulphate



$C_{46}H_{56}N_4O_{10},H_2SO_4$ Mol. Wt. 923.1

Vinblastine Sulphate is methyl (3a*R*,4*R*,5*S*,5a*R*,10b*R*,13a*R*) 4-acetoxy-3a-ethyl-9-[(5*S*,7*R*,9*S*)-5-ethyl-5-hydroxy-9- methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-2*H*-3,7- methanoazacycloundecino[(5,4-*b*)indol-9-yl]-6-formyl-5-hydroxy- 8-methoxy-3a,4,5,5a,6,11,12,13a-octahydro-*1H*- indolizino[8,1-*cd*]carbazole-5-carboxylate sulphate.

Vincristine Sulphate contains not less than 95.0 per cent and not more than 104.0 per cent of $C_{46}H_{56}N_4O_{10},H_2SO_4$, calculated on the dried basis.

Category. Anticancer.

Dose. By intravenous injection, according to the needs of the patient.

Description. A white to slightly yellowish, amorphous or crystalline powder; very hygroscopic.

CAUTION— Handle Vincristine Sulphate with great care since it is a potent cytotoxic agent. Avoid contact with eyes; irritant to tissues.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *vincristine sulphate RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to vincristine sulphate in the chromatogram obtained with reference solution (b).

C. A 10 per cent w/v solution gives the reaction for sulphates (2.3.1).

Tests

Appearance of solution. A 0.5 per cent w/v in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution YS7 (2.4.1).

pH (2.4.24). 3.5 to 4.5. Determined in a 0.1 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

NOTE— Keep the solutions in iced water before use.

Test solution. Dissolve about 50.0 mg of the substance under examination in 10.0 ml of *carbon dioxide-free water.* Dilute 1.0 ml of this solution to 5.0 ml with *water.*

Reference solution (a). A 0.1 per cent w/v solution of *vincristine sulphate RS* in *water.*

Reference solution (b). A 0.1 per cent w/v solution of *vinblastine sulphate RS* in reference solution (a).

Reference solution (c). Dilute 1.0 ml of the test solution to 50.0 ml with *water.*

Reference solution (d). Dilute 1.0 ml of reference solution (c) to 20.0 ml with *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 1.5 per cent v/v solution of *diethylamine*, adjusted to pH 7.5 with *orthophosphoric acid*

  B. *methanol*,
- a linear gradient programme using the condition given below,
- flow rate. 2 ml per minute,
- spectrophotometer set at 297 nm,
- injection volume. 20 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-12	38	62
12-27	38-8	62-92
27-29	8-38	92-62
29-34	38	62

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to vincristine and vinblastine is not less than 4.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (2.0 per cent) and the sum of areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Ignore any peak with an area less than 0.1 per cent the area of the principal peak in the chromatogram obtained with reference solution (d).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined by Method B, on an appropriately calibrated instrument using about 3.0 mg, accurately weighed. Heat the substance under examination at the rate of 5° per minute between ambient temperature and 200° in current of nitrogen for chromatography with a flow rate of 40 ml per minute.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *water.*

Reference solution (a). A solution containing 0.1 per cent w/v each of *vinblastine sulphate RS* and *vincristine sulphate RS* in *water.*

Reference solution (b). A 0.1 per cent w/v solution of *vincristine sulphate RS* in *water.*

Reference solution (c). A 0.002 per cent w/v solution of *vincristine sulphate RS* in *water.*

Reference solution (d). A 0.0001 per cent w/v solution of *vincristine sulphate RS* in *water.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm), (b) a guard column packed with a suitable silica gel placed between the pump and the injection device,
- mobile phase: a mixture of 70 volumes of *methanol* and 30 volumes of a 1.5 per cent v/v solution of *diethylamine* adjusted to pH 7.5 with *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 297 nm,
- injection volume. 10 µl.

NOTE — Store all solutions in ice before use.

Inject each solution and record the chromatograms for 3 times the retention time of the peak due to vincristine.

The assay is not valid unless the resolution between the peaks due to vincristine and vinblastine in the chromatogram obtained with reference solution (a) is at least 4 and the signal-to-noise ratio in the peak in the chromatogram obtained with reference solution (d) is at least 5.

Calculate the content of $C_{46}H_{56}N_4O_{10},H_2SO_4$.

Vincristine Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 62.5 Endotoxin Units per mg.

Vincristine Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light in a deep freezer (Below -18°). If the material is intended for use in the manufacture of parenteral preparations, it should be stored in sterile, tamper-evident glass containers and sealed so as to exclude micro-organisms.

Labelling. The label states whether or not the contents are suitable for use in the manufacture of parenteral preparations.

Vincristine Injection

Vincristine Sulphate Injection

Vincristine Injection is a sterile material consisting of Vincristine Sulphate with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile 0.9 per cent w/v solution of Sodium Chloride, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Vincristine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of vincristine sulphate, $C_{46}H_{56}N_4O_{10},H_2SO_4$.

Usual strengths. The equivalent of 1 mg, 2 mg and 5 mg of dried vincristine sulphate.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

CAUTION — Handle Vincristine Injection with great care since it is a potent cytotoxic agent. Avoid contact with eyes; irritant to tissues.

A. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (b).

B. Shake a quantity containing 1 mg of dried vincristine sulphate with 3 ml of *chloroform*, filter and wash the filter with 2 ml of *chloroform*. Reserve the residue for test D. Evaporate the combined chloroform solutions to dryness at 40°. Add 0.2 ml of a freshly prepared 1 per cent w/v solution of *vanillin* in *hydrochloric acid* to the residue; an orange colour is produced in about 1 minute (distinction from vinblastine sulphate).

Tests

Appearance of solution. A solution prepared by dissolving the contents of a sealed container in 10 ml of *carbon dioxide-free water* is clear (2.4.1).

pH (2.4.24). 3.5 to 5.0, determined in a solution containing 0.15 per cent w/v solution of the dried contents.

Related substances. Deteremine by liquid chromatography (2.4.14).

NOTE—Keep the solutions in ice before use.

Test solution. Dissolve the contents of a sealed container in *water* to produce a solution containing about 0.1 per cent w/v of anhydrous vincristine sulphate.

Reference solution (a). A solution containing 0.1 per cent w/v each of *vincristine sulphate RS* and *vinblastine sulphate RS* in *water*.

Reference solution (b). A 0.1 per cent w/v solution of *vincristine sulphate RS* in *water*.

Reference solution (c). A 0.002 per cent w/v solution of *vincristine sulphate RS* in *water*.

Reference solution (d). A 0.0001 per cent w/v solution of *vincristine sulphate RS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with endcapped octylsilane bonded to porous silica (5 µm), (Such as Zorbax C8),
- mobile phase: a mixture of 30 volumes of a 1.5 per cent v/v solution of *diethylamine* adjusted to pH 7.5 with *orthophosphoric acid* and 70 volumes of *methanol,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 297 nm,
- injection volume. 10 µl.

Inject reference solution (a). Run the chromatogram 3 times the retention time of the principal peak.

The test is not valid unless the resolution between the peaks due to vincristine and vinblastine in the chromatogram obtained with reference solution (a) is not less than 4 and signal-to-noise ratio in the principal peak in the chromatogram obtained with reference solution (d) is not less than 5.

Inject the test solution, reference solution (c) and (d). In the chromatogram obtained with the test solution the area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (c)

(2.0 per cent) and the sum of the areas of all the secondary peaks is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (5.0 per cent). Ignore any peak with an area less than the of the principal peak in the chromatogram obtained with reference solution (d) (0.1 per cent).

Uniformity of content. The content of anhydrous vincristine sulphate in each of 10 individual containers as determined in the Assay is not less than 90.0 per cent and not more than 110.0 per cent of the average except that in one container the content may be not less than 80.0 per cent and not more than 120.0 per cent of the average..

Bacterial endotoxins (2.2.3). Not more than 62.5 Endotoxin Units per mg of dried vincristine sulphate.

Assay. Dissolve the contents of a sealed container in a suitable volume of methanol to produce a solution containing about 0.005 per cent w/v of anhydrous vincristine sulphate. Measure the absorbance of the resulting solution at the maximum at 297 nm (2.4.7). Calculate the content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ taking 177 as the specific absorbance at 297 nm.

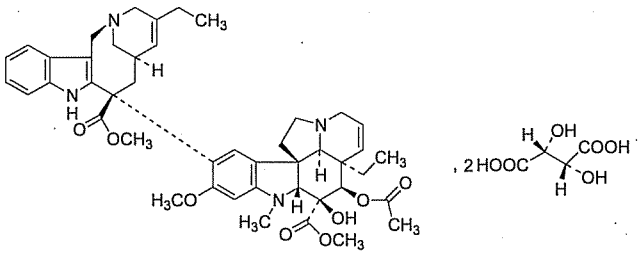
Repeat the procedure with a further nine containers.

Calculate the average content of $C_{46}H_{56}N_4O_{10},H_2SO_4$ in the sealed container.

Storage. Store in sealed containers in a deep freezer (Below -18°).

Labelling. The label states (1) the strength in terms of the weight of dried vincristine sulphate contained in it; (2) the names of auxilary substances, if any; (3) that the contents are to be used by intravenous injection only; (4) the storage conditions.

Vinorelbine Tartrate



$C_{45}H_{54}N_4O_8,(C_4H_6O_6)_2$ Mol Wt. 1079.1

Vinorelbine Tartrate is 3′,4′-didehydro-4′-deoxy-*C*′-norvincaleukoblastine ditartrate.

Vinorelbine Tartrate is the ditartrate salt of vinorelbine, a semisynthetic *Vinca* alkaloid; structurally relate to vinblastine.

Vinorelbine Tartrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{45}H_{54}N_4O_8.2C_4H_6O_6$, calculated on the anhydrous basis.

Category. Anticancer.

Description. A white to yellow or light brown amorphous powder.

CAUTION – Vinorelbine Tartrate is cytotoxic; extra care required to prevent inhaling particles and exposing the skin to it.

Identification

A. Dissolve 10 mg in 5 ml of *water*, add 0.5 ml of 5 *M sodium hydroxide*, and extract with 5 ml of *methylene chloride*. Filter the organic layer through *anhydrous sodium sulphate*, and evaporate to dryness.

Determine by Infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *vinorelbine tartrate RS* treated in the same manner.

B. In the test for Related substances, the principal peak in the chromatogram of the test solution corresponds to that due to vinorelbine tartarate in the chromatogram obtained with the refrence solution (a).

C. It gives the reactions for tartrate (2.3.1).

Tests

Appearance of solution. A 1.0 per cent w/v solution is clear (2.4.1).

Light absorption (2.4.7). The absorbance of 1.0 per cent w/v solution, at about 420 nm is not more than 0.03.

pH (2.4.24). 3.3 to 3.8, determined on a 1.0 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 35 mg of the substance under examination in 25 ml of the mobile phase.

Reference solution (a). A 0.14 per cent w/v solution of *vinorelbine tartrate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of the reference solution (a) to 100 ml with the mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.3 times the area of the peak

in the chromatogram obtained with reference solution (b) (0.3 per cent) and the sum of areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore any secondary peak having area less than 0.1 per cent.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 4.0 per cent, determined on 0.1 g.

Bacterial endotoxins (2.2.3). Not more than 1.5 Endotoxin Unit per mg of vinorelbine base.

Microbial contamination (2.2.9). Total viable aerobic count, not more than 100 cfu per g, total combined molds and yeast does not exceed 50 cfu per g. It also meets the requirement of the tests for the absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella species*, *Escherichia coli*, *Enterobacteria* and *Closteridia*.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 35 mg of the substance under examination in 25.0 ml of mobile phase.

Reference solution. A 0.14 per cent w/v solution of *vinorelbine tartrate RS* in mobile phase.

Chromatographic system

- a stainless steel column 15 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: 62 volumes of *methanol* containing 1.22 g of *sodium 1-decane sulphonate* and 38 volumes of *phosphate buffer solution,* prepared by dissolving 6.9 g of *monobasic sodium phosphate* in 900 ml of *water*, adjust the pH to 4.2 with *orthophosphoric acid* and dilute to 1000 ml with *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 267 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of $C_{45}H_{54}N_4O_8.2C_4H_6O_6$.

Storage. Store protected from light, in a deep freezer (below – 18°).

Vinorelbine Injection

Vinorelbine Tartrate Injection

Vinorelbine Injection is a sterile solution of vinorelbine tartrate in Water for Injection.

Vinorelbine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of vinorelbine, $C_{45}H_{54}N_4O_8$.

Usual strength. 10 mg per ml.

Description. A clear, colourless to pale yellow solution.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. When examined in the range 220 nm to 380 nm (2.4.7), a solution containing 0.01 per cent w/v of Vinorelbine Tartrate, exhibits the maxima at about 267 nm.

Tests

pH (2.4.24). 3.0 to 3.8.

Light absorption. The absorbance of the injection at about 420 nm (2.4.7), is not more than 0.06.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Accurately measured volume of injection containing 10 mg of Vinorelbine, dilute to 10 ml with the mobile phase.

Reference solution (a). A 0.14 per cent w/v solution of *vinorelbine tartrate RS* in the mobile phase.

Reference solution (b). Dilute 1 ml of the reference solution (a) to 100 ml with the mobile phase.

Chromatographic system as described under Assay.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and reference solution (b). Run the chromatograms three times the retention time of the peak due to vinorelbine. In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (b) (0.2 per cent) and the sum of areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Complies with the tests stated under Parenteral Preparation (Injections).

Bacterial endotoxins (2.2.3). Not more than 3.0 Endotoxin Unit per mg of vinorelbine tartrate.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Accurately measured volume of injection containing 10 mg of Vinorelbine, diluted to 10.0 ml with *water.*

Reference solution. A 0.14 per cent w/v solution of *vinorelbine tartrate RS* in *water.*

Chromatographic system
- a stainless steel column 15 cm × 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- column temperature 40°,
- mobile phase: a mixture of 62 volumes of *methanol* containing 1.22 g of *sodium1-decane sulphonate* and 38 volumes of phosphate buffer solution prepared by dissolving 6.9 g of *monobasic sodium phosphate* in 900 ml of *water,* adjusted the pH to 4.2 with *orthophosphoric acid* and dilute to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 267 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{45}H_{54}N_4O_8$.

Storage. Store protected from light, in a single-dose container, in a refrigerator (2° to 8°) ; do not freeze.

Vitamin A Concentrate Oil

Synthetic Vitamin A Concentrate (Oily Form); Synthetic Retinol Concentrate (Oily Form).

Vitamin A Concentrate Oil consists of an ester or a mixture of esters of retinol (as acetate, propionate or palmitate) prepared by synthesis. It may be diluted with a suitable vegetable oil. It may contain suitable stabilising agents such as antioxidants.

Vitamin A Concentrate Oil contains not less than 500,000 Units of Vitamin A per g, and not less than 95.0 per cent and not more than 110.0 per cent of the stated number of Units of Vitamin A per g.

Category. Antixerophthalmic vitamin.

Dose. Prophylactic, 5000 Units of Vitamin A, daily; therapeutic, 10,000 to 200,000 Units of Vitamin A, daily.

Description. A yellow to brownish yellow, oily liquid; odour, faint and characteristic.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a solution in *2-propanol* containing 10 to 15 Units per ml shows an absorption maximum at about 325 nm to 327 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 80 volumes of *cyclohexane* and 20 volumes of *ether.*

Test solution. Prepare a solution containing 5 Units per µl of the substance under examination in *cyclohexane.*

Reference solution (a). Prepare a solution containing 5 Units per µl of *retinyl acetate RS* in *cyclohexane.*

Reference solution (b). Prepare a solution containing 5 Units per µl of *retinyl propionate RS* in *cyclohexane.*

Reference solution (c). Prepare a solution containing 5 Units per µl of *retinyl palmitate RS* in *cyclohexane.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *antimony trichloride solution.* The principal spot or spots in the chromatogram obtained with the test solution correspond to one or more of the spots in the chromatograms obtained with reference solutions (a), (b) and (c).

C. Dissolve a quantity containing 10 to 15 Units in 1 ml of *chloroform* and add 5 ml of *antimony trichloride solution*; a transient bright blue colour is produced immediately.

Tests

Acid value (2.3.23). Not more than 2.0, determined on 2.0 g.

Peroxides. Add 0.3 g to 25.0 ml of a mixture of 6 volumes of *toluene* and 4 volumes of *methanol* (solution A). Add in a test-tube, in the following order, mixing after each addition, 0.3 ml of a 1.8 per cent w/v solution of *ammonium thiocyanate,* 10.0 ml of *methanol,* 0.3 ml of *ferrous sulphate solution* and 15.0 ml of *toluene* and add 1.0 ml of solution A. The colour produced after 5 minutes is not more intense than that obtained in a solution prepared at the same time and in the same manner but using a solution prepared in the following manner in place of solution A. Add 1.0 ml of a 27.0 per cent w/v solution of *ferric chloride hexahydrate* to 99 ml of a mixture of 6 volumes of *toluene* and 4 volumes of *methanol* and dilute 2.0 ml to 100.0 ml with the same solvent mixture.

Assay. Carry out the assay of vitamin A, Method A (2.3.41).

Storage. Store protected from light, in well-filled containers. Once the container has been opened, its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of an inert gas.

Labelling. The label states (1) the number of Units of Vitamin A per g; (2) the name of the ester or esters; (3) the name(s) of any added stabilising agent(s); (4) the method of restoring the solution if partial crystallisation has occurred.

Vitamin A Concentrate Powder

Synthetic Vitamin A Concentrate (Powder Form); Synthetic Retinol Concentrate (Powder Form).

Vitamin A Concentrate Powder consists of an ester or a mixture of esters of retinol (as acetate, propionate or palmitate) prepared by synthesis and dispersed in a matrix of Gelatin, Acacia or any other suitable material. It may contain suitable stabilising agents such as antioxidants.

Vitamin A Concentrate Powder contains not less than 250,000 Units of Vitamin A per g, and not less than 95.0 per cent and not more than 115.0 per cent of the stated number of Units of Vitamin A per g.

Category. Antixerophthalmic vitamin.

Dose. Prophylactic, 5000 Units of Vitamin A, daily; therapeutic, 10,000 to 200,000 Units of Vitamin A, daily. In multi-vitamin oral preparations, prophylactic, 1600 to 2500 Units of Vitamin A; therapeutic, 5000 to 10,000 Units of Vitamin A.

Description. A yellowish powder usually in the form of pellets or particles of almost uniform size.

Identification

To a quantity containing 50,000 Units of Vitamin A, add 1.5 ml of *2 M ammonia* previously heated to 60° and heat in a water-bath at 60°, shaking occasionally. After 10 minutes add 40 ml of *ethanol*, dilute to 200 ml with *ether* and shake. Allow to stand for a few minutes and use the supernatant liquid (solution A) for the following tests.

Certain samples may not react sufficiently during the course of the above treatment. In such cases the volume of solution A used in the following tests should be increased which may be as much as 10-fold.

A. When examined in the range 230 nm to 360 nm (2.4.7), a solution in *2-propanol* containing 10 to 15 Units per ml shows an absorption maximum at about 325 nm to 327 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *cyclohexane* and 20 volumes of *ether*.

Test solution. Evaporate 10 ml of solution A to dryness in a current of nitrogen and dissolve the residue in 0.5 ml of *cyclohexane*.

Reference solution (a). Prepare a solution containing 5 Units per µl of *retinyl acetate RS* in *cyclohexane*.

Reference solution (b). Prepare 5 Units per µl of *retinyl propionate RS* in *cyclohexane*.

Reference solution (c). Prepare a solution containing 5 Units per µl of *retinyl palmitate RS* in *cyclohexane*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *antimony trichloride solution*. The principal spot or spots in the chromatogram obtained with the test solution correspond to one or more of the spots in the chromatograms obtained with reference solutions (a), (b) and (c).

C. Dilute 2 ml of solution A to 50 ml with *n-pentane* and evaporate 1 ml of the solution to dryness in a current of nitrogen. Dissolve the residue in 1 ml of *chloroform* and add 5 ml of *antimony trichloride solution*; a transient bright blue colour is produced immediately.

Tests

Related substances and degradation products. Using the relative absorbances obtained in the Assay, the ratio A_{300}/A_{325} is not more than 0.612 and the sum of the ratios A_{300}/A_{325} and A_{350}/A_{325} is not more than 1.054, where A_{300}, A_{325} and A_{350} are the absorbances measured at about 300 nm, 325 nm and 350 nm respectively.

Assay. Carry out the assay of vitamin A, Method B (2.3.41), adding 5 ml of *water* to the mixture for saponification, using *2-propanol* as the blank and taking 0.612 as the maximum value of the ratio A_{300}/A_{325}.

Storage. Store protected from light. Once the container has been opened, its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of an inert gas.

Labelling. The label states (1) the number of Units of Vitamin A per g; (2) the name of the ester or esters; (3) the name of the principal excipient or excipients used; (4) the name of any added stabilising agents.

Water-Miscible Vitamin A Concentrate

Synthetic Vitamin A Concentrate (Water-dispersible Form); Synthetic Retinol Concentrate (Water-dispersible Form).

Water-miscible Vitamin A Concentrate consists of an ester or a mixture of esters of retinol (as acetate, propionate or palmitate) prepared by synthesis to which suitable solubilisers have been added. It may contain suitable stabilising agents such as antimicrobial preservatives and antioxidants.

Water-miscible Vitamin A Concentrate contains not less than 100,000 Units of Vitamin A per g, and not less than 95.0 per

cent and not more than 115.0 per cent of the stated number of Units of Vitamin A per g.

Category. Antixerophthalmic vitamin.

Dose. Prophylactic, 5000 Units of Vitamin A, daily; therapeutic, 10,000 to 200,000 Units of Vitamin A, daily. In multi-vitamin oral preparations, prophylactic, 1600 to 2500 Units of Vitamin A; therapeutic, 5000 to 10,000 Units of Vitamin A.

Description. A yellow or yellowish liquid of variable opalescence and viscosity; odour, characteristic. Highly concentrated solutions may become cloudy at low temperatures or gel at room temperature.

Identification

To a quantity containing about 10,000 Units of Vitamin A, add 5 ml of *water* and homogenise. Add 5 ml of *ethanol (95 per cent)* and 20 ml of *n-pentane* and shake vigorously for 30 seconds. Allow to stand for a few minutes and use the supernatant liquid (solution A) for the following tests.

A. Dilute solution A with sufficient *2-propanol* so that the absorbance at the wavelength of maximum absorption is 0.3 to 0.7 (2.4.7).

When examined in the range 230 nm to 360 nm (2.4.7), the solution shows an absorption maximum at 325 to 327 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 80 volumes of *cyclohexane* and 20 volumes of *ether*.

Test solution. Evaporate 10 ml of solution A to dryness in a current of *nitrogen* and dissolve the residue in 0.5 ml of *cyclohexane*.

Reference solution (a). Prepare a solution containing 5 Units per µl of *retinyl acetate RS* in *cyclohexane*.

Reference solution (b). Prepare a solution containing 5 Units per µl of *retinyl propionate RS* in *cyclohexane*.

Reference solution (c). Prepare a solution containing 5 Units per µl of *retinyl palmitate RS* in *cyclohexane*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *antimony trichloride solution*. The principal spot or spots in the chromatogram obtained with the test solution correspond to one or more of the spots in the chromatograms obtained with reference solutions (a), (b) and (c).

C. Evaporate 0.1 ml of solution A to dryness in a current of nitrogen, dissolve the residue in 1 ml of *chloroform* and add 5 ml of *antimony trichloride solution*; a transient bright blue colour is produced immediately.

Tests

Water miscibility. Mix about 1 g with 10 ml of *water* previously heated to 50° and cool to 20°. Immediately after cooling, a uniform, slightly opalescent and slightly yellow dispersion is obtained.

Related substances and degradation products. Using the relative absorbances obtained in the Assay, the ratio A_{300}/A_{325} is not more than 0.618 and the sum of the ratios A_{300}/A_{325} and A_{350}/A_{325} is not more than 1.060, where A_{300}, A_{325} and A_{350} are the absorbances measured at about 300 nm, 325 nm and 350 nm respectively.

Assay. Carry out the assay of vitamin A, Method B (2.3.41), using *2-propanol* as the blank and taking 0.618 as the maximum value of the ratio A_{300}/A_{325}.

Storage. Store protected from light at the temperature stated on the label. Once the container has been opened, its contents should be used as soon as possible; any part of the contents not used at once should be protected by an atmosphere of an inert gas.

Labelling. The label states (1) the number of Units of Vitamin A per g; (2) the name of the ester or esters; (3) the name of the principal excipient or excipients used; (4) the temperature at which it should be stored.

Vitamins A and D Capsules

Vitamins A and D Capsules contain Vitamin A Oil and a source of vitamin D such as Cholecalciferol or Ergocalciferol in an edible vegetable oil.

Vitamins A and D Capsules contain not less than 90.0 per cent of the stated number of Units of vitamin A and vitamin D.

Category. Antixerophthalmic and antirachitic vitamins.

Dose. Prophylactic, 1600 to 2500 Units of Vitamin A and 100 to 200 Units of vitamin D once a day; therapeutic, 5000 to 10,000 Units of Vitamin A and 400 to 1000 Units of vitamin D once a day.

Tests

Other tests. Comply with the tests stated under Capsules.

Assay. *For vitamin A* — Weigh accurately a portion of the mixed contents of 20 capsules containing about 500 Units of Vitamin A and carry out the assay of vitamin A, Method A (2.3.41).

For vitamin D — Weigh accurately a portion of the mixed contents of 20 capsules containing about 5000 Units of vitamin D and carry out the assay of vitamin D (2.3.42).

Storage. Store protected from light and moisture.

Labelling. The label states the number of Units of vitamin A and vitamin D per capsule.

Concentrated Vitamin D Solution

Concentrated Vitamin D Solution is a solution of Cholecalciferol or Ergocalciferol in an edible vegetable oil. It may contain suitable stabilising agents such as antioxidants.

Concentrated Vitamin D Solution contains not less than 10,000 Units of vitamin D and not less than 90.0 per cent of the stated number of Units of vitamin D.

Category. Antirachitic vitamin.

Dose. Prophylactic, 400 to 1000 Units daily; therapeutic, 5000 to 50,000 Units daily.

Description. A pale yellow to yellow, oily liquid; odour, faint but not rancid.

Identification

Dissolve a quantity containing about 1000 Units of vitamin D in 1 ml of *chloroform* and add 10 ml of *antimony trichloride solution*; a pinkish red colour appears at once.

Tests

Acid value (2.3.23). Not more than 2.5, determined on 2.0 g.

Assay. Carry out the assay of vitamin D (2.3.42).

Storage. Store protected from light, in well-filled containers at a temperature of 6° to 15°. The contents of an opened container should be used as soon as possible.

Labelling. The label states (1) the number of Units of vitamin D per g; (2) the storage conditions; (3) the nature and concentration of any stabilising agent added.

Concentrated Vitamins A and D Solution

Concentrated Vitamins A and D Solution is a solution of Vitamin A Oil and a source of vitamin D such as Cholecalciferol or Ergocalciferol in an edible vegetable oil. It may contain suitable stabilising agents such as antioxidants.

Concentrated Vitamins A and D Solution contains not less than 50,000 Units of vitamin A and 5000 Units of vitamin D per g, and not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of Vitamin A and vitamin D per g.

Category. Antixerophthalmic and antirachitic vitamins.

Dose. 2500 to 25,000 Units of Vitamin A and 250 to 2500 Units of vitamin D daily.

Tests

Acid value (2.3.23). Not more than 2.5, determined on 2.0 g.

Assay. *For vitamin A* — Carry out the assay of vitamin A, Method A (2.3.41).

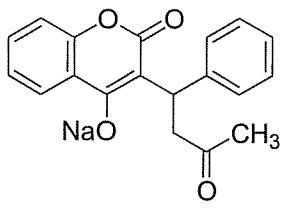
For vitamin D — Carry out the assay of vitamin D (2.3.42).

Storage. Store protected from light and moisture.

Labelling. The label states (1) the number of Units of vitamin A and vitamin D per gram; (2) the storage conditions.

W

Warfarin Sodium



$C_{19}H_{15}NaO_4$ Mol. Wt. 330.3

Warfarin Sodium is sodium 2-oxo-3-[(1*RS*)-3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-4-olate.

Warfarin Sodium contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{19}H_{15}NaO_4$, calculated on the anhydrous basis.

Category. Anticoagulant.

Dose. 10 to 15 mg.

Description. A white powder; hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Dissolve 1 g in 25 ml of *water*, add 2 ml of *2 M hydrochloric acid* and filter. Wash the residue with *water* and dry over *phosphorus pentoxide*.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *warfarin sodium RS* or with the reference spectrum of warfarin sodium.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (c).

C. Dissolve 1 g in 10 ml of *water*, add 5 ml of *nitric acid* and filter. To the filtrate add 2 ml of *potassium dichromate solution*, shake for 5 minutes and allow to stand for 20 minutes; the solution is not greenish blue when compared with a blank.

D. The residue obtained in test A, after washing with *water* and drying at 105°, melts at 159° to 163° (2.4.21).

E. The filtrate obtained in test A gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 7.6 to 8.6, determined in a 1.0 per cent w/v solution.

Phenolic ketones. Absorbance of a 12.5 per cent w/v solution in a 5.0 per cent w/v solution of *sodium hydroxide* at the maximum at about 385 nm, measured within 15 minutes of preparation, is not more than 0.20 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *methanol* and 75 volumes of *water*.

Test solution. Dissolve 40 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution (a). Dissolve 2 mg each of of *4-hydroxycoumarin* (warfarin impurity B) and *benzalacetone* (warfarin impurity C) in 25 ml of *methanol* and dilute to 100 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.08 per cent w/v solution of *warfarin sodium RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with spherical nitrile silica gel (5 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 25 volumes of *acetonitrile* and 75 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to warfarin impurity B and warfarin impurity C is not less than 2.0. The relative retention time with reference to warfarin for warfarin impurity B is about 0.4 and for warfarin impurity C is about 0.6.

Inject the test solution and reference solution (b). Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to warfarin impurity B and warfarin impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 4.0 per cent, determined on 0.75 g.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient *0.01 M sodium hydroxide* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with *0.01 M sodium hydroxide* and dilute 5.0 ml to 50.0 ml with *0.01 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{19}H_{15}NaO_4$ taking 431 as the specific absorbance at 308 nm.

Storage. Store protected from light and moisture.

Warfarin Sodium Clathrate

Warfarin Sodium Clathrate is a clathrate form of Warfarin Sodium consisting principally of Warfarin Sodium and Isopropyl Alcohol in a 2:1 molecular ratio.

Warfarin Sodium Clathrate contains not less than 97.0 per cent and more than 102.0 per cent of $C_{19}H_{15}NaO_4$ calculated on the anhydrous, isopropyl alcohol-free basis and not less than 8.0 per cent and not more than 8.5 per cent of isopropy alcohol.

Category. Anticoagulant.

Dose. 10 to 15 mg.

Description. A white crystalline powder; hygroscopic.

Identification

Test A may be omitted if test B, C, D and E are carried out. Tests B and D may be omitted if tests A, C and E are carried out.

A. Dissolve about 1 g in 25 ml of *water*, add 2 ml of *2 M hydrochloric acid* and filter. Wash the precipitate 5 to 6 times with *water*. Dry the residue over *phosphorus pentoxide*.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *warfarin sodium RS* or with the reference spectrum of warfarin sodium.

B. In the test for Related substances, the principal peak in the chromatogram obtained with the test solution corresponds to the principal peak in the chromatogram obtained with reference solution (c).

C. Dissolve 1 g in 10 ml of *water*, add 5 ml of *nitric acid* and filter. To the filtrate, add 2 ml of *potassium dichromate solution*, shake for 5 minutes and allow to stand for 20 minutes; the solution is not greenish-blue when compared with a blank.

D. The residue obtained in test A, after washing with *water* and drying at 105° melts at 159° to 163°.

E. The filtrate obtained in test A gives the reactions of sodium salts (2.3.1).

Tests

Appearance of solution. A 5.0 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 7.2 to 8.3, determined in a 1.0 per cent w/v solution.

Phenolic ketones. Absorbance of a 12.5 per cent w/v solution in a 5.0 per cent w/v solution of *sodium hydroxide* at the maximum at about 385 nm, measured within 15 minutes of preparation, is not more than 0.20 (2.4.7).

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 25 volumes of *methanol* and 75 volumes of *water.*

Test solution. Dissolve 40 mg of the substance under examination in 50.0 ml of the solvent mixture.

Reference solution (a). Dissolve 2 mg each of *4-hydroxycoumarin* (warfarin impurity B) and *benzalacetone* (warfarin impurity C) in 25 ml of *methanol* and dilute to 100 ml with *water*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (c). A 0.08 per cent w/v solution of *warfarin sodium RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 25 cm x 4.0 mm, packed with spherical nitrile silica gel (5 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid,* 25 volumes of *acetonitrile* and 75 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 260 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the resolution between the peaks due to warfarin impurity B and warfarin impurity C is not less than 2.0. The relative retention time with reference to warfarin for warfarin impurity B is about 0.4 and for warfarin sodium impurity C is about 0.6.

Inject the test solution and reference solution (b). Run the chromatogram twice the retention time of the principal peak. In the chromatogram obtained with the test solution the area of the peak due to warfarin impurity B and warfarin impurity C is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent). The sum of all the secondary peaks is not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent). Ignore any peak with an area less

than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Water (2.3.43). Not more than 4.0 per cent, determined on 0.75 g.

Isopropyl alcohol. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.5 g of the substance under examination in sufficient *water* to produce 10 ml.

Reference solution (a). A solution containing 5.0 per cent w/v of the substance under examination and 0.5 per cent v/v of *propan-1-ol* (internal standard).

Reference solution (b). A solution containing 0.5 per cent v/v each of *propan-2-ol* and the internal standard.

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with porous polymer beads (125 to 150 mm) (Such as Porapak Q),
- temperature:
  column 150°,
  inlet port at 180° and detector at 200°,
- flow rate. 40 ml per minute of the carrier gas.

The column temperature may be varied so that the resolution, R, between propan-1-ol and propanol-2 ol is not less than 2.0, the tailing factor. T, for the propan-2-ol is not less than 2.0 the tailing factor T, for the propan-2-ol peak is not more than 1.5 and the relative standard deviation of the ratio of the area due to the peak of propanol-2-ol to that due to propan-1-ol for five replicate injections of reference solution (b) is not more than 2.0 per cent.

Calculate the content of isopropyl alcohol.

Assay. Weigh accurately about 0.1 g and dissolve in sufficient *0.01 M sodium hydroxide* to produce 100.0 ml. Dilute 10.0 ml to 100.0 ml with *0.01 M sodium hydroxide* and dilute 5.0 ml to 50.0 ml with *0.01 M sodium hydroxide*. Measure the absorbance of the resulting solution at the maximum at about 308 nm (2.4.7). Calculate the content of $C_{19}H_{15}NaO_4$ taking 431 as the specific absorbance at 308 nm.

Storage. Store protected from light and moisture.

Warfarin Tablets

Warfarin Sodium Tablets

Warfarin Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of warfarin sodium, $C_{19}H_{15}NaO_4$.

Usual strengths. 1 mg; 3 mg; 5 mg; 10 mg.

Identification

A. Extract a quantity of the powdered tablets containing 0.1 g of Warfarin Sodium with 30 ml of *water*, add 0.1 ml of *2 M hydrochloric acid*, filter, wash the precipitate with water and dry. Warm the residue gently with 3 ml of *ethanol (95 per cent)*, filter and add the filtrate to 25 ml of water containing 0.1 ml of *2 M hydrochloric acid*. Filter, wash the precipitate with water and dry it at 105°.

On the residue, determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *warfarin sodium RS* or with the reference spectrum of warfarin sodium.

B. The final residue obtained in test A melts at about 159° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *chloroform*, 50 volumes of *cyclohexane* and 20 volumes of *glacial acetic acid*.

Test solution. Shake a quantity of the powdered tablets containing 40 mg of Warfarin Sodium with 30 ml of *water* for 15 minutes, add 0.1 ml of *hydrochloric acid* and extract with three quantities, each of 10 ml, of *chloroform*, drying each extract with *anhydrous sodium sulphate*. Evaporate the combined extracts at a temperature not exceeding 40° and dissolve the residue in 2 ml of *acetone*.

Reference solution (a). Dilute 1 volume of the test solution to 200 volumes with *acetone*.

Reference solution (b). A 0.002 per cent w/v solution of (E)-*4-phenylbut-3-en-2-one* in *acetone*.

Reference solution (c). A 0.02 per cent w/v solution of *4-hydroxycoumarin* in *acetone*.

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine immediately in visible light noting the position of any coloured spots and then examine in ultraviolet light at 254 nm, ignoring any spot that was noted in visible light. Any spots corresponding to *(E)*-4-phenylbut-3-en-2-one and 4-hydroxycoumarin in the chromatogram obtained with the test solution are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively and any other secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

Test solution. Shake one tablet with 10 ml of *0.01 M sodium hydroxide* for 15 minutes, add 10 ml of a 2 per cent v/v solution of *glacial acetic acid* in *acetonitrile*, centrifuge for 10 minutes and use the clear supernatant liquid.

Chromatographic system
- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 55 volumes of *acetonitrile*, 45 volumes of *water* and 1 volume of *glacial acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 283 nm,
- injection volume. 20 µl.

Determine the content of $C_{19}H_{15}NaO_4$ in the tablet.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of a 0.68 per cent w/v solution of *potassium dihydrogen phosphate* with the pH adjusted to 6.8 by the addition of *1 M sodium hydroxide,*
Speed and time. 100 rpm and 45 minutes.

For tablets containing 2 mg or less of warfarin sodium, use three tablets for each test; for tablets containing more than 2 mg of warfarin sodium, use a single tablet for each test.

Withdraw a suitable volume of the medium and filter. Measure the absorbance of a layer of suitable thickness of the filtrate, suitably diluted if necessary, at the maxima at about 307 nm and 360 nm (2.4.7), and calculate the difference between the two absorbances (DA). Calculate the total content of warfarin sodium, $C_{19}H_{15}NaO_4$, in the medium taking 428 as the specific absorbance value of DA.

D. Not less than 70 per cent of the stated amount of $C_{19}H_{15}NaO_4$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 20 mg of Warfarin Sodium and shake with 250.0 ml of *0.01 M sodium hydroxide* for 15 minutes and filter. To 20.0 ml of the filtrate add 0.15 ml of *hydrochloric acid* and extract with three quantities, each of 15 ml, of *chloroform*. Extract the combined chloroform layers with three quantities, each of 20 ml, of *0.01 M sodium hydroxide.* Dilute the combined aqueous layers to 100.0 ml with *0.01 M sodium hydroxide*, filter and measure the absorbance of the resulting solution at the maximum at about 307 nm (2.4.7). Calculate the content of $C_{19}H_{15}NaO_4$ taking 431 as the specific absorbance at 307 nm.

Storage. Store protected from light.

Purified Water

H_2O Mol. Wt. 18.0

Purified Water is prepared by distillation, by means of ion exchange or by any other appropriate means from suitable potable water that complies with all relevant statutory regulations.

During production and subsequent storage, it is recommended that adequate measures are taken to ensure that the microbial quality is controlled and monitored. Appropriate alert and action limits are set so as to detect adverse trends. Under controlled conditions, an appropriate action limit is a total viable count (2.2.9) of 100 micro-organisms per ml, determined by membrane filtration. In addition, the test for oxidisable substances (given below) is carried out. The adequacy of these measures may be determined by carrying out the test for conductivity (2.4.9) off-line or on-line.

Category. Pharmaceutical aid (solvent).

Description. A clear, colourless and odourless liquid.

Tests

Acidity or alkalinity. To 10 ml, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution*; the resulting solution is not red. To 10 ml add 0.1 ml of *bromothymol blue solution*; the resulting solution is not blue.

Ammonium. To 20 ml add 1 ml of *alkaline potassium mercuri-iodide solution,* and allow to stand for 5 minutes. When viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 ml of *alkaline potassium mercuri-iodide solution* to a mixture of 4.0 ml of *ammonium standard solution (1 ppm NH_4)* and 16.0 ml of *ammonia-free water* (0.2 ppm).

Calcium and magnesium. To 100 ml add 2 ml of *ammonia buffer pH 10.0*, 50 mg of *mordant black II mixture* and 0.5 ml of *0.01 M disodium edetate*; a pure blue colour is produced.

Heavy metals (2.3.13). Evaporate 150 ml to 15 ml on a water-bath; 12 ml of the solution complies with the limit test for heavy metals, Method D (0.1 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Chlorides. To 10 ml add 1 ml of *2 M nitric acid* and 0.2 ml of *0.1 M silver nitrate*; the appearance of the solution does not change for at least 15 minutes.

Nitrates. To 5 ml in a test-tube immersed in ice add 0.4 ml of a 10 per cent w/v solution of *potassium chloride*, 0.1 ml of *diphenylamine solution* and, dropwise with shaking, 5 ml of *sulphuric acid*. Transfer the tube to a water-bath at 50° and allow to stand for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 ml of

nitrate-free water and 0.5 ml of *nitrate standard solution (2 ppm NO_3)* (0.2 ppm).

Sulphates. To 10 ml add 0.1 ml of *2 M hydrochloric acid* and 0.1 ml of *barium chloride solution*. The appearance of the solution does not change for at least 1 hour.

Oxidisable substances. To 100 ml add 10 ml of *1 M sulphuric acid* and 0.1 ml of *0.02 M potassium permanganate* and boil for 5 minutes; the solution remains faintly pink.

Residue on evaporation. Evaporate 100 ml to dryness on a water-bath and dry to constant weight at 105°. The residue weighs not more than 1 mg (0.001 per cent).

Purified Water intended for use in the manufacture of dialysis solutions and also without a further procedure for the removal of bacterial endotoxins complies with the following additional requirements.

Aluminium (2.3.8). Not more than 10 ppb, determined using the following solutions.

Test solution. To 400 ml of the water under examination, add 10 ml of *acetate buffer solution pH 6.0* and 100 ml of *distilled water*.

Reference solution. Mix 2 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer solution pH 6.0* and 98 ml of *distilled water*.

Blank solution. Mix 10 ml of *acetate buffer solution pH 6.0* and 100 ml of *distilled water*.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per ml.

Water for Injections

H_2O Mol. Wt. 18.0

Water for Injections is water intended for use in the preparations of medicines for parenteral administration when water is used as a vehicle (Water for Injections in bulk) and for dissolving or diluting substances or preparations for injectable preparations (Sterile Water for Injections).

Water for Injections in Bulk

Production

Water for Injections in bulk is obtained by distilling potable water or Purified Water from a neutral glass, quartz or suitable metal still fitted with an effective device for preventing the entrainment of droplets; the still must be suitably maintained to ensure the production of apyrogenic water. The first portion of the distillate is discarded and the remainder is collected and stored in conditions designed to prevent the growth of micro-organisms and to avoid any other contamination.

During production and subsequent storage, it is recommended that adequate measures are taken to ensure that the microbial quality is controlled and monitored. Appropriate action limit of total viable count (2.2.9) of 10 micro-organisms per ml, determined by membrane filtration using at least 200 ml of water for injection in bulk. Appropriate alert and action limits are set so as to detect adverse trends. The adequacy of these measures is determined by the following tests that may be done off-line or on-line.

Total organic carbon (2.4.30). Not more than 0.5 mg per litre.

Conductivity (2.4.9). Meets the requirements of the test.

Description. A clear, colourless and odourless liquid.

Tests

Acidity or alkalinity. To 10 ml, freshly boiled and cooled in a borosilicate glass flask, add 0.05 ml of *methyl red solution*; the resulting solution is not red. To 10 ml add 0.1 ml of *bromothymol blue solution*; the resulting solution is not blue.

Ammonium. To 20 ml, add 1 ml of *alkaline potassium mercuri-iodide solution* and allow to stand for 5 minutes. When viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 ml of *alkaline potassium mercuri-iodide* solution to a mixture of 4.0 ml of *ammonium standard solution (1 ppm NH_4)* and 16.0 ml of *ammonia-free water* (0.2 ppm).

Calcium and magnesium. To 100 ml, add 2 ml of *ammonia buffer pH 10.0*, 50 mg of mordant black II mixture and 0.5 ml of *0.01 M disodium edetate*; a pure blue colour is produced.

Heavy metals (2.3.13). Evaporate 150 ml to 15 ml on a water-bath. 12 ml of the solution complies with the limit test for heavy metals, Method D (0.1 ppm). Use *lead standard solution* (1 ppm Pb) to prepare the standard.

Chlorides. To 10 ml, add 1 ml of *2 M nitric acid* and 0.2 ml of *0.1 M silver nitrate*; the appearance of the solution does not change for at least 15 minutes.

Nitrates. To 5 ml in a test-tube immersed in ice add 0.4 ml of a 10 per cent w/v solution of *potassium chloride*, 0.1 ml of *diphenylamine solution* and, dropwise with shaking, 5 ml of *sulphuric acid*. Transfer the tube to a water-bath at 50° and allow to stand for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 ml of *nitrate-free water* and 0.5 ml of *nitrate standard solution (2 ppm NO_3)* (0.2 ppm).

Sulphates. To 10 ml, add 0.1 ml of *2 M hydrochloric acid* and 0.1 ml of *barium chloride solution*. The appearance of the solution does not change for at least 1 hour.

Aluminium (2.3.8) *For water for injections intended for use in the manufacture of dialysis solutions.*

Not more than 10 ppb, determined using the following solutions.

Test solution. To 400 ml of the water under examination add 10 ml of *acetate buffer solution pH 6.0* and 100 ml of *distilled water.*

Reference solution. Mix 2 ml of *aluminium standard solution (2 ppm Al)*, 10 ml of *acetate buffer solution pH 6.0* and 98 ml of *distilled water.*

Blank solution. Mix 10 ml of *acetate buffer solution pH 6.0* and 100 ml of *distilled water.*

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per ml.

Storage. Store in containers designed to prevent the growth of micro-organisms.

Labelling. The label on the container in which the bulk has been distributed states that the contents have not been sterilised.

Sterile Water for Injections

Sterile Water for Injections is Water for Injections in bulk that has been distributed in suitable containers of glass or any other material, sealed and sterilised by heat under conditions that ensure that the water complies with the test for bacterial endotoxins. It is free from any added substances. Each container contains a sufficient quantity of Water for Injections to permit the withdrawal of the nominal volume.

Description. A clear, colourless liquid; odourless.

Tests

Appearance of solution. When examined in suitable conditions of visibility, it is clear (2.4.1) colourless (2.4.1) and practically free from suspended particles.

Acidity or alkalinity. To 20 ml, add 0.05 ml of *phenol red solution.* If the solution is yellow, it becomes red on the addition of 0.1 ml of *0.01 M sodium hydroxide*; if red, it becomes yellow on the addition of 0.15 ml of *0.01 M hydrochloric acid.*

Ammonium. To 20 ml, add 1 ml of *alkaline potassium mercuri-iodide solution* and allow to stand for 5 minutes. When viewed vertically the solution is not more intensely coloured than a solution prepared at the same time by adding 1 ml of *alkaline potassium mercuri-iodide* solution to a mixture of 4.0 ml of *ammonium standard solution (1 ppm NH_4)* and 16.0 ml of *ammonia-free water* (0.2 ppm).

Calcium and magnesium. To 100 ml, add 2 ml of *ammonia buffer pH 10.0*, 50 mg of *mordant black II mixture* and 0.5 ml of *0.01 M disodium edetate*; a pure blue colour is produced.

Heavy metals (2.3.13). Evaporate 150 ml to 15 ml on a water-bath. 12 ml of the solution complies with the limit test for heavy metals, Method D (0.1 ppm). Use *lead standard solution (1 ppm Pb)* to prepare the standard.

Chlorides. To 10 ml, add 1 ml of *2 M nitric acid* and 0.2 ml of *0.1 M silver nitrate*; the appearance of the solution does not change for at least 15 minutes.

For containers with a nominal volume of 100 ml or less, 15 ml complies with the limit test for chlorides (2.3.12) (0.5 ppm), using a standard solution prepared by mixing 1.5 ml of *chloride standard solution (5 ppm Cl)* and 13.5 ml of *water.*

Nitrates. To 5 ml in a test-tube immersed in ice add 0.4 ml of a 10 per cent w/v solution of *potassium chloride*, 0.1 ml of *diphenylamine solution* and, dropwise with shaking, 5 ml of *sulphuric acid.* Transfer the tube to a water-bath at 50° and allow to stand for 15 minutes. Any blue colour in the solution is not more intense than that in a solution prepared at the same time and in the same manner using a mixture of 4.5 ml of *nitrate-free water* and 0.5 ml of *nitrate standard solution (2 ppm NO_3)* (0.2 ppm).

Sulphates. To 10 ml, add 0.1 ml of *2 M hydrochloric acid* and 0.1 ml of *barium chloride solution.* The appearance of the solution does not change for at least 1 hour.

Oxidisable substances. Boil 100 ml with 10 ml of *1 M sulphuric acid*, add 0.4 ml of *0.02 M potassium permanganate* (for Sterile Water for Injection in containers with fill volume of less than 50 ml) or 0.2 ml of *0.02 M potassium permanganate* (for Sterile Water for Injection in containers with fill volume of 50 ml or more) and boil for 5 minutes. If a precipitate forms, cool in an ice-bath to room temperature and filter through a sintered glass filter (porosity No.3). The pink colour of the solution does not disappear completely.

Residue on evaporation. Evaporate 100 ml to dryness on a water-bath and dry the residue to constant weight at 105°. For containers with a nominal volume of 10 ml or less, the residue weighs not more than 4 mg (0.004 per cent) and for containers with a nominal volume greater than 10 ml, the residue weighs not more than 3 mg (0.003 per cent).

Particulate contamination (2.5.9). Complies with the requirements of Method 1 or Method 2.

Bacterial endotoxins (2.2.3). Not more than 0.25 Endotoxin Unit per ml.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store in single dose containers of not larger than one litre size.

Wool Fat

Anhydrous Lanolin

Wool Fat is purified, anhydrous, waxy material obtained from the wool of sheep. It may contain Butylated Hydroxytoluene as an antioxidant.

Category. Pharmaceutical aid (absorbent ointment base).

Description. A pale yellow, unctuous substance; odour, characteristic.

Identification

A. To a solution of 0.5 g in 5 ml of *chloroform* add 1 ml of *acetic anhydride* and 0.1 ml of *sulphuric acid*; a green colour develops.

B. To a solution of 50 mg in 5 ml of *chloroform* add 5 ml of *sulphuric acid* and shake; a red colour is produced and a strong fluorescence appears in the lower layer.

Tests

Melting range (2.4.21). 34° to 44°, determined by Method IV. To fill the metal cup, melt the substance under examination on a water-bath, cool to about 50°, pour into the cup and allow to stand at 15° to 20° for 24 hours.

Acid value (2.3.23). Not more than 1.0, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Peroxide value (2.3.35). Not more than 20.

Saponification value. (2.3.37). 90 to 105. Heat for 4 hours.

Water-absorption capacity. Weigh 10.0 g into a mortar. Add *water* in quantities of 0.2 to 0.5 ml from a burette and stir vigorously, incorporating all the water before proceeding to the next addition. The end-point is reached when visible droplets remain that cannot be incorporated; not less than 20 ml of water is absorbed.

Water-soluble acidic or alkaline substances. Shake vigorously 5.0 g, previously melted on a water-bath, for 2 minutes with 75 ml of *water* previously heated to 90° to 95°. Allow to cool and filter through filter paper previously washed with *water*. To 60 ml of the filtrate, which may not be clear, add 0.25 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.02 M hydrochloric acid* or 0.15 ml of *0.02 M sodium hydroxide* is required to change the colour of the solution.

Water-soluble oxidisable substances. To 10 ml of the filtrate obtained in the test for Water-soluble acidic or alkaline substances add 1 ml of *1 M sulphuric acid* and 0.1 ml of *0.02 M potassium permanganate*; the solution is not completely decolorised within 10 minutes.

Ammonia. To 10 ml of the filtrate obtained in the test for Water-soluble acidic or alkaline substances, add 1 ml of *1 M sodium hydroxide* and boil; the vapours do not turn red *litmus paper* blue.

Chlorides. Boil 1.0 g with 20 ml of *ethanol (90 per cent)* under a reflux condenser for 5 minutes, cool, add 40 ml of *water* and 0.5 ml of *nitric acid* and filter. To the filtrate add 0.15 ml of a 1 per cent w/v solution of *silver nitrate* in *ethanol (90 per cent)*. After 5 minutes, protected from light; any opalescence produced is not more intense than that obtained by adding 0.15 ml of a 1 per cent w/v solution of *silver nitrate* in *ethanol (90 per cent)* to a mixture of 0.2 ml of *0.02 M hydrochloric acid*, 20 ml of *ethanol (90 per cent)*, 40 ml of *water* and 0.5 ml of *nitric acid (150 ppm)*.

Paraffins. Prepare an alumina column 23 cm x 2 cm by adding a slurry of *anhydrous aluminium oxide* and *light petroleum (40° to 60°)* to a glass tube fitted with a tap and containing the light petroleum; the tap and absorbent cotton plugs should be free from grease. Allow to settle and reduce the depth of the solvent above the column to about 4 cm. Dissolve 3.0 g of the substance under examination in 50 ml of warm *light petroleum (40° to 60°)*, cool, pass the solution through the column at a rate of 3 ml per minute and wash with 250 ml of the light petroleum. Distil the combined eluate and washings to low bulk, evaporate to dryness on a water-bath and heat the residue at 105° for periods of 10 minutes until the difference between two successive weighings is not greater than 1 mg; the residue weighs not more than 30 mg.

Butylated hydroxytoluene *(if present)*. Not more than 200 ppm, determined by gas chromatography (2.4.13).

Test solution (a). A 10 per cent w/v solution of the substance under examination in *carbon disulphide*.

Test solution (b). A solution containing 10 per cent w/v of the substance under examination and 0.002 per cent w/v of *methyl n-decanoate* (internal standard) in *carbon disulphide*.

Reference solution. A solution containing 0.002 per cent w/v each of *butylated hydroxytoluene* and the internal standard.

Chromatographic system

- a glass column 1.5 m × 4 mm, packed with silanised diatomaceous support (80 to 100 mesh) (such as Diatomite) impregnated with 10 per cent w/w of silicone gum rubber (methyl) (such as SE-30),
- temperature:
  column 150°,
  inlet port at 180° and detector at 300°,
- flow rate. 40 ml per minute of the carrier gas.

Calculate the content of butylated hydroxytoluene in the substance under examination from the heights or areas of the peaks due to butylated hydroxytoluene and the internal standard in the chromatograms obtained with test solution (b) and the reference solution.

Sulphated ash (2.3.18). Not more than 0.15 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105° for 1 hour.

Storage. Store protected from moisture.

Labelling. The label states the proportion of any butylated hydroxytoluene present.

Hydrous Wool Fat

Hydrous Wool Fat is a mixture of 75 per cent w/w of Wool Fat and 25 per cent w/w of Purified Water.

Category. Pharmaceutical aid (water-in-oil emulsion ointment base).

Description. A pale yellow, unctuous substance; odour, faint and characteristic. On heating, it separates at first into two layers; with continued heating with stirring, water is driven off and the residue which is transparent while warm, cools to form a yellowish, tenacious, soft mass.

Identification

A. To a solution of 0.5 g in 5 ml of *chloroform* add 1 ml of *acetic anhydride* and 0.1 ml of *sulphuric acid*; a green colour develops.

B. To a solution of 50 mg in 5 ml of *chloroform* add 5 ml of *sulphuric acid* and shake; a red colour is produced and a strong fluorescence appears in the lower layer.

Tests

Melting range (2.4.21). 34° to 44°, determined by Method IV. To fill the metal cup, melt the substance under examination on a water-bath, cool to about 50°, pour into the cup and allow to stand at 15° to 20° for 24 hours.

Acid value (2.3.23). Not more than 1.0, determined on 5.0 g dissolved in 25 ml of the prescribed mixture of solvents.

Paraffins. Prepare an alumina column 23 cm x 2 cm by adding a slurry of *anhydrous aluminium oxide* and *light petroleum (40° to 60°)* to a glass tube fitted with a tap and containing the light petroleum; the tap and absorbent cotton plugs should be free from grease. Allow to settle and reduce the depth of the solvent above the column to about 4 cm. Dissolve 3 g of the substance under examination in 50 ml of warm *light petroleum (40° to 60°)*, cool, pass the solution through the column at a rate of 3 ml per minute and wash with 250 ml of the light petroleum. Distil the combined eluate and washings to low bulk, evaporate to dryness on a water-bath and heat the residue at 105° for periods of 10 minutes until the difference between two successive weighings is not greater than 1 mg; the residue weighs not more than 30 mg.

Peroxide value (2.3.35). Not more than 15.

Saponification value (2.3.37). 67 to 79. Heat for 4 hours.

Water-absorption capacity. Weigh 10.0 g of the residue obtained in the test for Wool fat content into a mortar. Add *water* in quantities of 0.2 to 0.5 ml from a burette and stir vigorously, incorporating all the water before proceeding to the next addition. The end-point is reached when visible droplets remain that cannot be incorporated; not less than 20 ml of water is absorbed.

Water-soluble acidic or alkaline substances. Shake vigorously 6.7 g, previously melted on a water-bath, for 2 minutes with 75 ml of *water* previously heated to 90° to 95°. Allow to cool and filter through filter paper previously washed with *water*. To 60 ml of the filtrate, which may not be clear, add 0.25 ml of *bromothymol blue solution*. Not more than 0.2 ml of *0.02 M hydrochloric acid* or 0.15 ml of *0.02 M sodium hydroxide* is required to change the colour of the solution.

Water-soluble oxidisable substances. 10 ml of the filtrate obtained in the test for Water-soluble acidic or alkaline substances, add 1 ml of *1 M sulphuric acid* and 0.1 ml of *0.02 M potassium permanganate*; the solution is not completely decolorised within 10 minutes.

Ammonia. To 10 ml of the filtrate obtained in the test for Water-soluble acidic or alkaline substances add 1 ml of *1 M sodium hydroxide* and boil; the vapours do not turn red *litmus paper* blue.

Chlorides. Boil 1.0 g with 20 ml of *ethanol (90 per cent)* under a reflux condenser for 5 minutes, cool, add 40 ml of *water* and 0.5 ml of *nitric acid* and filter. To the filtrate, add 0.15 ml of 1.0 per cent w/v solution of *silver nitrate* in *ethanol (90 per cent)*. After 5 minutes, protected from light, any opalescence produced is not more intense than that obtained by adding 0.15 ml of a 1 per cent w/v solution of *silver nitrate* in *ethanol (90 per cent)* to a mixture of 0.2 ml of *0.02 M hydrochloric acid*, 20 ml of *ethanol (90 per cent)*, 40 ml of *water* and 0.5 ml of *nitric acid (150 ppm)*.

Wool fat content. 72.5 to 77.5 per cent.

Weigh accurately about 30 g in a tared porcelain dish containing a glass rod, heat on a water-bath with continuous stirring to constant weight and weigh the residue.

Storage. Store protected from light and moisture.

X

Xanthan Gum

Xanthan Gum is high-molecular-mass anionic polysaccharide produced by fermentation of carbohydrates with *Xanthomonas campestris*. It consists of a principal chain of b(1→4)-linked D-glucose units with trisaccharide side chains, on alternating anhydroglucose units, consisting of 1 glucuronic acid unit included between 2 mannose units. Most of the terminal units contain a pyruvate moiety and the mannose unit adjacent to the principal chain may be acetylated at C-6.

Xanthan Gum has a relative molecular mass of approximately 1×10^6. It exists as the sodium, potassium or calcium salt.

Xanthan Gum contains not less than 1.5 per cent of pyruvoyl groups, ($C_3H_3O_2$; Mol. Wt. 71.1), calculate on the dried basis.

Category. Pharmaceutical aid (Excipient).

Description. A white or yellowish-white, free-flowing powder.

Identification

A. Suspend 1 g in 15 ml of *0.1 M hydrochloric acid* in a flask, close the flask with a fermentation bulb containing *barium hydroxide solution* and heat carefully for 5 minutes. The barium hydroxide solution shows a white turbidity.

B. To 300 ml of *water*, previously heated to 80° and stirred rapidly with a mechanical stirrer in a 400 ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of *carob bean gum* and 1.5 g of the substance under examination. Stir until the mixture forms a solution, and then continue stirring for 30 minutes or longer. Do not allow the water temperature to drop below 60° during stirring. Discontinue stirring and allow the mixture to stand for at least 2 hours. A firm rubbery gel forms after the temperature drops below 40° but no such gel forms in a 1 per cent control solution of the sample prepared in the same manner but omitting the carob bean gum.

Tests

pH (2.4.24). 6.0 to 8.0 determined in 1 per cent w/v solution.

Viscosity (2.4.28). Not less than 600 mPas.

Add 3.0 g within 45-90 seconds into 250 ml of a 1.2 per cent w/v solution of *potassium chloride* in a 500 ml beaker stirring with a low-pitch propeller-type stirrer rotating at 800 rpm. When adding the substance take care that agglomerates are destroyed. Add an additional quantity of 44 ml of *water*, to rinse any adhering residue from the walls of the beaker. Stir the preparation at 800 rpm for 2 hours whilst maintaining the temperature at 24 ± 1°. Determine the viscosity within 15 min at 24 ± 1° using a rotating viscosimeter set at 60 rpm and equipped with a rotating spindle 12.7 mm in diameter and 1.6 mm high which is attached to a shaft 3.2 mm in diameter. The distance from the top of the cylinder to the lower tip of the shaft being 25.4 mm, and the immersion depth being 50.0 mm.

2-Propanol. Not more than 750 ppm

Determine by gas chromatography (2.4.13).

Internal standard solution. Dilute 0.50 g of *2-methyl-2-propanol* to 500 ml with *water*.

Test solution. To 200 ml of *water* in a 1000-ml round bottomed flask, add 5.0 g of the substance under examination and 1 ml of a 1.0 per cent w/v emulsion of *dimeticone* in *liquid paraffin*, stopper the flask and shake for 1 hour. Distil about 90.0 ml, mix the distillate with 4.0 ml of the internal standard solution and dilute to 100.0 ml with *water*.

Reference solution. Dilute a suitable quantity of *2-propanol*, accurately weighed, with water to obtain a solution having a known concentration of 2-propanol of about 1 mg per ml. To 4.0 ml of this solution add 4.0 ml of the internal standard solution and dilute to 100.0 ml with *water*.

Chromatographic system
- a glass or stainless steel column 1.8 m x 4 mm, packed with styrene-divinylbenzene copolymer (149-177 µm),
- temperature:
  column. 165°,
  injection port and detector 200°,
- a flame ionisation detector,
- flow rate. 30 ml per minute of nitrogen as carrier gas.

Inject 5 µl of the reference solution. The test is not valid unless the relative retention times with reference to 2-propanol for 2-methyl-2-propanol is about 1.5.

Inject 5 µl of the test solution and the reference solution.

Other polysaccharides. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel*.

Mobile phase. A mixture of 10 volumes of 1.6 per cent w/v solution of *sodium dihydrogen phosphate*, 40 volumes of *butanol* and 50 volumes of *acetone*.

Test solution. To 10 mg of the substance under examination in a thick-walled centrifuge test tube add 2 ml of a 23 per cent w/v solution of *trifluoroacetic acid*, shake vigorously to dissolve the forming gel, stopper the test tube, and heat the mixture at 120° for 1 hour. Centrifuge the hydrolysate, transfer the clear supernatant liquid carefully into a 50 ml flask, add 10 ml of *water* and evaporate the solution to dryness under reduced pressure. Take up the residue thus obtained in 10 ml of *water* and evaporate to dryness under reduced pressure. Wash 3 times with 20 ml of *methanol* and evaporate under reduced pressure. To the resulting clear film which has no odour of acetic acid, add 0.1 ml of *water* and 1 ml of *methanol*. Centrifuge to separate the amorphous precipitate. Dilute the supernatant liquid, if necessary, to 1 ml with *methanol*.

Reference solution. Dissolve 10 mg of *glucose* and 10 mg of *mannose* in 2 ml of *water* and dilute to 10 ml with *methanol*.

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 15 cm. Dry the plate in air and spray with 2 per cent w/v solution of *diphenylamine* in *methanol* to which added 0.5 ml of *aniline* and 2.5 ml of *orthophosphoric acid*. Heat the plate at 120° for 5 minutes and examine in day light. The principal spot in the chromatogram obtained with the test solution shows 2 spots corresponding to the spots due to glucose and mannose in the chromatogram obtained with the reference solution. In addition, 1 weak reddish and 2 faint bluish-grey bands may be visible just above the starting line. 1 or 2 bluish-grey bands may also be seen in the upper quarter of the chromatogram. No other bands are visible.

The test is not valid unless the chromatogram obtained with the reference solution shows two clearly separated greyish-brown spots due to glucose and mannose in the middle third.

Total ash (2.3.19). Not less than 6.5 and not more than 16.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105° for 2.5 hours.

Microbial contamination (2.2.9). Total microbial count is not more than 10^3 bacteria and 10^2 fungi per gram, determined by plate count. 1 g is free from *Escherichia coli.*

Assay.

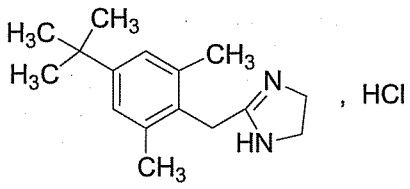
Test solution. Dissolve a quantity of the substance under examination containing 120 mg of the dried substance in 20 ml of *water.*

Reference solution. Dissolve 45 mg of *pyruvic acid* in 500 ml of *water.*

Place 10.0 ml of the test solution in a 50 ml round-bottomed flask, add 20.0 ml of *0.1 M hydrochloric acid* and weigh. Boil on a water-bath under a reflux condenser for 3 hours. Weigh and adjust to the initial mass with *water.* In a separating funnel mix 2.0 ml of the solution with 1.0 ml of *dinitrophenylhydrazine-hydrochloric solution.* Allow to stand for 5 minutes and add 5.0 ml of *ethyl acetate.* Shake and allow the solids to settle. Collect the upper layer and shake with three quantities, each of 5.0 ml, of *sodium carbonate solution.* Combine the aqueous layers and dilute to 50.0 ml with *sodium carbonate solution,* mix. Treat 10.0 ml of the reference solution at the same time and in the same manner as for the test solution.

Immediately measure the absorbance of the two solutions at the maximum at about 375 nm (2.4.7), using *sodium carbonate solution* as the compensation liquid. The absorbance of the test solution is not less than that of the reference solution, which corresponds to a content of pyruvic acid of not less than 1.5 per cent.

Xylometazoline Hydrochloride



$C_{16}H_{24}N_2,HCl$ Mol. Wt. 280.8

Xylometazoline Hydrochloride is 2-(4-*tert*-butyl-2,6-dimethylbenzyl)-2-imidazoline hydrochloride.

Xylometazoline Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{16}H_{24}N_2$, HCl, calculated on the dried basis.

Category. Sympathomimetic amine.

Description. A white or almost white, crystalline powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *xylometazoline hydrochloride RS* or with the reference spectrum of xylometazoline hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.05 per cent w/v solution in *0.1 M hydrochloric acid* shows an absorption maximum at about 265 nm and a minimum at about 257 nm with two inflections at about 270 nm and 275 nm; absorbance at about 265 nm, about 0.5.

C. To 1 ml of a 0.05 per cent w/v solution, add 0.2 ml of a 5 per cent w/v solution of *sodium nitroprusside* and 0.1 ml of *5 M sodium hydroxide*, allow to stand for 10 minutes and add 2 ml of *sodium bicarbonate solution*; a violet colour is produced.

D. Gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 5.0 to 6.6, determined in a 5.0 per cent w/v solution.

N-(2-Aminoethyl)-4-tert-butyl-2, 6-xylylacetamide. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 200 volumes of *methanol* and 3 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol.*

Reference solution. A 0.01 per cent w/v solution of *N-(2-aminoethyl)-4-tert-butyl-2,6-xylyl- acetamide RS in methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.3 g of *ninhydrin* in a mixture of 100 ml of *1-butanol* and 3 ml of *glacial acetic acid*. Heat at 100° for 10 minutes, allow to cool, and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to *N-(2-aminoethyl)-4-tert-butyl-2,6-xylyl-acetamide* in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of *water*. Allow to stand for 1 hour before injection.

Reference solution (a). Dilute 5.0 ml of the test solution to 100.0 ml with *water*. Dilute 2.0 ml of this solution to 100.0 ml with *water*.

Reference solution (b). Dissolve 5 mg each of *N-(2-aminoethyl)-2-[4-(1,1-dimethylethyl)-2,6- dimethylphenyl] acetamide RS (xylometazoline impurity A RS)* and the substance under examination in 50.0 of *water*. Dilute 10.0 ml of this solution to 50.0 ml with *water*.

Reference solution (c). Dilute 5.0 ml of reference solution (b) to 50.0 ml with *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a 0.14 per cent w/v solution of *potassium dihydrogen phosphate* adjusted to pH 3.0 with *orthophosphoric acid*,
  B. *acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 10 µl.

Time (min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-5	70	30
5-20	70 → 15	30 → 85
20-35	15	85
35-37	15 → 70	85 → 30
37-47	70	30

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to xylometazoline and xylometazoline impurity A is not less than 2.5. The relative retention time with reference to xylometazoline for xylometazoline impurity A is about 0.79.

Inject the test solution, reference solution (a) and (c). In the chromatogram obtained with the test solution the area of the peak due to xylometazoline impurity A is not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent). The sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent). Ignore any peak with an area less than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Iron (2.4.14). Moisten the residue obtained in the test for Sulphated ash with 5 ml of *hydrochloric acid*, evaporate to dryness and dissolve in sufficient *water* to produce 50 ml. 10 ml of the resulting solution complies with the limit test for iron (50 ppm).

Sulphates (2.3.17). 0. 75 g complies with the limit test for sulphates (200 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 50 ml of *anhydrous glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02808 g of $C_{16}H_{24}N_2$, HCl.

Storage. Store protected from light and moisture.

Xylometazoline Nasal Drops

Xylometazoline Hydrochloride Nasal Drops

Xylometazoline Nasal Drops are a solution of Xylometazoline Hydrochloride in Purified Water.

Xylometazoline Nasal Drops contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of xylometazoline hydrochloride, $C_{16}H_{24}N_2$, HCl.

Usual strengths. 0.05 per cent w/v; 0.1 per cent w/v.

Identification

A. To a volume containing 50 mg of Xylometazoline Hydrochloride add 5 ml of *1 M sodium hydroxide*, extract with 10 ml of *dichloromethane*, evaporate to dryness and dissolve the residue in 0.5 ml of *dichloromethane*.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *xylometazoline hydrochloride RS* treated in the same manner or with the reference spectrum of xylometazoline.

B. To a volume containing 0.5 mg of Xylometazoline Hydrochloride add 0.2 ml of a 5 per cent w/v solution of *sodium nitroprusside* and 0.1 ml of *5 M sodium hydroxide*, allow to stand for 10 minutes and add 1 ml of *sodium bicarbonate solution*; a violet colour is produced.

Tests

pH (2.4.24). 5.6 to 6.6.

N-(2-Aminoethyl)-4-tert-butyl-2,6-xylylacetamide. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 200 volumes of *methanol* and 3 volumes of *strong ammonia solution*.

Test solution. Add a volume containing 10 mg of Xylometazoline Hydrochloride to 30 ml of *water*, add 5 ml of *5 M sodium hydroxide*, mix, extract with three quantities, each of 20 ml, of *dichloromethane*, evaporate the combined extracts to dryness and dissolve the residue in 1 ml of *dichloromethane*.

Reference solution. A 0.03 per cent w/v solution of *N-(2-aminoethyl)-4-tert-butyl-2,6-xylylacetamide RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air, spray with a solution containing 0.3 g of *ninhydrin* in a mixture of 100 ml of *1-butanol* and 3 ml of *glacial acetic acid*. Heat at 100° for 10 minutes, allow to cool, and spray with *dilute potassium iodobismuthate solution*. Any spot corresponding to *N-(2-aminoethyl)-4-tert-butyl-2,6-xylyl- acetamide* in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution.

Other tests. Comply with the tests stated under Nasal Preparations.

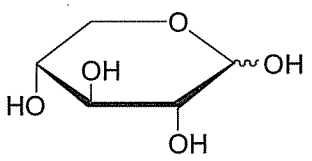
Assay. To a volume containing 10 mg of Xylometazoline Hydrochloride add 5 ml of *water*, 10 ml of *2 M hydrochloric acid* and 10 ml of *dichloromethane* and shake for 1 minute. Discard the dichloromethane layer and repeat the extraction with two further quantities, each of 10 ml, of *dichloromethane*. Add to the aqueous extract 10 ml of *5 M sodium hydroxide* and 10 ml of *dichloromethane*, shake for 1 minute and allow to separate. Filter the dichloromethane extract through glass wool and repeat the extraction with four further quantities, each of 10 ml, of dichloromethane. Evaporate the combined dichloromethane extracts almost to dryness on a water-bath, remove the final traces of solvent in a current of air and dissolve the residue in 10.0 ml of *0.01 M hydrochloric acid*. To 2.0 ml of this solution add 3 ml of *water*, 2.5 ml of *1 M sodium hydroxide* and 2.5 ml of a 5 per cent w/v solution of *sodium nitroprusside*, mix and allow to stand protected from light for 10 minutes. Add 10 ml of a freshly prepared 8.3 per cent w/v solution of *sodium bicarbonate*, dilute to 100.0 ml with *water*, allow to stand protected from light for 10 minutes and measure the absorbance of the resulting solution at the maximum at about 560 nm (2.4.7), using as blank a solution prepared by treating 5 ml of *water* and 2.5 ml of *1 M sodium hydroxide* in the same manner beginning at the words "and 2.5 ml of a 5 per cent w/v solution of *sodium nitroprusside*,.....".

Calculate the content of $C_{16}H_{24}N_2$, HCl from the absorbance obtained by repeating the operation using a 0.1 per cent w/v solution of *xylometazoline hydrochloride RS* in place of the nasal drops.

Storage. Store protected from light and moisture.

Xylose

D-Xylose; D-Xylopyranose



$C_5H_{10}O_5$ Mol. Wt. 150.1

Xylose contains not less than 98.0 per cent and not more than 102.0 per cent of $C_5H_{10}O_5$, calculated on the dried basis.

Category. Diagnostic aid in intestinal malfunction.

Description. Colourless needles or a white, crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a suspension of *silica gel G* in a 0.3 per cent w/v solution of *sodium acetate* to form a uniform layer 0.5 mm thick.

Mobile phase. A mixture of 70 volumes of *glacial acetic acid*, 60 volumes of *chloroform* and 10 volumes of *water*.

Test solution. Dissolve 0.5 g of the substance under examination in 10 ml of *water*.

Reference solution (a). A 5.0 per cent w/v solution of *xylose RS* in *water*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 2 µl of each solution. Develop the plate in a continuous elution tank for about 4 hours. Dry the plate in warm air, spray with a solution in *acetone* containing 1 per cent w/v solution of *diphenylamine*, 1 per cent v/v of *aniline* and 1 per cent v/v of *phosphoric acid* and heat for 10 minutes at 130°. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

B. When heated with *potassium cupri-tartrate solution* it produces a copious precipitate of cuprous oxide.

Tests

Appearance of solution. A 10.0 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and colourless (2.4.1).

Acidity. Dissolve 5.0 g in 50 ml of *carbon dioxide-free water*. Not more than 0.2 ml of *0.1 M sodium hydroxide* is required to neutralise the solution using *dilute phenolphthalein solution* as indicator.

Specific optical rotation (2.4.22). +18.5° to +19.5°, determined at 20° in a 10.0 per cent w/v solution containing 0.4 per cent v/v of *5 M ammonia*.

Arsenic (2.3.10). Dissolve 10.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Iron (2.3.14). A solution of 4.0 g in 25 ml of *water* complies with the limit test for iron (10 ppm).

Chlorides (2.3.12). Dissolve 3.0 g in 20 ml of *water*. 5 ml of the resulting solution complies with the limit test for chlorides (330 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 100° at a pressure not exceeding 0.7 kPa.

Assay. *Carry out the following procedure keeping strict control of time between steps.*

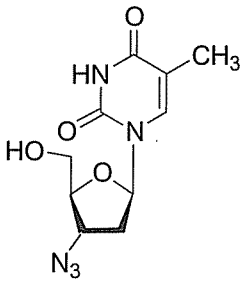
Weigh accurately about 1.0 g of the substance under examination, dissolve in a saturated solution of *benzoic acid* in a 100-ml volumetric flask and dilute to volume with the same solvent. Dilute 1.0 ml of this solution to 100.0 ml with the same solvent. To 1.0 ml of the resulting solution, in two different test-tubes, add 5 ml of *4-bromoaniline solution* into each tube and mix. Loosely stopper one tube, place in a water-bath maintained at 70° for 10 minutes, remove, cool rapidly to room temperature and mix. Keep the tube in the dark for 70 minutes and measure the absorbance at the maximum at about 520 nm (2.4.7), using the untreated solution in the second test tube as the blank. Simultaneously, carry out the operation using 1.0 ml of a 0.01 per cent w/v solution of *xylose RS* in a saturated solution of *benzoic acid* beginning at the words "add 5 ml of *4-bromoaniline solution*.....".

Calculate the content of $C_5H_{10}O_5$.

Storage. Store protected from moisture.

Z

Zidovudine



$C_{10}H_{13}N_5O_4$ Mol. Wt. 267.2

Zidovudine is 1-(3-azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione.

Zidovudine contains not less than 97.0 per cent and not more than 102.0 per cent of $C_{10}H_{13}N_5O_4$, calculated on the anhydrous basis.

Category. Antiretroviral.

Dose. 300 mg twice daily.

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zidovudine RS* or with the reference spectrum of zidovudine.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to zidovudine in the chromatogram obtained with reference solution (a).

C. Melting range (2.4.21). 122° to 125°.

Tests

Specific optical rotation (2.4.22). +60.5° to +63.0°, determined in a 1.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of *methanol*.

Reference solution. A solution containing 0.01 per cent w/v each of *zidovudine RS* and *triphenylmethanol* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise to about three-fourths of the height of the plate. Dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spots observed in the chromatogram obtained with the test solution correspond to those of the principal spots in the chromatogram obtained with the reference solution. No secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with the reference solution (0.5 per cent).

Spray the plate with a mixture of 0.5 g of *carbazole* in 95 ml of *ethanol (95 per cent)* and 5 ml of *sulphuric acid*, heat for 10 minutes at 120° and compare the intensities of any secondary spots observed in the chromatogram obtained with the test solution with those of the principal spots in the chromatogram obtained with the reference solution. No spot corresponding to triphenylmethanol (R_f value about 2.3 relative to the R_f of zidovudine) is more intense than the corresponding spot in the chromatogram obtained with the reference solution (0.5 per cent). No secondary spot in the chromatogram obtained with the test solution is more intense than the principal spot in the chromatogram obtained with the reference solution.

B. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.001 per cent w/v solution of *zidovudine RS* in *methanol*.

Reference solution (b). A solution containing 0.1 per cent w/v of *zidovudine RS* and 0.001 per cent w/v each of *zidovudine-related compound B RS* and *zidovudine- related compound C RS* in *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. *water*
  B. *methanol*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
15	35	65
30	35	65
35	5	95
40	5	95
45	90	10

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to zidovudine and zidovudine-related compound B is not less than 1.5 and the tailing factor for zidovudine is not more than 1.5.

Separately inject the test solution and reference solution (a). The area of the peak corresponding to zidovudine- related compound B is not greater than 1.0 per cent and of that to zidovudine-related compound C is not greater than 2.0 per cent.

The sum of the percentages of related substances by tests A and B is not greater than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.25 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 100 mg of the substance under examination, dissolve in a suitable quantity of *methanol* in a 50-ml volumetric flask and make up to volume with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a). Weigh accurately about 100 mg of *zidovudine RS*, dissolve in a suitable quantity of *methanol* in a 50-ml volumetric flask and make up to volume with the mobile phase (solution A). Dilute 5.0 ml of solution A to 50.0 ml with the mobile phase.

Reference solution (b). Transfer 2.0 ml of a 0.005 per cent w/v solution of *zidovudine-related compound B RS* in the mobile phase to a 50-ml volumetric flask, add 5.0 ml of solution A and make up to volume with the mobile phase.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 70 volumes of *water* and 30 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to zidovudine and zidovudine-related compound B is not less than 2.0.

Inject reference solution (a). The relative standard deviation for replicate injections is not more than 2.0 per cent.

Separately inject the test solution and reference solution (a) and measure the responses for the principal peak.

Calculate the content of $C_{10}H_{13}N_5O_4$.

Storage. Store protected from light.

Zidovudine Capsules

Zidovudine Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strength. 100 mg.

Identification

A.Transfer a quantity of the mixed contents of the capsules containing about 15 mg of Zidovudine to a 100-ml volumetric flask. Add about 80 ml of a mixture of 75 volumes of *methanol* and 25 volumes of *water*, shake for 10 minutes and dilute to volume with the same solvent mixture, mix and filter. Dilute 10 ml of the filtrate to 100 ml with the same solvent mixture.

When examined in the range 200 nm to 300 nm (2.4.7), the resulting solution shows an absorption maximum at about 265 nm.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to zidovudine in the chromatogram obtained with reference solution (c).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the mixed contents of 20 capsules containing about 100 mg of Zidovudine and transfer to a 100-ml volumetric flask. Add about 60 ml of the mobile phase, mix with the aid of ultrasound for 10 minutes, dilute to volume with the mobile phase, mix and filter. Dilute 5.0 ml of the filtrate to 50.0 ml with the mobile phase.

Reference solution (a). Weigh accurately about 100 mg of *zidovudine RS* and transfer to a 100-ml volumetric flask. Dissolve in about 60 ml of the mobile phase and dilute to volume with the mobile phase.

Reference solution (b). Weigh accurately about 20 mg of *thymine* and transfer to a 200-ml volumetric flask, dissolve and make up to volume with *methanol*.

Reference solution (c). Transfer 10.0 ml of the reference solution (a) and 2.0 ml of reference solution (b) to a 100-ml volumetric flask and make up to the volume with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol* and 80 volumes of *water,*
- flow rate. 1 ml per minute,

- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention times are about 0.2 for thymine and 1.0 for zidovudine, the resolution between zidovudine and thymine is not less than 5.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and reference solution (c) and measure the responses for the thymine peak. The content of thymine in the capsules should not be more than 3.0 per cent.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *water,*
Speed and time. 50 rpm and 45 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate, if necessary with *water*.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A known quantity of *zidovudine RS* is dissolved in 1 ml of *methanol* and suitably diluted with *water* to obtain a solution having a similar concentration as that of the test solution.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 20 volumes of *methanol* and 80 volumes of *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject the reference solution. The tailing factor is not more than 2.0 for zidovudine peak and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the peak responses of the major peak.

Calculate the content of $C_{10}H_{13}N_5O_4$ in the medium.

D. Not less than 75 per cent of the stated amount of $C_{10}H_{13}N_5O_4$.

Other tests. Comply with the tests stated under Capsules.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances. Inject separately the test solution and reference solution (c) and measure the responses for the principal peak.

Calculate the content of $C_{10}H_{13}N_5O_4$ in the capsules.

Storage. Store protected from moisture.

Zidovudine Injection

Zidovudine Injection is a sterile solution of Zidovudine in Water for Injections.

Zidovudine Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of $C_{10}H_{13}N_5O_4$.

Usual strengths. 10 mg per ml.

Description. A clear, colourless solution.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), a 0.0015 per cent w/v solution in a mixture of 75 volumes of *methanol* and 25 volumes of *water* shows absorption maxima similar to those obtained with a solution of *zidovudine RS* of the same concentration.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 3.5 to 7.0, in a mixture containing a volume of injection containing 150 mg of zidovudine and 5 ml of *0.12 M potassium chloride.*

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dilute an accurately measured volume of the injection containing 25 mg of Zidovudine, to 25 ml with *methanol.*

Reference solution (a). A 0.1 per cent w/v solution of *zidovudine RS* in *methanol.*

Reference solution (b). A solution containing 0.1 per cent w/v of *zidovudine RS* and 0.001 per cent w/v of *zidovudine related compound C (thymine).*

Chromatographic system
- a stainless steel column 25 cm × 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 80 volumes of *water* and 20 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject reference solution (b). The test is not valid unless the resolution between zidovudine and thymine is not less than 4.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of any peak corresponding to thymine is not greater than twice the area of the corresponding peak in the chromatogram obtained with reference solution (b) (2.0 per cent).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 1.0 Endotoxin Unit per mg of zidovudine.

Assay. Determine by liquid chromatography (2.4.14), as given under the test for Related substances.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{10}H_{13}N_5O_4$ in the injection.

Storage. Store protected from light and moisture.

Zidovudine Oral Solution

Zidovudine Oral Solution is a solution of Zidovudine in a suitable flavoured vehicle.

Zidovudine Oral Solution contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strength. 50 mg in 5 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *1-butanol*, 30 volumes of *heptane*, 30 volumes of *acetone* and 10 volumes of *strong ammonia solution*.

Test solution. Dilute the preparation under examination with *methanol* to obtain a solution containing 5 mg of zidovudine per ml.

Reference solution. A 0.5 per cent w/v solution of *zidovudine RS* in a mixture of 75 volumes of *methanol* and 25 volumes of *water*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to zidovudine in the chromatogram obtained with reference solution (c).

Tests

pH (2.4.24). 3.0 to 4.0, determined in a mixture containing a volume of the preparation under examination containing 150 mg of zidovudine and 5 ml of *0.12 M potassium chloride*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Transfer an accurately measured volume of the preparation under examination containing about 100 mg of zidovudine to a 100-ml volumetric flask, dissolve and dilute to volume with the mobile phase and mix. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a). Weigh accurately about 100 mg of *zidovudine RS* and transfer to a 100-ml volumetric flask, add about 50 ml of the mobile phase, mix with the aid of ultrasound to dissolve, dilute to volume with the mobile phase and mix.

Reference solution (b). Weigh accurately about 20 mg of *thymine RS* and transfer to a 200-ml volumetric flask, add about 150 ml of the mobile phase, mix with the aid of ultrasound to dissolve, dilute to volume with the mobile phase and mix.

Reference solution (c). Transfer 10.0 ml of the reference solution (a) and 2.0 ml of reference solution (b) to a 100-ml volumetric flask and make up to the volume with the mobile phase.

Chromatographic system
- a stainless steel column 12.5 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 90 volumes of *0.04 M sodium acetate*, 9 volumes of *methanol*, 1 volume of *acetonitrile* and 0.2 volume of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 240 nm,
- injection volume. 10 µl.

Inject reference solution (c). The test is not valid unless the relative retention times are about 0.12 for thymine and 1.0 for zidovudine, the resolution between zidovudine and thymine is not less than 4.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject separately the test solution and reference solution (c) and measure the responses for the thymine peak. The content of thymine in the capsules should not be more than 3.0 per cent.

Other tests. Complies with the tests stated under Oral Liquids.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances. Inject the test solution and reference solution (c) and measure the responses for the major peak.

Calculate the content of $C_{10}H_{13}N_5O_4$ in the preparation under examination.

Storage. Store protected from light and moisture.

Zidovudine Tablets

Zidovudine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of zidovudine, $C_{10}H_{13}N_5O_4$.

Usual strengths. 100 mg; 300 mg.

Identification

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak due to Zidovudine in the chromatogram obtained with reference solution (a).

B. Remove the coating from a few tablets and crush them in a mortar so that no large pieces remain.

On the powder determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zidovudine RS* or with the reference spectrum of zidovudine.

Tests

Dissolution (2.5.2).

Apparatus No. 1,

Medium. 900 ml of *water*,

Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate, if necessary with *water*.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. A known quantity of *zidovudine RS* is dissolved in 1 ml of *methanol* and suitably diluted with *water* to obtain a solution having a similar concentration as that of the test solution.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- mobile phase: a mixture of 90 ml of *methanol* and 40 ml of *acetonitrile* and a buffer prepared by dissolving 3.0 g of *sodium acetate* and 3.0 g of *sodium 1-octanesulphonate* in 900 ml of *water* and adjusting the pH to 5.3 with *glacial acetic acid,*
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 10 µl.

Inject the reference solution. The tailing factor is not more than 2.0 for zidovudine peak and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the peak responses of the major peak.

Calculate the content of $C_{10}H_{13}N_5O_4$ in the medium.

D. Not less than 80 per cent of the stated amount of $C_{10}H_{13}N_5O_4$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse the powdered tablets containing about 1500 mg of Zidovudine in 50 ml of *water*, shake for 30 minutes. Add about 150 ml of *methanol*, sonicate for 10 minutes and dilute to 500.0 ml with *water*. Dilute 4.0 ml of this solution to 100.0 ml with *water*, filter.

Reference solution (a). A 0.01 per cent w/v solution of *3'-chloro-3'-deoxythymidine (zidovudine impurity B RS)* in *methanol*.

Reference solution (b). A 0.02 per cent w/v solution of *thymine RS (zidovudine impurity C RS)* in *methanol*.

Reference solution (c). Dissolve about 30 mg of *zidovudine RS* in 3.0 ml of *methanol*, add 2.5 ml of reference solution (a), 5.0 ml of reference solution (b) and dilute to 250.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: dissolve 3.0 g of *sodium acetate* and 1.3 g of *sodium 1-octanesulphonate* in 900 ml of *water*. Add 90 ml of *methanol* and 40 ml of *acetonitrile*, adjusted to pH 5.3 with *glacial acetic acid*, filter,
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 265 nm,
- injection volume. 20 µl.

Inject reference solution (c). The test is not valid unless the resolution between the peaks due to zidovudine and zidovudine impurity B is not less than 2.5, the tailing factor of the principal peak is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to zidovudine for

zidovudine impurity C is about 0.17 and for zidovudine impurity B is about 1.2.

Inject reference solution (c) and the test solution. In the chromatogram obtained the test solution, the area of the peak due to zidovudine impurity C is not more than 1.5 per cent, multiplying with response factor of 1.7 and the area of other secondary peaks is not more than 0.2 per cent. The sum of areas of all the secondary peaks is not more than 2.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances. Separately inject the test solution and reference solution (c) and measure the peak responses for the major peak.

Calculate the content of $C_{10}H_{13}N_5O_4$ in the tablets.

Storage. Store protected from light and moisture.

Zidovudine, Lamivudine and Nevirapine Tablets

Zidovudine, Lamivudine and Nevirapine Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of zidovudine, $C_{10}H_{13}N_5O_4$, lamivudine, $C_8H_{11}N_3O_3S$ and nevirapine, $C_{15}H_{14}N_4O$.

Usual strength. Zidovudine, 300 mg, Lamivudine, 150 mg and Nevirapine , 200 mg.

Identification

A. In the Assay, the three principal peaks in the chromatogram obtained with the test solution correspond to the peaks due to zidovudine, lamivudine and nevirapine in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately a quantity of the powdered tablets containing about 100 mg of Zidovudine and transfer to a 200-ml volumetric flask. Add about 150 ml of *water*, mix with the aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter.

Reference solution. Weigh accurately about 100 mg of *zidovudine RS*, 50 mg of *lamivudine RS* and 65 mg of *nevirapine RS* and transfer to a 200-ml volumetric flask. Add about 20 ml of *methanol*, mix with the aid of ultrasound to dissolve, dilute to volume with *water* and mix.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm) (Such as Hypersil C8),
- mobile phase: A. *0.1 M ammonium acetate,*
  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min.)	*0.1 M ammonium acetate* (per cent v/v)	*acetonitrile* (per cent v/v)
0	95	5
5	95	5
25	20	80
30	20	80
31	95	5
35	95	5

Inject the reference solution. The test is not valid unless the column efficiency determined from the zidovudine, lamivudine and nevirapine peaks is not less than 3000 theoretical plates and the tailing factor for the same peaks is not more than 2.0.

Inject separately *water* and the test solution. Examine the chromatogram obtained with water for any extraneous peaks and ignore the corresponding peaks observed in the chromatogram obtained with the test solution.

Any secondary peak observed in the chromatogram obtained with the test solution corresponding to a relative retention time of 0.35 should not be more than 1.0 per cent. Any other secondary peak observed in the chromatogram obtained with the test solution should not be more than 0.5 per cent and the sum of the areas of all the secondary peaks should not be more than 2.5 per cent when calculated by percentage area normalisation.

Dissolution (2.5.2).

Apparatus No. 1,
Medium. 900 ml of *0.1 M hydrochloric acid,*
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter promptly through a membrane filter disc having an average pore diameter not greater than 1.0 µm, rejecting the first few ml of the filtrate and dilute a suitable volume of the filtrate, if necessary with *water*.

Determine by liquid chromatography (2.4.14).

Test solution. The filtrate obtained as given above.

Reference solution. Weigh accurately about 300 mg of *zidovudine RS*, 150 mg of *lamivudine RS* and 200 mg of

nevirapine RS and transfer to a 100 ml volumetric flask. Add about 20 ml of *methanol*, mix with the aid of ultrasound to dissolve and dilute to volume with a solvent mixture of equal volumes of *methanol* and *water*. Dilute 5.0 ml of this solution to 50.0 ml with *0.1 M hydrochloric acid*.

Chromatographic system
- a stainless steel column 15 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS C18),
- mobile phase: a mixture of 35 volumes of *methanol* and 65 volumes of a buffer solution prepared by dissolving 0.68 g of *potassium dihydrogen phosphate* and 1.0 g of *sodium octanesulphonate* in 1000.0 ml of *water* to which 1 ml of *triethylamine* is added and the pH of which is adjusted to 2.5 with *phosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 266 nm,
- injection volume. 10 µl.

Inject the reference solution. The tailing factor for the individual zidovudine, lamivudine and nevirapine peaks is not more than 2.0 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the peak responses of the major peaks due to zidovudine, lamivudine and nevirapine.

Calculate the contents of $C_{10}H_{13}N_5O_4$, $C_8H_{11}N_3O_3S$ and $C_{15}H_{14}N_4O$ in the medium.

D. Not less than 70 per cent of the stated amounts of $C_{10}H_{13}N_5O_4$, $C_8H_{11}N_3O_3S$ and $C_{15}H_{14}N_4O$.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 100 mg of Zidovudine and transfer to a 200-ml volumetric flask. Add about 20 ml of *methanol*, mix with the aid of ultrasound for 10 minutes, dilute to volume with *water*, mix and filter. Further dilute 10.0 ml of the filtrate to 25.0 ml with *water*.

Reference solution. Weigh accurately about 100 mg of *zidovudine RS*, 50 mg of *lamivudine RS* and 65 mg of *nevirapine RS* and transfer to a 200-ml volumetric flask. Add about 20 ml of *methanol*, mix with the aid of ultrasound to dissolve, dilute to volume with *water* and mix. Further dilute 10.0 ml of this solution to 25.0 ml with *water*.

Follow the chromatographic procedure described under Dissolution.

Inject the reference solution. The tailing factor for the individual peaks due to zidovudine, lamivudine and nevirapine is not more than 2.0 and the relative standard deviation for replicate injections of all the analyte peaks is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the major peaks.

Calculate the contents of $C_{10}H_{13}N_5O_4$, $C_8H_{11}N_3O_3S$ and $C_{15}H_{14}N_4O$ in the tablets.

Storage. Store protected from moisture.

Zinc Chloride

$ZnCl_2$ Mol. Wt. 136.3

Zinc Chloride contains not less than 95.0 per cent and not more than 100.5 per cent of $ZnCl_2$.

Category. Pharmaceutical aid.

Description. A white or practically white, crystalline powder; odourless; very deliquescent.

Identification

A. To 2 g add 38 ml of *carbon dioxide-free water* prepared from *distilled water* and add *2 M hydrochloric acid* dropwise until solution is complete and dilute to 40 ml with *carbon dioxide-free water* prepared from *distilled water* (solution A). Solution A gives the reaction of zinc salts (2.3.1).

B. A 5 per cent w/v solution in *2 M nitric acid* gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 4.6 to 6.0, determined in a solution prepared by dissolving 1.0 g in 9 ml of freshly boiled and cooled *water*, ignoring any slight turbidity.

Aluminium, calcium, heavy metals, iron and magnesium. To 8 ml of solution A add 2 ml of *strong ammonia solution* and shake; the solution is clear (2.4.1), and colourless (2.4.1). Add 1 ml of a 9 per cent w/v solution of *disodium hydrogen phosphate*; the resulting solution remains clear for at least 5 minutes. Add 0.2 ml of *sodium sulphide solution*; a white precipitate is produced and the supernatant liquid remains colourless.

Ammonium salts. To 5 ml of a 10 per cent w/v solution add *1 M sodium hydroxide* until the precipitate first formed is redissolved and then warm the solution; no odour of ammonia is perceptible.

Oxychlorides. Dissolve 1.5 g in 1.5 ml of *carbon dioxide-free water*; the solution is not more opalescent than opalescence standard OS2 (2.4.1). Add 7.5 ml of *ethanol (95 per cent)*; the solution may become cloudy within 10 minutes but becomes clear on the addition of 0.2 ml of *2 M hydrochloric acid*.

Sulphates (2.3.17). 15 ml of solution A complies with the limit test for sulphates (225 ppm).

Assay. Weigh accurately about 3.0 g, dissolve in 125 ml of *water*, add 3 g of *ammonium chloride* and add sufficient *water* to produce 250.0 ml. To 25.0 ml of the resulting solution add 100 ml of *water* and 10 ml of *strong ammonia-ammonium chloride solution*. Titrate with *0.1 M disodium edetate*, using *eriochrome black T solution* as indicator until a deep blue colour is obtained.

1 ml of *0.1 M disodium edetate* is equivalent to 0.01363 g of $ZnCl_2$.

Storage. Store protected from moisture, in non-metallic containers.

Zinc Oxide

ZnO Mol. Wt. 81.4

Zinc Oxide contains not less than 99.0 per cent and not more than 100.5 per cent of ZnO, calculated on the ignited basis.

Category. Mild astringent; topical protectant.

Description. A soft, white or faintly yellowish white amorphous powder, free from grittiness. It gradually absorbs carbon dioxide from air.

Identification

A. It becomes yellow when strongly heated; the yellow colour disappears on cooling.

B. Dissolve 0.1 g in 1.5 ml of *2 M hydrochloric acid* and dilute to 5 ml with *water*. The solution gives the reaction of zinc salts (2.3.1).

Tests

Alkalinity. Shake 1.0 g with 10 ml of boiling *water*, add 0.1 ml of *phenolphthalein solution* and filter. If the filtrate is red, not more than 0.3 ml of *0.1 M hydrochloric acid* is required to discharge the colour.

Carbonate and substances insoluble in acids. Dissolve 1.0 g in 15 ml of *2 M hydrochloric acid*; no effervescence is produced and the solution is not more opalescent than opalescence standard OS2 (2.4.1), and colourless (2.4.1).

Arsenic (2.3.10). Dissolve 2.0 g in 15 ml of *brominated hydrochloric acid AsT* and 45 ml of *water* and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (5 ppm).

Iron (2.3.14). Dissolve 0.1 g in a mixture of 5 ml of *water* and 1 ml of *hydrochloric acid* and dilute to 40 ml with *water*. The resulting solution complies with the limit test for iron (400 ppm). Use 0.5 ml of *thioglycollic acid* in the test.

Lead. Dissolve 2 g in a mixture of 20 ml *water* and 5 ml of *glacial acetic acid* and add 0.25 ml of *potassium chromate solution*; the solution remains clear.

Loss on ignition (2.4.20). Not more than 1.0 per cent, determined on 2.0 g by igniting at 500°.

Assay. Dissolve 0.15 g in 10 ml of *2 M acetic acid* and dilute to 50 ml with *water*. To the resulting solution add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.1 M disodium edetate* until the solution becomes yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.008138 g of ZnO.

Storage. Store protected from moisture.

Zinc Oxide Cream

Zinc Cream

Zinc Oxide Cream contains 32 per cent w/v of Zinc Oxide in a suitable water-in-oil emulsified base.

Zinc Oxide Cream contains not less than 30.0 per cent and not more than 34.0 per cent w/w of zinc oxide, ZnO.

Identification

The residue obtained in the Assay is yellow when hot and white when cool.

Tests

Other tests. Complies with the tests stated under Creams.

Assay. Weigh accurately about 0.5 g in a porcelain dish, heat gently over a small flame until the base is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10 ml of *2 M acetic acid* and add sufficient *water* to produce 50 ml. To the resulting solution add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.1 M disodium edetate* until the solution becomes yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.008138 g of ZnO.

Zinc Stearate

$(C_{17}H_{35}COO)_2Zn$ Mol. Wt. 632.3

Zinc Stearate consists mainly of zinc stearate but many contain variable proportions of zinc palmitate $(C_{15}H_{31}COO)_2$ Zn, and zinc oleate $(C_{17}H_{33}COO)_2Zn$.

Zinc Stearate contains not less than 10.0 per cent and not more than 12.0 per cent of zinc, Zn.

Category. Pharmaceutical aid (dusting powder).

Description. A fine, white, bulky, amorphous powder, free from grittiness; odour, faint and characteristic.

Identification

A. To 5.0 g add 50 ml of *ether* and 40 ml of a 7.5 per cent v/v solution of *nitric acid* in *distilled water* and heat under a reflux condenser until dissolution is complete. Allow to cool, separate the aqueous layer and shake the ether layer with two quantities, each of 4 ml, of *distilled water*. Combine the washings with the aqueous layer, wash with 15 ml of *ether* and heat on a water-bath until ether is completely eliminated. Allow to cool and dilute to 50.0 ml with *distilled water* (solution A). Evaporate the ether layer to dryness and dry the residue at 105°. The freezing point of the residue is not lower than 53° (2.4.11).

B. Neutralise 5 ml of solution A to red *litmus paper* with *10 M sodium hydroxide*. The solution gives the reactions of zinc salts (2.3.1).

Tests

Appearance of solution. Solution A is not more intensely coloured than reference solution YS6 (2.4.1).

Acidity or alkalinity. Shake 1.0 g with 5 ml of *ethanol (95 per cent)* and add 20 ml of *carbon dioxide-free water* and 0.1 ml of *phenol red solution*. Not more than 0.3 ml of *0.1 M hydrochloric acid* or 0.1 ml of *0.1 M sodium hydroxide* is required to change the colour of the solution.

Alkalis and alkaline earths. Add 1.0 g to a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid*, boil, filter immediately and wash with 25 ml of hot *water*. Add *dilute ammonia solution* to make the filtrate just alkaline and then add *ammonium sulphide solution* in excess to precipitate the zinc as zinc sulphide completely. Filter, add 0.5 ml of *sulphuric acid* to the filtrate, evaporate to dryness and ignite to constant weight; the residue weighs not more than 20 mg.

Arsenic (2.3.10). Mix 5.0 g with 10 ml of *bromine solution* and evaporate to dryness on a water-bath. Ignite gently, dissolve the cooled residue, ignoring any carbon, in 50 ml of *water* and 14 ml of *brominated hydrochloric acid AsT* and remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (2 ppm).

Heavy metals (2.3.13). Heat 5.0 g with 40 ml of *2 M acetic acid* and allow to cool. Filter, wash the residue with two successive quantities, each of 5 ml, of warm *water* and dilute the combined filtrate and washings to 100.0 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (20 ppm). Use 1.0 ml of *lead standard solution (10 ppm Pb)* to prepare the standard.

Chlorides (2.3.12). 10 ml of solution A complies with the limit test for chlorides (250 ppm).

Sulphates (2.3.17). 2.5 ml of solution A complies with the limit test for sulphates (0.6 per cent).

Assay. Weigh accurately about 1.0 g and boil with 50 ml of *2 M acetic acid* until the fatty acid layer which separates is clear, adding more *water* if necessary to maintain the original volume. Cool, filter and wash the filter and the flask thoroughly with *water* until the last washing is not acidic to blue *litmus paper*. To the combined filtrate and washings add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.1 M disodium edetate* until the colour changes to yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.00654 g of Zn.

Zinc Sulphate

$ZnSO_4, 7H_2O$ Mol. Wt. 287.5

Zinc Sulphate contains not less than 99.0 per cent and not more than 104.0 per cent of $ZnSO_4, 7H_2O$.

Category. Astringent.

Description. Colourless, transparent crystals or a white, crystalline powder; odourless; efflorescent.

Identification

Dissolve 2.5 g in sufficient *carbon dioxide-free water* to produce 50 ml (solution A). Solution A gives the reactions of zinc salts and sulphates (2.3.1).

Tests

Appearance of solution. Solution A is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 4.4 to 5.6, determined in solution A.

Arsenic (2.3.10). Dissolve 1.0 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (10 ppm).

Iron (2.3.14). Dissolve 0.4 g in 20 ml of *water*. The resulting solution complies with the limit test for iron (100 ppm).

Chlorides (2.3.12). 20 ml of solution A complies with the limit test for chlorides (250 ppm).

Assay. Weigh accurately about 0.5 g and dissolve in 5 ml of *2 M acetic acid* and dilute to 50 ml with *water*. To the resulting solution add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce violet-pink colour. Further, add 2 g of *hexamine* and titrate with *0.1 M disodium edetate* until the colour changes to yellow.

1 ml *of 0.1 M disodium edetate* is equivalent to 0.02875 g of $ZnSO_4, 7H_2O$.

Storage. Store protected from moisture, in non-metallic containers.

Zinc Sulphate Eye Drops

Zinc Sulphate Eye Drops are a sterile solution containing 0.25 per cent w/v of Zinc Sulphate in Purified Water.

Zinc Sulphate Eye Drops contain not less than 0.22 per cent and not more than 0.28 per cent w/v of zinc sulphate, $ZnSO_4, 7H_2O$.

Usual strenghts. 200 mg; 500 mg; 1 g.

Description. A clear, colourless solution.

Identification

Give the reactions of zinc salts and of sulphates (2.3.1).

Tests

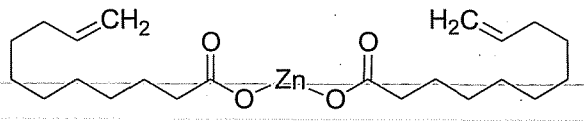
Other tests. Comply with the tests stated under Eye Drops.

Assay. To 5.0 ml add 50 ml of *water* and 5 ml of *ammonia buffer pH 10.9* and titrate with *0.01 M disodium edetate* using *mordant black II solution* as indicator.

1 ml of *0.01 M disodium edetate* is equivalent to 0.002875 g of $ZnSO_4, 7H_2O$.

Storage. Store in containers of glass or any other non-metallic material and sealed so as to exclude micro-organisms.

Zinc Undecenoate



$C_{22}H_{38}O_4Zn$ Mol. Wt. 431.9

Zinc Undecenoate is zinc di(undec-10-enoate).

Zinc Undecenoate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{38}O_4Zn$, calculated on the dried basis.

Category. Antifungal (topical).

Description. A white or almost white, fine powder.

Identification

A. To 2.5 g add 10 ml of *water* and 10 ml of *1 M sulphuric acid* and extract with two quantities, each of 10 ml, of *ether*. Reserve the aqueous layer for test C. Wash the combined ether extracts with *water* and evaporate to dryness. To the residue add 2 ml of freshly distilled *aniline* and boil under a reflux condenser for 10 minutes, cool and add 30 ml of *ether*. Extract with three quantities, each of 20 ml, of *2 M hydrochloric acid* and then with 20 ml of *water*. Evaporate the ether extract to dryness on a water-bath. The residue, after recrystallising twice from *ethanol (70 per cent)* and drying at a pressure not exceeding 2 kPa for 3 hours, melts at about 67° (2.4.21).

B. Dissolve 0.1 g in a mixture of 2 ml of *1 M sulphuric acid* and 5 ml of *glacial acetic acid* and add, dropwise, 0.25 ml of *potassium permanganate solution*; the pink colour of permanganate is discharged.

C. A mixture of 1 ml of the aqueous layer reserved in test A and 4 ml of *water* gives the reaction of zinc salts (2.3.1).

D. Melting range (2.4.21). 115° to 121°.

Tests

Alkalinity. Mix 1.0 g with 5 ml of *ethanol (95 per cent)* and 0.5 ml of *phenol red solution*, add 50 ml of *carbon dioxide-free water* and examine immediately; no reddish colour is produced.

Alkalis and alkaline earths. Add 1.0 g to a mixture of 25 ml of *water* and 5 ml of *hydrochloric acid*, boil, filter immediately and wash with 25 ml of hot *water*. Add *dilute ammonia solution* to make the filtrate just alkaline and then add *ammonium sulphate solution* in excess to precipitate the zinc as zinc sulphide completely. Filter, add 0.5 ml of *sulphuric acid* to the filtrate, evaporate to dryness and ignite to constant weight; the residue weighs not more than 20 mg.

Sulphates (2.3.17). To 0.25 g add a mixture of 10 ml of *distilled water* and 5 ml of *2 M hydrochloric acid*. Cool, filter and dilute to 20 ml with *distilled water*. The resulting solution complies with the limit test for sulphates (600 ppm).

Degree of unsaturation. Dissolve 0.1 g in a mixture of 5 ml of *2 M hydrochloric acid* and 30 ml of *glacial acetic acid* and titrate with *0.05 M bromine* using 0.05 ml of *ethoxychrysoidine hydrochloride solution*, added towards the end of the titration, as indicator. Not less than 9.1 ml and not more than 9.4 ml of *0.05 M bromine* is required to discharge the red colour.

Loss on drying (2.4.19). Not more than 1.5 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.35 g, add 25 ml of *2 M acetic acid*, heat to boiling, cool and dilute to 50 ml with *water*. Add about 50 mg of *xylenol orange triturate* and sufficient *hexamine* to produce a violet-pink colour. Add a further 2 g of *hexamine* and titrate with *0.1 M disodium edetate* until the colour changes to yellow.

1 ml of *0.1 M disodium edetate* is equivalent to 0.04319 g of $C_{22}H_{38}O_4Zn$.

Storage. Store protected from light and moisture.

Zinc Undecenoate Ointment

Zinc Undecylenate Ointment

Zinc Undecenoate Ointment contains 20 per cent w/v of Zinc Undecenoate in a suitable ointment basis.

Zinc Undecenoate Ointment contains not less than 18.0 per cent and not more than 22.0 per cent of zinc undecenoate, $C_{22}H_{38}O_4Zn$, and not less than 4.5 per cent and not more than 5.5 per cent of free undecenoic acid, $C_{11}H_{20}O_2$.

Tests

Other tests. Complies with the tests stated under Ointments.

Assay. *For zinc undecenoate* — Weigh accurately about 2.0 g, add 20 ml of *dilute hydrochloric acid* and boil under a reflux condenser for at least 20 minutes or until the fatty layer is clear. Filter while hot and wash the residue with hot *water*. Cool the combined filtrate and washings, neutralise to *litmus paper* with *dilute ammonia solution*, add 3 ml of *dilute hydrochloric acid* and 5 g of *hexamine* and titrate with *0.05 M disodium edetate* using *xylenol orange solution* as indicator, until the colour changes to yellow.

1 ml of *0.05 M disodium edetate* is equivalent to 0.02160 g of $C_{22}H_{38}O_4Zn$.

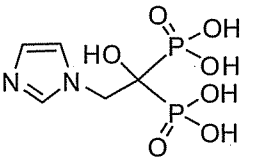
For free undecenoic acid — Weigh accurately about 5.0 g, add 100 ml of *dilute hydrochloric acid* and heat to 70° with constant stirring. Cool and transfer to a separator with the aid of four quantities, each of 25 ml of *ether* and add the rinsings to the mixture in the separator. Dilute the aqueous phase to 300 ml, saturate it with *sodium chloride* and shake the mixture. Transfer the aqueous layer to a second separator and extract with another 100 ml of *ether*. Wash the combined ether extracts with successive quantities, each of 10 ml, of *water* until the washings are free from chloride. Transfer the ether solution to a beaker and evaporate on a water-bath to about 5 ml. Add 20 ml of *carbon tetrachloride*, mix, transfer the mixture to a small separator and drain the *carbon tetrachloride* layer into a 100-ml volumetric flask. Rinse the beaker with three quantities, each of 5 ml, of *carbon tetrachloride* and transfer the rinsings to the volumetric flask, dilute to volume with carbon tetrachloride and mix. Evaporate 50.0 ml of the resulting solution to about 5 ml, add 100 ml of *ethanol (95 per cent)*, previously neutralised, and 0.15 ml of *phenolphthalein solution* and titrate the total undecenoic acid with *0.1 M sodium hydroxide*.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01843 g of $C_{11}H_{20}O_2$.

Calculate the content of free undecenoic acid from the difference between the total undecenoic acid and the undecenoic acid equivalent to the determined zinc undecenoate (the content of zinc undecenoate multiplied by 0.8533 gives the equivalent of undecenoic acid).

Storage. Store protected from light and moisture.

Zoledronic acid



$C_5H_{10}N_2O_7P_2$ Mol. Wt. 272.1

Zoledronic Acid is [1-hydroxy-2-(1*H*-imidazol-1-yl)ethylidene]diphosphonic acid.

Zoledronic Acid contains not less than 98.0 per cent and not more than 102.0 per cent of $C_5H_{10}N_2O_7P_2$, calculated on the dried basis.

Category. Bone resorption inhibitor.

Description. A white to almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zoledronic acid RS* or with the reference spectrum of zoledronic acid.

B. In the assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 2.0 to 4.0, determined in a 0.02 per cent w/v solution.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution. Dilute 1 ml of the test solution to 100 ml with the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: mix 1 ml of *triethylamine* with 500 ml of *water*, adjust the pH to 3.2 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 2000 theoretical plates and the tailing factor is not more than 2.0.

Inject the test solution and the reference solution. Run the chromatogram 3 times the retention time of the principal peak. In the chromatogram obtained with the test solution, area of any secondary peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (1.0 per cent). The sum of areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with the reference solution (2.0 per cent). Ignore any peak with an area less than 0.1 times the area of the peak in the chromatogram obtained with the reference solution (0.1 per cent).

Loss on drying (2.4.19). 5.0 to 8.0 per cent, determined on 1.0 g by drying in an oven at 105° for 6 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 50.0 ml of the mobile phase. Dilute 20 ml of the solution to 100 ml with the mobile phase.

Reference solution. A 0.02 per cent w/v solution of *zoledronic acid RS* in the mobile phase.

Use the chromatographic system as described under Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_5H_{10}N_2O_7P_2$.

Storage. Store protected from light and moisture.

Labelling. The label states whether or not the material is intended for use in the manufacture of parenteral preparations.

Zoledronic Acid Injection

Zoledronic Acid Injection is prepared by dissolving the contents of a sealed container containing Zoledronic Acid with or without auxiliary substances in the requisite amount of water for injection as directed on the label.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Usual strength. 4 mg per ml.

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Zoledronic Acid Injection contains not less than 90.0 per cent and not more than 110.0 per cent of stated amount of zoledronic acid, $C_5H_{10}N_2O_7P_2$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Identification

In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 4.5 to 7.0, determined in a solution constituted as directed on the label, in Water for Injections.

Other tests. Complies with the tests stated under Parental Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 17.0 Endotoxin Units per mg of Zoledronic acid.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve the contents of sealed container in sufficient mobile phase to give a concentration 0.16 mg per ml.

Reference solution. A 0.016 per cent w/v solution of *zoledronic acid RS* in the mobile phase.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Hypersil BDS),
- mobile phase: mix 1 ml of *triethylamine* with 500 ml of *water*, adjusted to pH 3.2 with *orthophosphoric acid,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

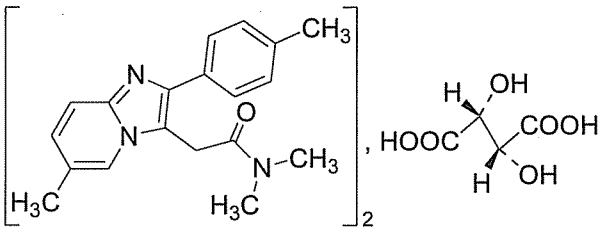
Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_5H_{10}N_2O_7P_2$ in the Injection.

Storage. Store the sealed container at a temperature not exceeding 30°. Use the prepared solution within the period recommended by the manufacturer and stored strictly in accordance with the instructions of the manufacturer.

Zolpidem Tartrate



$(C_{19}H_{21}N_3O)_2, C_4H_6O_6$ Mol. Wt. 765.0

Zolpidem Tartrate is bis[*N*,*N*-dimethyl-2-[6-methyl-2-(4-methylphenyl)imidazo[1,2-*a*]pyridine-3-yl]acetamide] tartrate.

Zolpidem Tartrate contains not less than 98.5 per cent and not more than 101.0 per cent of $(C_{19}H_{21}N_3O)_2$, $C_4H_6O_6$, calculated on the anhydrous basis.

Category. Sedatives.

Description. A white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zolpidem tartrate RS* or with the reference spectrum of zolpidem tartrate.

B. A 10 per cent w/v solution in *methanol* gives reaction (c) of tartrates (2.3.1).

Tests

Appearance of solution. Dissolve 1.0 g of the substance under examination in 100 ml of a 0.5 per cent w/v solution of *tartaric acid*. The solution is clear (2.4.1), and not more intensely coloured than reference solution YS6 or BYS6 (2.4.1).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 25 mg of the substance under examination in 50.0 ml of the mobile phase.

Reference solution (a). A 0.01 per cent w/v solution of *N,N-dimethyl-2-[7-methyl-2-(4-methylphenyl) imidazo[1,2-a] pyridin-3-yl acetamide RS (zolpidem impurity A RS)* in the mobile phase.

Reference solution (b). A 0.05 per cent w/v solution of *zolpidem tartrate RS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d). To 2.0 ml of reference solution (b), add 10.0 ml of reference solution (a).

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: 18 volumes of *acetonitrile*, 23 volumes of *methanol* and 59 volumes of 0.56 per cent w/v solution of *orthophosphoric acid* adjusted to pH 5.5 with *triethylamine*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (d). Adjust the sensitivity of the system so that the height of the peak due to zolpidem impurity A is at least 50 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks due to zolpidem impurity A and zolpidem tartrate is at least 2.0.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.2 times the area of the peak in the chromatogram obtained with reference solution (c) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the peak in the chromatogram obtained with reference solution (c) (1.0 per cent). Ignore any peak with a relative retention time of 0.16 to the zolpidem peak, corresponding to tartaric acid and any secondary peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 3.0 per cent, determined on 0.5 g.

Assay. Weigh accurately about 0.3 g, dissolve in a mixture of 20 ml of *anhydrous acetic acid* and 20 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, determining the end point potentiometrically (2.4.25). Carry out a blank titration

1 ml of *0.1 M perchloric acid* is equivalent to 0.03824 g of $(C_{19}H_{21}N_3O)_2, C_4H_6O_6$.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

Zolpidem Tablets

Zolpidem Tartrate Tablets

Zolpidem Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of zolpidem tartrate, $C_{42}H_{48}N_6O_8$.

Usual strengths. 5 mg; 10 mg.

Identification

Shake a quantity of the powdered tablets containing 0.1 g of Zolpidem Tartrate with 40 ml of *methanol*, filter and evaporate the filtrate to dryness. The residue complies with the following test.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *zolpidem tartrate RS* or with the reference spectrum of zolpidem tartrate.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Dissolution (2.5.2).

Apparatus No.1,
Medium. 900 ml of *0.01 M hydrochloric acid*,
Speed and time. 50 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Dilute the filtrate, if necessary, with the dissolution medium.

Reference solution. Dissolve 0.01 g of *zolpidem tartrate RS* in sufficient *methanol* to produce 100 ml. Dilute the resulting solution with the dissolution medium to obtain a solution containing 0.0005 per cent w/v of zolpidem tartrate.

Use the chromatographic system described in the test for Related substances.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{42}H_{48}N_6O_8$.

D. Not less than 75 per cent of the stated amount of $C_{42}H_{48}N_6O_8$.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of the powdered tablets containing 25 mg of Zolpidem Tartrate with 25 ml of the mobile phase and dilute to 50.0 ml with the same solvent and filter.

Reference solution (a). A 0.01 per cent w/v solution of *N,N-dimethyl-2-[7-methyl-2-(4-methylphenyl) imidazo[1,2-a] pyridin-3-yl acetamide RS (zolpidem impurity A RS)* in the mobile phase.

Reference solution (b). A 0.05 per cent w/v solution of *zolpidem tartrate RS* in the mobile phase.

Reference solution (c). Dilute 1.0 ml of the reference solution (b) to 100.0 ml with the mobile phase.

Reference solution (d). To 2.0 ml of reference solution (b), add 10.0 ml of reference solution (a).

Chromatographic system
- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (4 µm),
- mobile phase: 18 volumes of *acetonitrile*, 23 volumes of *methanol* and 59 volumes of 0.56 per cent w/v solution of *orthophosphoric acid* adjusted to pH 5.5 with *triethylamine*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (d). Adjust the sensitivity of the system so that the height of the peak due to zolpidem impurity A is at least 50 per cent of the full scale of the recorder. The test is not valid unless the resolution between the peaks due to zolpidem impurity A and zolpidem tartrate is at least 2.0.

Inject the test solution and reference solution (c). In the chromatogram obtained with the test solution, the area of any secondary peak is not more than 0.5 times the area of the peak in the chromatogram obtained with reference solution (c) (0.5 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the peak in the chromatogram obtained with reference solution (c) (2.0 per cent). Ignore any peak with a relative retention time of 0.16 to the zolpidem peak, corresponding to tartaric acid and any secondary peak with an area less than 0.05 times the area of the peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

Uniformity of content. Comply with the test stated under Tablets.

Determine by liquid chromatography (2.4.14), as described in the Assay, using the following solution as the test solution.

Test solution. Disperse one tablet in the mobile phase, mix and dilute to obtain a solution containing 0.005 per cent w/v of zolpidem tartrate and filter.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of powder containing 25 mg of Zolpidem Tartrate, disperse in 25 ml of the mobile phase and dilute to 50.0 ml with same solvent and filter. Dilute 5.0 ml of the solution to 50.0 ml with the mobile phase.

Reference solution. A 0.005 per cent w/v solution of *zolpidem tartrate RS* in the mobile phase.

Use the chromatographic system described in the test for Related substances.

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of $C_{42}H_{48}N_6O_8$ in the tablets.

Storage. Store protected from light and moisture, at a temperature not exceeding 30°.

VACCINES AND IMMUNOSERA FOR HUMAN USE

General Requirements

Monographs

Vaccines : General Requirements

Vaccines are preparations of antigenic substances that are administered for the purpose of inducing in the recipient a specific and active immunity against the infective agent or toxin produced by it.

Vaccines may contain living micro-organisms suitably treated to attenuate their virulence but retain their immunogenicity or they may consist of pathogenic organisms which have been killed or inactivated. Some vaccines consist of antigenic fractions or substances produced by the same pathogenic organisms but rendered harmless whilst retaining their immunogenicity. Vaccines may be prepared from one species only or from a mixture of two or more species.

Vaccines may be prepared by the method described in the individual monographs or by the general methods given below or in any other manner provided the identity of the antigens is maintained and the vaccines are free from microbial contamination and extraneous agents. Suitable adjuvants may be added during the preparation but streptomycin, penicillin or other β-lactam antibiotics may not be added at any stage of manufacture or in the final vaccine. A suitable microbicide may be added to sterile and inactivated vaccines. The final products are distributed aseptically into sterile containers which are then sealed to exclude extraneous micro-organisms. Unless otherwise indicated in the monograph, the final vaccine may be filled into single dose or multiple dose containers but vaccines in multiple dose containers must invariably contain a microbicide.

Bacterial Vaccines. Bacterial vaccines are either sterile suspensions of live or killed bacteria or sterile extracts of derivatives of bacteria. They may be simple vaccines prepared from one species or may be mixed vaccines prepared by blending two or more simple vaccines from different species or strains.

Bacterial vaccines may be prepared from cultures grown on suitable solid or liquid media. The whole culture or parts of it may be used in preparing the vaccine. The identity, antigenic potency and purity of each bacterial culture must be carefully controlled.

Vaccines containing killed organisms may be prepared by killing the organisms by chemical or physical means provided the immunogenicity of the vaccine is preserved. Vaccines containing living bacteria may be prepared from strains which are avirulent for humans but can stimulate the production of antibodies active against pathogenic strains of the same species. The final vaccines must be free from any substance known to cause toxic, allergic or other undesirable immunological reactions in humans.

Bacterial vaccines are suspensions of varying degrees of opacity in colourless or slightly coloured liquids or they may be freeze-dried so that the water content is not more than 3.0 per cent w/w unless otherwise stated in the individual monograph. They may be standardized in terms of interopacity units or, where appropriate, by numbers of living or killed bacteria determined by direct cell count or by viable count.

Bacterial toxoids. Bacterial toxoids are toxins or material derived therefrom, the toxicity of which has been reduced to a very low level or completely eliminated by chemical or physical means without destroying their immunizing potency. The toxins are obtained from selected strains of specific micro-organisms, grown in media free from ingredients known to cause toxic, allergic or other undesirable immunological reactions in humans. Toxoids produced by the action of formaldehyde are known as formol toxoids.

Bacterial toxoids may be liquid or may be prepared by adsorbing on mineral carriers such as *aluminium phosphate, aluminium hydroxide* or any other suitable adsorbent; the adsorbed product may be separated, washed and suspended in a *saline* or other appropriate solution isotonic with blood.

Bacterial toxoids are clear or slightly opalescent liquids, colourless or slightly yellow. Adsorbed toxoids may be white or greyish white suspensions or pale-yellow liquids with a sediment at the bottom of the container. Freeze-dried preparations are greyish white or yellowish white powders or pellets.

Viral and rickettsial vaccines. Viral and rickettsial vaccines are suspensions of viruses or rickettsiae and are prepared from infected tissues or blood obtained from artificially infected animals, from cultures in fertile eggs, or from cell or tissue culture. Viral vaccines may be live or killed and they may be freeze-dried. Live vaccines are usually prepared using attenuated strains of the specific organisms. Killed vaccines may be inactivated by suitable chemical or physical means.

Mixed Vaccines. Mixed vaccines are mixtures of two or more vaccines. A suitable antibacterial substance may be added to inactivated or live viral and rickettsial vaccines provided that it has no action against the specific organisms.

Production

General provisions. Requirements for production including in-process testing are included in individual monographs. Where justified and authorized, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test.

Unless otherwise justified and authorized, vaccines are produced using a seed-lot system. The methods of preparation are designed to maintain adequate immunogenic properties, to render the preparation harmless and to prevent contamination with extraneous agents.

Unless otherwise justified and authorized, in the production of a final lot of vaccine, the number of passages of a virus, or the number of subcultures of a bacterium, from the master seed lot shall not exceed that used for production of the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Vaccines are as far as possible free from ingredients known to cause toxic, allergic or other undesirable reactions in man. Suitable additives, including stabilizers and adjuvants may be incorporated. Penicillin and streptomycin are neither used at any stage of production nor added to the final product; however, master seed lots prepared with media containing penicillin or streptomycin may, where justified and authorized, be used for production.

Substrates for propagation. Substrates for propagation comply with the relevant requirements of the Pharmacopoeia or in the absence of such requirements with those of the competent authority. Processing of cell banks and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Serum and trypsin used in the preparation of cell suspensions shall be shown to be free from extraneous agents.

Seed lot. The strain of bacterium or virus used in a master seed lot is identified by historical records that include information on the origin of the strain and its subsequent manipulation. No micro-organism other than the seed strain shall be present in a seed lot.

Culture media. Culture media are as far as possible free from ingredients known to cause toxic, allergic or other undesirable reactions in man; if inclusion of such ingredients is necessary, it shall be demonstrated that the amount present in the final lot is reduced to such a level as to render the product safe. Approved animal (but not human) serum may be used in the growth medium for cell cultures but the medium used for maintaining cell growth during virus multiplication shall not contain serum, unless otherwise stated. Cell culture media may contain a pH indicator such as *phenol red* and approved antibiotics at the lowest effective concentration although it is preferable to have a medium free from antibiotics during production.

Propagation and harvest. The seed cultures are propagated and harvested under defined conditions. The purity of the harvest is verified by suitable tests as defined in the monograph.

Control cells. For vaccines produced in cell cultures, control cells are maintained and tested as prescribed. In order to provide a valid control, these cells must be maintained in conditions that are rigorously identical with those used for the production cell cultures, including use of the same batches of media and media changes.

Control eggs. For live vaccines produced in SPF eggs, control eggs are incubated and tested as prescribed in the monograph.

Purification. Where applicable, validated purification procedures may be applied.

Inactivation. Inactivated vaccines are produced using a validated inactivation process whose effectiveness and consistency have been demonstrated. Where there are recognised potential contaminants of a harvest, for example in vaccines produced in eggs from healthy, non-SPF flocks, the inactivation process is also validated with respect to the potential contaminants. A test for inactivation is carried out as soon as possible after the inactivation process, unless otherwise justified and authorised.

Intermediates. Where applicable, the stability of intermediates in given storage conditions shall be evaluated and a period of validity established.

Final bulk. The final bulk is prepared by aseptically blending the ingredients of the vaccine.

Adsorbents. Vaccines may be adsorbed on *aluminium hydroxide, aluminium phosphate, calcium phosphate* or other suitable adsorbent; the adsorbents are prepared in special conditions which confer the appropriate physical form and adsorptive properties.

Antimicrobial preservative. A suitable antimicrobial preservative may be included in sterile and inactivated vaccines and is invariably added if these preparations are issued in multidose containers, unless otherwise stated. If an antimicrobial preservative is used, it shall be shown that it does not impair the safety or efficacy of the vaccine and its effectiveness throughout the period of validity shall be demonstrated.

Final lot. For vaccines for parenteral administration, the final lot is prepared by aseptically distributing the final bulk into sterile tamper-proof containers which, after freeze-drying where applicable, are closed so as to exclude contamination. For vaccines for administration by a non-parenteral route, the final lot is prepared by distributing the final bulk under suitable conditions into sterile, tamper-proof containers.

Stability. Maintenance of potency of the final lot throughout the period of validity shall be demonstrated by validation studies; the loss of potency in the recommended storage conditions is assessed and excessive loss even within the limits of acceptable potency may indicate that the vaccine is unacceptable.

Degree of adsorption. During development of an adsorbed vaccine, the degree of adsorption is evaluated as part of the consistency testing. A release specification for the degree of adsorption is established in the light of results found for batches used in clinical testing. From the stability data

generated for the vaccine it must be shown that at the end of the period of validity the degree of adsorption will not be less than for batches used in clinical testing.

Tests

Vaccines, reconstituted where necessary, comply with the following requirements unless otherwise stated in the individual monograph.

Phenol (*If present*) (2.3.36). Not more than 0.25 per cent w/v.

Thiomersal (*If present*) (2.3.48). Between 0.005 per cent w/v and 0.02 per cent w/v.

Free formaldehyde (*If present*) (2.3.20). Maximum 0.2 g/l.

Aluminium (*If present*) (2.3.9). Not more than 1.25 mg per dose.

Sterility (2.2.11). Unless otherwise stated all vaccines comply with tests for sterility, except that for living bacterial vaccines, growth of the organism from which the vaccine was prepared is permitted (sterility means absence of bacterial and fungal contaminants except where specified in the individual monograph).

Abnormal toxicity (2.2.1). Unless otherwise stated, all vaccines comply with the test for abnormal toxicity, Method B. In vaccines containing phenol as preservative, the test in mice may be inappropriate.

NOTE — The statements given in this general chapter is intended to be read in conjunction with the monographs on the individual vaccine in this Pharmacopoeia which refer to preparations for human use; they do not necessarily apply to vaccines for use in veterinary practice.

Storage. Liquid vaccines must be stored at a temperature between 2° and 8° and should not be allowed to freeze unless otherwise specified in the individual monograph. Freeze-dried preparations must be stored at temperatures between 2° and 8° and for long term storage a temperature of -20° protected. At higher temperatures vaccines deteriorate rapidly.

Labelling. The label states (1) for liquid vaccines, the total number of ml in the container and, for freeze dried vaccines, the number of doses in the container; (2) unless otherwise indicated the minimum number of Units per dose or per ml or, for viral vaccines, the minimum viral titre; (3) the dose and route of administration; (4) the name and proportion of any antibacterial preservative or other auxiliary substances added to the vaccine; (5) the date after which the vaccine is not intended to be used; (6) the conditions under which it should be stored; (7) for freeze dried vaccines, the liquid to be used for reconstitution and its volume; (8) that the vaccine should be used immediately after reconstitution; (9) unless otherwise directed, that the vaccine should be shaken well before use; (10) any contraindication to the use of the vaccine.

Antisera

Immunosera

Antisera are native (unconcentrated) sera or preparations from native sera containing specific immunoglobulins that have prophylactic or therapeutic action when injected into persons exposed to or suffering from a disease caused by a specific micro-organism.

Antisera are prepared by injecting antigens which are preparations of cultures of the specific organisms or venoms or their products into healthy humans or animals such as horses so as to produce in them antibodies which are normally associated with the globulin fraction of serum. Antigens commonly used for this purpose are toxins, toxoids, venoms, bacterial and viral vaccines. Non-lethal amounts of the toxin or the corresponding toxoid/vaccine are injected in gradually increasing doses into animals. Specific antitoxins are formed in the serum and the animals become actively immune. During the process of immunisation, the animals should not be treated with penicillin. When a satisfactory degree of the immunity is produced, larger volumes of blood are withdrawn from the animals and the plasma or serum is processed to produce specific antisera. The globulins may be obtained from the immune serum by enzyme treatment and fractional precipitation or by other physical or chemical methods.

Antiviral sera with the exception of Rabies Antiserum are usually obtained from the plasma or serum of human patients who have recovered from the viral disease or who have been artificially immunised. Rabies Antiserum is obtained from animals by injecting gradually increasing doses of a rabies vaccine, a killed vaccine being used first and when some immunity is established living virus being used as an antigen. When sufficient virus-neutralising titre is reached the blood is collected and processed.

Antisera in liquid form are distributed under aseptic conditions into sterile containers which are then sealed so as to exclude micro-organisms. A suitable antibacterial substance may be added and is invariably added when the final product is filled in multiple dose containers. The product may be freeze-dried by a procedure which reduces the water content of the final product to less than 1.0 per cent w/w. Antisera are almost colourless or very faintly yellow liquids free from turbidity. Freeze-dried antisera consist of white or pale-yellow powders or friable masses which dissolve in water to form colourless or pale yellow liquids having the same characteristics as the corresponding liquid preparations.

Tests

The following tests refer to liquid antisera and to the reconstituted freeze-dried preparations.

pH (2.4.24). 6.0 to 7.0.

Total protein. Not more than 17.0 per cent w/v, determined by carrying out the determination of nitrogen, Method C (2.3.30) and multiplying the result by 6.25.

Foreign proteins. When examined by precipitation reactions with specific antisera, they are shown to consist exclusively of protein of the declared animal species.

Phenol (2.3.36) *(if present)*. Not more than 0.25 per cent w/v.

Sterility (2.2.11). Comply with the tests for sterility.

Abnormal toxicity (2.2.1). Comply with the test for abnormal toxicity. In antisera containing phenol as preservative, the test in mice may be inappropriate.

NOTE — The statements given in this general monograph are intended to be read in conjunction with the monographs on the individual antiserum in the Pharmacopoeia which refer to preparations for human use; they do not necessarily apply to antisera for use in veterinary practice.

Storage. Antisera should be stored at a temperature between 2° and 8° and should not be allowed to freeze unless otherwise stated in the individual monograph.

Labelling. The label states the name and quantity of antibacterial substance added, if any.

Adsorbed Diphtheria, Tetanus and Hepatitis B (rDNA) Vaccine

Diphtheria, Tetanus and Hepatitis B (rDNA) Vaccine (Adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; hepatitis B surface antigen (HBsAg); a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

HBsAg is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines, and with the following test for specific toxicity of the diphtheria and tetanus components: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

The content of bacterial endotoxins in the bulk purified diphtheria toxoid and tetanus toxoid is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxin is less than the limit approved for the particular vaccine and in any case the contents are such that the final vaccine contains less than 100 IU per single human dose.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed) and Hepatitis B Vaccine (rDNA).

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid and HBsAg onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the test for antimicrobial preservative and the assays for the diphtheria and tetanus components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant liquid obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The assay or, where applicable, the electrophoretic profile, serves also to identify the hepatitis B component of the vaccine.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject the equivalent of 1 human dose into each rabbit.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Hepatitis B component

It complies with the assay of Hepatitis B Vaccine.

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the amount of HBsAg per single human dose; (3) the type of cells used for production of the HBsAg component; (4) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (5) the name and the amount of the adsorbent; (6) that the vaccine must be shaken before use; (7) that the vaccine is not to be frozen.

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Haemophilus Type b Conjugate Vaccine

Diphtheria, Tetanus, Pertussis (Acellular Component) and Haemophilus Type b Conjugate Vaccine (Adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral absorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product may be presented with the haemophilus type b component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by

expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter two antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

If the vaccine is presented with the haemophilus component in a separate vial, as part of consistency studies the assays of the diphtheria, tetanus and pertussis components are carried out on a suitable number of batches of vaccine reconstituted for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

The content of bacterial endotoxins in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine; if the vaccine is presented with the haemophilus component in a separate container, the contents of the diphtheria, tetanus and pertussis antigens are in any case such that the final vial for these components contains less than 100 IU per single human dose.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell dependent B-cell immune response to PRP.

Reference vaccine

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the tests of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Acellular Component, Adsorbed) and Haemophilus Type b Conjugate Vaccine.

FINAL BULK VACCINE

Different methods of preparation may be used: a final bulk vaccine may be prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and PRP conjugate onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate; or 2 final bulks may be prepared and filled separately, one containing the diphtheria, tetanus and pertussis components, the other containing the haemophilus component, which may be freeze-dried. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid and antimicrobial preservative and the assays have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

pH (2.4.24). The pH of the vaccine, reconstituted if necessary, is within the range approved for the particular product.

Free PRP. Unbound PRP is determined after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography (2.4.16), ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

Identification

If the vaccine is presented with the haemophilus component in a separate vial: identification tests A, B and C are carried out using the vial containing the diphtheria, tetanus and pertussis components; identification test D is carried out on the vial containing the haemophilus components.

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in Identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in Identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The haemophilus component is identified by a suitable immunochemical method (2.2.14) for PRP.

Tests

If the product is presented with the haemophilus component in a separate container: the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus and pertussis components; the tests for PRP content, water (where applicable), sterility and pyrogens are carried out on the container with the haemophilus component.

If the haemophilus component is freeze-dried, some tests may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component under test.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not less than 5 histamine-sensitive mice. Inject intraperitoneally into each mouse of the first group twice the single human dose of the vaccine stored at 2° to 8°. Inject intraperitoneally into each mouse of the second group twice the single human dose of the vaccine incubated at 37° for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5.0 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously three-fold dilutions of a *reference pertussis toxin* preparation in *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine shows symptoms of sensitisation.

PRP. Minimum 80.0 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.7.1) or phosphorus (2.7.1), by an immunochemical method (2.2.14) or by anion-exchange liquid chromatography with pulsed-amperometric detection.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Water (2.3.43). Maximum 3.0 per cent for the freeze-dried haemophilus component.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to: 1 mg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 mg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 mg of PRP for a vaccine with OMP as carrier.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay as the stated Adsorbed Pertussis Vaccine (Acellular Component).

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the names and amounts of the pertussis components per single human dose; (3) the number of micrograms of PRP per single human dose; (4) the type and nominal amount of carrier protein per single human dose; (5) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (6) the name and the amount of the adsorbent; (7) that the vaccine must be shaken before use; (8) that the vaccine is not to be frozen; (9) where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Hepatitis B (rDNA) Vaccine

Diphtheria, Tetanus, Pertussis (Acellular Component) and Hepatitis B (rDNA) Vaccine (Adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; hepatitis B surface antigen; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties, produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Hepatitis B surface antigen is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The content of bacterial endotoxins in the bulk purified diphtheria toxoid, tetanus toxoid and pertussis components is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine

(Acellular Component, Adsorbed) and Hepatitis B Vaccine (rDNA).

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.2.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The assay or, where applicable, the electrophoretic profile, serves also to identify the hepatitis B component of the vaccine.

Tests

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not less than 5 histamine-sensitive mice. Inject intraperitoneally into each mouse of the first group twice the single human dose of the vaccine stored at 2° to 8°. Inject intraperitoneally into each mouse of the second group twice the single human dose of the vaccine incubated at 37° for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in phosphate-buffered saline solution containing 0.2 per cent w/v of gelatin and challenge with histamine as above; the strain is suitable if more than 50.0 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine shows symptoms of sensitisation.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not

greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject the equivalent of 1 human dose into each rabbit.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay as stated under Adsorbed Pertussis Vaccine (Acellular Component).

Hepatitis B component

The vaccine complies with the assay as stated under Hepatitis B Vaccine (rDNA).

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the names and amounts of the pertussis components per single human dose; (3) the amount of HBsAg per single human dose; (4) the type of cells used for production of the hepatitis B component; (5) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (6) the name and the amount of the adsorbent; (7) that the vaccine must be shaken before use; (8) that the vaccine is not to be frozen (9) where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component), Inactivated Poliomyelitis Vaccine and Haemophilus Type b Conjugate Vaccine

Diphtheria, Tetanus, Pertussis (Acellular Component), Inactivated Poliomyelitis Vaccine and Haemophilus Type b Conjugate Vaccine (Adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strain of human polioviruses 1,2, and 3 grown in suitable cell cultures and inactivated by validated method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral absorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product may be presented with the haemophilus type b component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The acellular pertussis component may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be co-purified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The content of bacterial endotoxins in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components, purified, inactivated monovalent poliovirus harvests and bulk PRP conjugate is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

The production method is validated to demonstrate that the product, if tested, would comply with the following test. Inject

subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell dependent B-cell immune response to PRP.

As part of consistency studies the assays of the diphtheria, tetanus, pertussis and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Acellular Component, Adsorbed), Poliomyelitis Vaccine (Inactivated) and Haemophilus Type b Conjugate Vaccine.

FINAL BULK VACCINE

The final bulk of the diphtheria, tetanus, pertussis and poliomyelitis components is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid and bulk purified acellular pertussis components onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of purified, monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added.

The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin. Determine on the poliomyelitis components by a suitable immunochemical method (2.2.14) during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will not be more than 50 ng per single human dose.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk of the haemophilus component is freeze-dried. Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the test for absence of residual pertussis toxin and irreversibility of pertussis toxoid, the test for antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens and the purified monovalent harvests or the trivalent pool of polioviruses or the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

Free PRP

Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange,

size-exclusion or hydrophobic chromatography (2.4.16), ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

Identification

Identification tests A, B, C and D are carried out using the vial containing the diphtheria, tetanus, pertussis and poliomyelitis components; identification test E is carried out on the vial containing the haemophilus component.

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by suitable immunochemical methods (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained during identification test A reacts with specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14), such as determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

E. The haemophilus component is identified by a suitable immunochemical method (2.2.14) for PRP.

Tests

The tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus, pertussis and poliomyelitis components; the tests for PRP content, water, sterility and pyrogens are carried out on the container with the haemophilus component.

Some tests for the haemophilus component may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component under test.

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into each mouse of the first group twice the single human dose of the vaccine stored at 2° to 8°. Inject intraperitoneally into each mouse of the second group twice the single human dose of the vaccine incubated at 37° for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following *histamine* challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a *reference pertussis toxin preparation* in *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as above; the strain is suitable if more than 50.0 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

PRP. Minimum 80.0 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.7.1) or phosphorus (2.7.1), by an immunochemical method (2.2.14) or by anion-exchange liquid chromatography with pulsed-amperometric detection.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Water (2.3.43). Maximum 3.0 per cent for the haemophilus component.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to: 1 mg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 mg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 mg of PRP for a vaccine with OMP as a carrier.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

Unless otherwise justified and authorised, the lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

It complies with the assay as stated under Adsorbed Pertussis Vaccine (Acellular Component).

Poliomyelitis component

D-antigen content

As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) using a reference preparation calibrated in units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) reference preparation* is calibrated in Units and intended for use in the assay of D-antigen. The Unit and the International Unit are equivalent.

In vivo test

The vaccine complies with the *in vivo* assay as stated under Inactivated Poliomyelitis Vaccine.

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose (2) the names and amounts of the pertussis components per single human dose; (3) the nominal amount of poliovirus of each type (1, 2 and 3), expressed in units of D-antigen per single human dose; (4) the type of cells used for production of the poliomyelitis component; (5) the number of micrograms of PRP per single human dose; (6) the type and nominal amount of carrier protein per single human dose; (7) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (8) the name and the amount of the adsorbent; (9) that the vaccine must be shaken before use; (10) that the vaccine is not to be frozen; (11) where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Adsorbed Diphtheria, Tetanus, Pertussis (Acellular Component) and Inactivated Poliomyelitis Vaccine

Diphtheria, Tetanus, Pertussis (Acellular Component) and Poliomyelitis (Inactivated) Vaccine is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; individually purified antigenic components of *Bordetella pertussis*; suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

The vaccine contains either pertussis toxoid or a pertussis-toxin-like protein free from toxic properties produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the toxin harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines.

The content of bacterial endotoxins in bulk purified diphtheria toxoid, tetanus toxoid, pertussis components and purified, inactivated monovalent poliovirus harvests is determined to monitor the purification procedure and to limit the amount in the final vaccine. For each component, the content of bacterial endotoxins is less than the limit approved for the particular vaccine and, in any case, the contents are such that the final vaccine contains less than 100 IU per single human dose.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between

the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Acellular Component, Adsorbed) and Poliomyelitis Vaccine (Inactivated).

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, acellular pertussis components and admixture of suitable quantities of purified monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Bovine serum albumin. Determine on the poliomyelitis components by a suitable immunochemical method (2.2.14) after virus harvest and before addition of the adsorbent in the preparation of the final bulk vaccine, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided the tests for absence of residual pertussis toxin, irreversibility of pertussis toxoid and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided the free formaldehyde content has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the determination of D-antigen content has been carried out with satisfactory results during preparation of the final bulk before addition of the adsorbent, it may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 h and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in Identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis components are identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained as described in Identification test A reacts with a specific antisera to the pertussis components of the vaccine.

D. The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

Tests

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not less than 5 histamine-sensitive mice. Inject intraperitoneally into each mouse of the first group twice the single human dose of the vaccine stored

at 2° to 8°. Inject intraperitoneally into each mouse of the second group twice the single human dose of the vaccine incubated at 37° for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of histamine base intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5.0 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously three-fold dilutions of a *reference pertussis toxin preparation* in *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as above; the strain is suitable if more than 50.0 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine shows symptoms of sensitisation.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than the minimum potency stated on the label.

Unless otherwise justified and authorised, the minimum potency stated on the label is 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose.

Pertussis component

The vaccine complies with the assay as stated under Adsorbed Pertussis Vaccine (Acellular Component).

Poliomyelitis component

D-antigen content

As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) following desorption using a reference preparation calibrated in units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) reference preparation* is calibrated in Units and intended for use in the assay of D-antigen. The Unit and the International Unit are equivalent.

In vivo test

The vaccine complies with the *in vivo* assay as stated under Inactivated Poliomyelitis Vaccine.

Labelling. The label complies with the requirements stated under Vaccine and also states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the names and amounts of the pertussis components per single human dose; (3) the nominal amount of poliovirus of each type (1, 2 and 3), expressed in units of D-antigen per single human dose; (4) the type of cells used for production of the poliomyelitis component; (5) the number of micrograms of PRP per single human dose; (6) the type and nominal amount of carrier protein per single human dose; (7) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (8) the name and the amount of the adsorbent; (9) that the vaccine must be shaken before use; (10) that the vaccine is not to be frozen; (11) where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification.

Adsorbed Diphtheria, Tetanus, Pertussis and Poliomyelitis (Inactivated) Vaccine

Diphtheria, Tetanus, Pertussis and Poliomyelitis (Inactivated) Vaccine (Adsorbed) is a combined vaccine containing: diphtheria formol toxoid; tetanus formol toxoid; an inactivated suspension of *Bordetella pertussis*; suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines, and with the following test for specific toxicity of the diphtheria and tetanus components : inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Adsorbed) and Poliomyelitis Vaccine (Inactivated).

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, separately or together, of suitable quantities of bulk purified diphtheria toxoid and bulk purified tetanus toxoid and admixture of suitable quantities of an inactivated suspension of *B. pertussis* and purified monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such purified monovalent harvests. Suitable antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Specific toxicity

Use not less than 5 healthy mice each weighing between 14 and 16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 hours before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 0.9 per cent sterile solution of *sodium chloride*, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 hours and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 hours the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Bovine serum albumin. Determine on the poliomyelitis components by a suitable immunochemical method (2.2.14) during preparation of the final bulk vaccine; before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will be not more than 50 ng per single human dose.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for specific toxicity and antimicrobial preservative, and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens, the inactivated *B. pertussis* suspension and the purified monovalent harvests or the trivalent pool of polioviruses or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The centrifugation residue obtained in identification A may be used. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay of the pertussis component prescribed under Assay.

D. The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

If the test is carried out in guinea pigs, the lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit (P = 0.95) of the estimated potency is not less than 60 IU per single human dose.

Pertussis component

Carry out the assay as stated under Pertussis Vaccine.

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Poliomyelitis component

D-antigen content

As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) using a reference preparation calibrated in Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) reference preparation* is calibrated in Units and is intended for use in the assay of D-antigen. The Unit and the IU are equivalent.

In vivo test

The vaccine complies with the *in vivo* assay as stated under Poliomyelitis Vaccine (Inactivated).

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the minimum number of International Units of pertussis vaccine per single human dose; (3) the nominal amount of poliovirus of each type (1, 2 and 3), expressed in units of D-antigen per single human dose; (4) the type of cells used for production of the poliomyelitis component; (5) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (6) the name and the amount of the adsorbent; (7) that the vaccine must be shaken before use; (8) that the vaccine is not to be frozen.

Adsorbed Diphtheria, Tetanus, Pertussis, Poliomyelitis (Inactivated) and Haemophilus Type b Conjugate Vaccine

Diphtheria, Tetanus, Pertussis, Poliomyelitis (Inactivated) and Haemophilus Type b Conjugate Vaccine (Adsorbed) is a combined vaccine composed of: diphtheria formol toxoid; tetanus formol toxoid; an inactivated suspension of *Bordetella pertussis*; suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a suitable method; polyribosylribitol phosphate (PRP) covalently bound to a carrier protein; a mineral adsorbent such as aluminium hydroxide or hydrated aluminium phosphate. The product is presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before use.

The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively.

PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines, and with the following test for specific toxicity of the diphtheria and tetanus components: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal dies in the second test, the vaccine does not comply with the test.

During development studies and wherever revalidation is necessary, it shall be demonstrated by tests in animals that the vaccine induces a T-cell dependent B-cell immune response to PRP.

As part of consistency studies the assays of the diphtheria, tetanus, pertussis and poliomyelitis components are carried out on a suitable number of batches of vaccine reconstituted for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilised by a method that has been shown to have no effect on the assay procedure.

Production of components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Adsorbed), Poliomyelitis Vaccine (Inactivated) and Haemophilus Type b Conjugate Vaccine.

FINAL BULK VACCINE

The final bulk of the diphtheria, tetanus, pertussis and poliomyelitis components is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, and bulk purified tetanus toxoid onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate and admixture of suitable quantities of an inactivated suspension of *B. pertussis* and of purified, monovalent harvests of human polioviruses 1, 2 and 3 or a suitable quantity of a trivalent pool of such monovalent harvests. Suitable antimicrobial preservatives may be added.

The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabiliser may be added.

Only final bulk that complies with the following requirements may be used in the preparation of the final lot.

Specific toxicity

Use not less than 5 healthy mice each weighing between 14 and 16 g, for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 hours before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with

0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 0.9 per cent sterile solution of *sodium chloride*, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection and 72 hours and 7 days after the injection. The vaccine complies with the test if (a) at the end of 72 hours the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Bovine serum albumin. Determine on the poliomyelitis components by a suitable immunochemical method (2.2.14) during preparation of the final bulk vaccine, before addition of the adsorbent, the amount of bovine serum albumin is such that the content in the final vaccine will not be more than 50 ng per single human dose.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk of the haemophilus component is freeze-dried.

Only a final lot that is satisfactory with respect to the test for osmolality shown below and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for specific toxicity and antimicrobial preservative, and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Provided that the free formaldehyde content has been determined on the bulk purified antigens, the inactivated *B. pertussis* suspension and the purified monovalent harvests or the trivalent pool of polioviruses or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Provided that the *in vivo* assay for the poliomyelitis component has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine, reconstituted where applicable, is within the limits approved for the particular preparation.

Free PRP

Unbound PRP is determined on the haemophilus component after removal of the conjugate, for example by anion-exchange, size-exclusion or hydrophobic chromatography (2.4.16), ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

Identification

Identification tests A, B, C and D are carried out using the vial containing the diphtheria, tetanus, pertussis and poliomyelitis components; identification test E is carried out on the vial containing the haemophilus component.

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The centrifugation residue obtained in identification A may be used. Other suitable methods for separating the bacteria from the adsorbent may also be used. Identify pertussis vaccine by agglutination of the bacteria from the resuspended precipitate by antisera specific to *B. pertussis* or by the assay of the pertussis component prescribed under Assay.

D. The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14), such as determination of D-antigen by enzyme linked immunosorbent assay (ELISA).

E. The haemophilus component is identified by a suitable immunochemical method (2.2.14) for PRP.

Tests

The tests for specific toxicity, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with diphtheria, tetanus, pertussis and poliomyelitis components; the tests for PRP content, water, sterility and pyrogens are carried out on the container with the haemophilus component.

Some tests for the haemophilus component may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component under test.

PRP. Minimum 80.0 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.7.1) or

phosphorus (2.7.1), by an immunochemical method (2.2.14) or by anion-exchange liquid chromatography with pulsed-amperometric detection.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Water (2.3.43). Maximum 3.0 per cent for the haemophilus component.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to 1 mg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 mg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 mg of PRP for a vaccine with OMP as carrier.

Assay

Diphtheria component

Carry out one of the prescribed methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

If the test is carried out in guinea-pigs, the lower confidence limit (P = 0.95) of the estimated potency is not less than 40 IU per single human dose; if the test is carried out in mice, the lower confidence limit (P = 0.95) of the estimated potency is not less than 60 IU per single human dose.

Pertussis component

Carry out the assay as stated under Pertussis Vaccine.

The estimated potency is not less than 4 IU per single human dose and the lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Poliomyelitis component

D-antigen content

As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) using a reference preparation calibrated in Units of D-antigen. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product. *Poliomyelitis vaccine (inactivated) reference preparation* is calibrated in Units and intended for use in the assay of D-antigen. The Unit and the IU are equivalent.

In vivo test

The vaccine complies with the *in vivo* assay as stated under Poliomyelitis Vaccine (Inactivated).

Labelling. The label states (1) the minimum number of International Units of diphtheria and tetanus toxoid per single human dose; (2) the minimum number of International Units of pertussis vaccine per single human dose; (3) the nominal amount of poliovirus of each type (1, 2 and 3), expressed in Units of D-antigen per single human dose; (4) the type of cells used for production of the poliomyelitis component; (5) the number of micrograms of PRP per single human dose; (6) the type and nominal amount of carrier protein per single human dose; (7) where applicable, that the vaccine is intended for primary vaccination of children and is not necessarily suitable for reinforcing doses or for administration to adults; (8) the name and the amount of the adsorbent; (9) that the vaccine must be shaken before use; (10) that the vaccine is not to be frozen.

Adsorbed Pertussis Vaccine (Acellular Component)

Pertussis Vaccine (Acellular Component, Adsorbed) is a preparation of individually prepared and purified antigenic components of *Bordetella pertussis* adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

The vaccine contains either pertussis toxoid or a pertussis toxin, like protein free from toxic properties, produced by expression of a genetically modified form of the corresponding gene. Pertussis toxoid is prepared from pertussis toxin by a method that renders the latter harmless while maintaining adequate immunogenic properties and avoiding reversion to toxin. The vaccine may also contain filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. The latter 2 antigens may be copurified. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine

A batch of vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine is preferably stabilised by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently individual components that comply with the following requirements; after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylate cyclase. Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin. Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography (2.4.14).

Absence of residual dermonecrotic toxin. Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of component or antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 hours. No dermonecrotic reaction is demonstrable.

Specific properties. The components of the vaccine are analysed by one or more of the methods shown below in order to determine their identity and specific properties (activity per unit amount of protein) in comparison with reference preparations.

Pertussis toxin

Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as *in vitro* methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as *in vivo* methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin

Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens. Reactivity with specific antibody.

Pertussis toxoid

The toxoid induces in animals production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED COMPONENTS

Production of each component is based on a seed-lot system. The seed cultures from which toxin is prepared are managed to conserve or where necessary restore toxinogenicity by deliberate selection.

None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed lots and inocula may contain blood or blood products of animal origin.

Pertussis toxin and, where applicable, filamentous haemagglutinin and pertactin are purified and, after appropriate characterisation, detoxified using suitable chemical reagents, by a method that avoids reversion of the toxoid to toxin, particularly on storage or exposure to heat. Other components such as fimbrial-2 and fimbrial-3 antigens are purified either separately or together, characterised and shown to be free from toxic substances. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.

The content of bacterial endotoxins is determined to monitor the purification procedure and to limit the amount in the final vaccine. The limits applied for the individual components are such that the final vaccine contains less than 100 IU per single human dose.

Before detoxification, the purity of the components is determined by a suitable method such as polyacrylamide gel electrophoresis (PAGE) or liquid chromatography. SDS-PAGE or immunoblot analysis with specific monoclonal or polyclonal antibodies may be used to characterise subunits. Requirements are established for each individual product.

Only purified components that comply with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility (2.2.11). Carry out the test for sterility using for each medium a quantity of purified component equivalent to not less than 100 doses.

Absence of residual pertussis toxin

This test is not necessary for the product obtained by genetic modification. Use a group of not fewer than 5 histamine-sensitive mice each weighing between 18 and 26 g. Inject into each mouse the equivalent of 1 human dose intravenously or twice the human dose intraperitoneally, diluted to not more than 0.5 ml with *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin*. Inject diluent into a second group of control mice. After 5 days, inject 2 mg of *histamine base* intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. If no animal dies, the preparation complies with the test.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject three-fold dilutions of a

reference pertussis toxin preparation in *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

A validated test based on the clustering effect of the toxin for Chinese hamster ovary (CHO) cells may be used instead of the test on mice.

Residual detoxifying agents and other reagents

The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content

Determine the antigen content by a suitable immunochemical method (2.2.14) and protein nitrogen by sulphuric acid digestion (2.2.30) or another suitable method. The ratio of antigen content to protein nitrogen is within the limits established for the product.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of suitable quantities of purified components, separately or together, onto aluminium hydroxide or hydrated aluminium phosphate. A suitable antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for absence of residual pertussis toxin and irreversibility of pertussis toxoid, antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

Identification

Subject the vaccine to a suitable desorption procedure such as the following: dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution; maintain at 37° for about 16 hours and centrifuge until a clear supernatant liquid is obtained. Examined by a suitable immunochemical method (2.2.14), the clear supernatant liquid reacts with specific antisera to the components stated on the label.

Tests

Absence of residual pertussis toxin and irreversibility of pertussis toxoid

This test is not necessary for the product obtained by genetic modification. Use 3 groups each of not fewer than 5 histamine-sensitive mice. Inject intraperitoneally into the first group twice the single human dose of the vaccine stored at 2° to 8°. Inject intraperitoneally into the second group twice the single human dose of the vaccine incubated at 37° for 4 weeks. Inject diluent into the third group of mice. After 5 days, inject into each mouse 2 mg of *histamine base* intraperitoneally in a volume not exceeding 0.5 ml and observe for 24 hours. The test is invalid if 1 or more control mice die following histamine challenge. The vaccine complies with the test if no animal in the first or second group dies following histamine challenge. If 1 mouse dies in either or both of the first and second groups, the test may be repeated with the same number of mice or with a greater number and the results of valid tests combined; the vaccine complies with the test if, in both of the groups given the vaccine, not more than 5.0 per cent of the total number of mice die following histamine challenge.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject intravenously threefold dilutions of a reference pertussis toxin preparation in *phosphate-buffered saline solution* containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as above; the strain is suitable if more than 50.0 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Assay

The capacity of the vaccine to induce the formation of specific antibodies is compared with the same capacity of a reference

preparation examined in parallel; antibodies are determined using suitable immunochemical methods (2.2.14) such as enzyme-linked immunosorbent assay (ELISA). The test on mice shown below uses a three-point model but, after validation, for routine testing a single-dilution method may be used.

Requirement

The capacity to induce antibodies is not significantly (P = 0.95) less than that of the reference vaccine.

The following test model is given as an example of a method that has been found to be satisfactory.

Selection and distribution of test animals

Use in the test healthy mice (for example, CD1 strain) of the same stock 4 to 8 weeks old. Distribute the animals in 6 groups of a number appropriate to the requirements of the assay. Use 3 dilutions of the vaccine under examination and 3 dilutions of a *reference preparation* and attribute each dilution to a group of mice. Inject intraperitoneally or subcutaneously into each mouse 0.5 ml of the dilution attributed to its group.

Collection of serum samples

4 to 5 weeks after vaccination, bleed the mice individually under anaesthesia. Store the sera at -20° until tested for antibody content.

Antibody determination

Assay the individual sera for content of specific antibodies to each component using a validated method such as the ELISA test shown below.

ELISA

Microtitre plates (polyvinyl chloride or polystyrene as appropriate for the specific antigen) are coated with the purified antigen at a concentration of 100 ng per well. After washing, unreacted sites are blocked by incubating with a *solution of bovine serum albumin* and then washed. Two-fold dilutions of sera from mice immunised with test or reference vaccines are made on the plates. After incubation at 22° to 25° for 1 hour, the plates are washed. A suitable solution of *anti-mouse IgG enzyme conjugate* is added to each well and incubated at 22° to 25° for 1 hour. After washing, a substrate is added from which the bound enzyme conjugate liberates a chromophore which can be quantified by measurement of absorbance. The test conditions are designed to obtain a linear response for absorbance with respect to antibody content over the range of measurement used and absorbance values within the range 0.1 to 2.0.

A reference antiserum of assigned potency is used in the test and serves as the basis for calculation of the antibody levels in test sera. A standardised control serum is also included in the test.

The test is not valid if (a) the value found for the control serum differs by more than 2 standard deviations from the assigned value; (b) the confidence interval of the potency estimate is greater than 50.0 per cent to 200.0 per cent.

Calculation

The antibody titres in the sera of mice immunised with reference and test vaccines are calculated and from the values obtained the potency of the test vaccine in relation to the reference vaccine is calculated by the usual statistical methods.

Labelling. The label states (1) the names and amounts of the components present in the vaccine; (2) where applicable, that the vaccine contains a pertussis toxin-like protein produced by genetic modification; (3) the name and amount of the adsorbent; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen.

Adsorbed Pertussis Vaccine (Acellular, Co-Purified)

Pertussis Vaccine (Acellular, Co-Purified, Adsorbed) is a preparation of antigenic components of *Bordetella pertussis* adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

The vaccine contains an antigenic fraction purified without separation of the individual components. The antigenic fraction is treated by a method that transforms pertussis toxin to toxoid, rendering it harmless while maintaining adequate immunogenic properties of all the components and avoiding reversion to toxin. The antigenic fraction is composed of pertussis toxoid, filamentous haemagglutinin, pertactin (a 69 kDa outer-membrane protein) and other defined components of *B. pertussis* such as fimbrial-2 and fimbrial-3 antigens. It may contain residual pertussis toxin up to a maximum level approved by the competent authority. The antigenic composition and characteristics are based on evidence of protection and freedom from unexpected reactions in the target group for which the vaccine is intended.

Production

General provisions

The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

Reference vaccine. A batch of vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine is preferably stabilised, by a method that has been shown to have no significant effect on the assay procedure when the stabilised and non-stabilised batches are compared.

CHARACTERISATION OF COMPONENTS

During development of the vaccine, the production process shall be validated to demonstrate that it yields consistently an antigenic fraction that complies with the following requirements; after demonstration of consistency, the tests need not be applied routinely to each batch.

Adenylate cyclase. Not more than 500 ng in the equivalent of 1 dose of the final vaccine, determined by immunoblot analysis or another suitable method.

Tracheal cytotoxin. Not more than 2 pmol in the equivalent of 1 dose of the final vaccine, determined by a suitable method such as a biological assay or liquid chromatography (2.4.14).

Absence of residual dermonecrotic toxin. Inject intradermally into each of 3 unweaned mice, in a volume of 0.1 ml, the amount of antigenic fraction equivalent to 1 dose of the final vaccine. Observe for 48 hours. No dermonecrotic reaction is demonstrable.

Specific properties. The antigenic fraction is analyzed by one or more of the methods shown below in order to determine the identity and specific properties (activity per unit amount of protein) of its components in comparison with reference preparations.

Pertussis toxin

Chinese hamster ovary (CHO) cell-clustering effect and haemagglutination as *in vitro* methods; lymphocytosis-promoting activity, histamine-sensitising activity and insulin secretory activity as *in vivo* methods. The toxin shows ADP-ribosyl transferase activity using transducin as the acceptor.

Filamentous haemagglutinin

Haemagglutination and inhibition by specific antibody.

Pertactin, fimbrial-2 and fimbrial-3 antigens. Reactivity with specific antibody.

Pertussis toxoid

The toxoid induces in animals the production of antibodies capable of inhibiting all the properties of pertussis toxin.

PURIFIED ANTIGENIC FRACTION

Production of the antigenic fraction is based on a seed-lot system. The seed cultures are managed to conserve or, where necessary, restore toxinogenicity by deliberate selection.

None of the media used at any stage contains blood or blood products of human origin. Media used for the preparation of seed batches and inocula may contain blood or blood products of animal origin.

The antigenic fraction is purified and, after appropriate characterisation, detoxified using suitable reagents by a method that ensures minimal reversion of toxoid to toxin, particularly on or exposure to heat. The purification procedure is validated to demonstrate appropriate clearance of substances used during culture or purification.

The content of bacterial endotoxins is determined to monitor the purification procedure and to limit the amount in the final vaccine. The limits applied are such that the final vaccine contains not more than 100 IU per single human dose.

Before detoxification, the purity of the antigenic fraction is determined by a suitable method such as polyacrylamide gel electrophoresis (PAGE) (2.4.12) or liquid chromatography (2.4.14). SDS-PAGE or immunoblot analysis with specific monoclonal or polyclonal antibodies may be used to characterise subunits. Requirements are established for each individual product.

Only a purified antigenic fraction that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility (2.2.11). Carry out the test for sterility using for each medium a quantity of purified antigenic fraction equivalent to not less than 100 doses of the final vaccine.

Test for residual pertussis toxin. Use 3 groups of not fewer than 5 histamine-sensitive mice each weighing between 18 and 26 g. Using *phosphate-buffered saline* containing 0.2 per cent of *gelatin*, prepare a series of dilutions of the *purified antigenic fraction* that have been shown to yield a graded response and attribute each dilution to a separate group of mice. Inject intraperitoneally into each mouse the dilution attributed to its group. Inject *diluent* into a fourth group of control mice. After 5 days, inject intraperitoneally into each mouse 1 mg of *histamine base* in a volume not exceeding 0.5 ml. Record the number of animals that die within 24 hours of histamine challenge. Calculate the weight or volume of a preparation that sensitises 50.0 per cent of the mice injected using a suitable statistical method such as probit analysis. The residual activity of pertussis toxin does not exceed that of batches shown to be safe in clinical studies.

The histamine sensitivity of the strain of mice used is verified at suitable intervals as follows: inject threefold dilutions of a *reference pertussis toxin preparation* in *phosphate-buffered saline* solution containing 0.2 per cent w/v of *gelatin* and challenge with *histamine* as described above; the strain is suitable if more than 50 per cent of the animals are sensitised by 50 ng of pertussis toxin and none of the control animals injected with only diluent and challenged similarly with histamine show symptoms of sensitisation.

A validated test based on the clustering effect of the toxin for Chinese hamster ovary (CHO) cells may be used instead of the test on mice.

Residual detoxifying agents and other reagents. The content of residual detoxifying agents and other reagents is determined and shown to be below approved limits unless validation of the process has demonstrated acceptable clearance.

Antigen content. Determine the complete quantitative antigen composition of the antigenic fraction by suitable immunochemical methods (2.2.14) and protein nitrogen by sulphuric acid digestion or another suitable method. The ratio of total antigen content to protein nitrogen is within the limits established for the product.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of a suitable quantity of the antigenic fraction onto aluminium hydroxide or hydrated aluminium phosphate. A suitable antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for residual pertussis toxin, reversibility of toxoid, antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

Identification

Subject the vaccine to a suitable desorption procedure such as the following: dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution; maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. Examine by a suitable immunochemical method (2.2.14), the clear supernatant reacts with specific antisera to the components in the vaccine.

Tests

Test for residual pertussis toxin. Use 3 groups of not fewer than 5 histamine-sensitive mice (see under Production) each weighing between 18 and 26 g. Using *phosphate-buffered saline* containing 0.2 per cent w/v of *gelatin*, prepare a series of dilutions of the vaccine under examination that have been shown to yield a graded response and attribute each dilution to a separate group of mice. Inject intraperitoneally into each mouse the dilution attributed to its group. Inject *diluent* into a fourth group of control mice. After 5 days, inject intraperitoneally into each mouse 1 mg of *histamine base* in a volume not exceeding 0.5 ml. Note the number of animals that die within 24 hours of histamine challenge. Calculate the weight or volume of a preparation that sensitises 50 per cent of the mice injected using a suitable statistical method such as probit analysis. The residual activity of pertussis toxin does not exceed that of batches shown to be safe in clinical studies.

Reversibility of toxoid. Carry out the test for residual pertussis toxin described above using the vaccine incubated at 37° for 4 weeks in parallel with a sample stored at 2° to 8°. The degree of reversibility does not exceed that of batches shown to be safe in clinical studies.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Sterility (2.2.11). Complies with the test for sterility.

Assay

The vaccine complies with the assay as stated under Adsorbed Pertussis Vaccine (Acellular Component).

Labelling. The label states (1) the names and amounts of the antigenic components present in the vaccine; (2) the maximum amount of residual pertussis toxin present in the vaccine; (3) the maximum degree of reversion of toxoid to toxin during the period of validity; (4) the name and amount of the adsorbent; (5) that the vaccine must be shaken before use; (6) that the vaccine is not to be frozen.

Bacillus Calmette-Guerin Vaccine (Freeze-Dried)

Freeze-dried BCG Vaccine is a preparation of live bacteria derived from a culture of the bacillus of Calmette and Guérin (*Mycobacterium bovis* BCG) capacity of which to protect against tuberculosis has been established.

Vaccine complies with the requirements stated under Vaccines with the following modifications.

Production

General provisions

The production method is validated to demonstrate that the product, if tested, would comply with the tests for safety and efficacy.

BCG vaccine shall be produced by a staff consisting of healthy persons who do not work with other infectious agents; in particular they shall not work with virulent strains of *Mycobacterium tuberculosis*, during the course of production cycle nor shall they be exposed to a known risk of tuberculosis infection. BCG vaccine is susceptible to sunlight: the procedures for the preparation of the vaccine shall be so designed that all cultures and vaccines are protected from direct sunlight and from ultraviolet light at all stages of manufacture, testing and storage.

Production of the vaccine is based on a seed-lot system. The production method shall have been shown to yield consistently BCG vaccines that induce adequate sensitivity to tuberculin in man, that have acceptable protective potency in animals and are safe. The vaccine is prepared from cultures which are derived from the master seed lot by as few subcultures as possible and in any case not more than 12 subcultures e.g. If the secondary seed lot is 4 culture passages removed from the primary seed lot, the number of passages from the secondary seed lot must not exceed 8.

The capacity of the working seed lot to induce sensitivity to tuberculin in guinea-pigs is demonstrated.

If a bioluminescence test or other biochemical method is used instead of viable count, the method is validated against the viable count for each stage of the process at which it is used.

SEED LOT

The strain used to establish the master seed lot is chosen for and maintained to preserve its stability, its capacity to sensitise man and guinea-pigs to tuberculin and to protect animals against tuberculosis, and its relative absence of pathogenicity for man and laboratory animals. The strain used shall be identified by historical records that include information on its origin and subsequent manipulation.

A suitable batch of vaccine is prepared from the first working seed lot and is reserved for use as the comparison/ in-house reference vaccine. When a new working seed lot is established, a suitable test for delayed hypersensitivity in guinea-pigs is carried out on a batch of vaccine prepared from the new working seed lot; the vaccine is shown to be not significantly different in activity from the comparison vaccine.

Only a working seed lot that complies with the following requirements may be used for propagation.

Identification

The bacteria in the working seed lot are identified as *Mycobacterium bovis* BCG using microbiological techniques, which may be supplemented by molecular biology techniques (for example, nucleic acid amplification and restriction-fragment-length polymorphism).

Sterility (2.2.11). Complies with the test for sterility, carried out using 10 ml for each medium. The working seed lot complies with the test for sterility except for the presence of mycobacteria.

Virulent mycobacteria

Examine the working seed lot as prescribed under Tests, using 10 guinea pigs.

PROPAGATION AND HARVEST

The bacteria are grown in a suitable medium for not more than 21 days by surface or submerged culture. The culture medium shall contain no substances known to cause toxic or allergic reactions in human beings or to cause the bacteria to become virulent for guinea-pigs. The culture is harvested and suspended in a sterile liquid medium that protects the viability of the vaccine as determined by a suitable method of viable count.

Test for purity. Purity is checked by acid fast staining.

FINAL BULK VACCINE

The final bulk vaccine is prepared from a single harvest or by pooling a number of single harvests. A stabiliser may be added; if the stabiliser interferes with the determination of bacterial concentration on the final bulk vaccine, the determination is carried out before addition of the stabiliser.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Virulent mycobacteria. Examine as prescribed under Tests.

Sterility (2.2.11). Complies with the test for sterility using 10 ml for each medium except for the presence of mycobacteria.

Count of viable units

Determine the number of viable units per ml by viable count on solid medium using a method suitable for the vaccine under examination or by determination of adenosine triphosphate by a bioluminescence reaction. Carry out the test in parallel on a *reference preparation* of the same strain.

Bacterial concentration

Determine the total bacterial concentration by a suitable method, either directly by determining the mass of the micro-organisms, or indirectly by an opacity method that has been

calibrated in relation to the mass of the organisms; if the bacterial concentration is determined before addition of a stabiliser, the concentration in the final bulk vaccine is established by calculation. The total bacterial concentration is within the limits approved for the particular product by National Regulatory Authority.

The ratio of the count of viable units to the total bacterial concentration is not less than that approved for the particular product by National Regulatory Authority.

FINAL LOT

The final bulk vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine; the containers are closed either under vacuum or under a gas that is not deleterious to the vaccine.

Except where the filled and closed containers are stored at a temperature of -20° or lower, the expiry date is not later than 4 years from the date of harvest.

Only a final lot that complies with the following requirement for count of viable units and with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the test for virulent mycobacteria has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot. Provided the test for excessive dermal reactivity has been carried out with satisfactory results on the working seed lot and on 5 consecutive final lots produced from it, the test may be omitted on the final lot.

Count of viable units

Determine the number of viable units per ml of the reconstituted vaccine by viable count on solid medium using a method suitable for the vaccine under examination or by determination of adenosine triphosphate by a bioluminescence reaction. The ratio of the count of viable units after freeze-drying to that before is not less than that approved for the particular product.

Identification

BCG vaccine is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property and by the characteristic appearance of colonies grown on solid medium. Alternatively, molecular biology techniques (like nucleic acid amplification) may be used.

Tests

Virulent mycobacteria

If this test is satisfactory at final bulk stage it can be omitted at the final lot.

Inject subcutaneously or intramuscularly into each of 6 guinea-pigs, each weighing between 250 and 400 g and having received no treatment likely to interfere with the test, a quantity of vaccine equivalent to at least 50 human doses. Observe the animals for at least 42 days. At the end of this period, kill the guinea-pigs and examine by autopsy for signs of infection with tuberculosis, ignoring any minor reactions at the site of injection. Animals that die during the observation period are also examined for signs of tuberculosis. The vaccine complies with the test if none of the guinea-pigs shows signs of tuberculosis and if not more than one animal dies during the observation period. If 2 animals die during this period and autopsy does not reveal signs of tuberculosis repeat the test on 6 other guinea-pigs. The vaccine complies with the test if not more than one animal dies during the 42 days following the injection and autopsy does not reveal any sign of tuberculosis.

Sterility (2.2.11). The reconstituted vaccine complies with the test for sterility except for the presence of mycobacteria.

Excessive dermal reactivity

Use 6 healthy white or pale-coloured guinea-pigs, each weighing not less than 250 g and having received no treatment likely to interfere with the test. Inject intradermally into each guinea-pig, according to a randomised plan, 0.1 ml of the reconstituted vaccine and of 2 tenfold serial dilutions of the vaccine and identical doses of the comparison vaccine. Observe the lesions formed at the site of the injection for 4 weeks. The vaccine complies with the test if the reaction it produces is not markedly different from that produced by the comparison vaccine.

Temperature stability

Maintain samples of the freeze-dried vaccine at 37° for 4 weeks. Determine the number of viable units in the heated vaccine and in unheated vaccine as described below. The number of viable units in the heated vaccine is not less than 20.0 per cent of that in unheated vaccine.

Water (2.3.43). Not more than 3.0 per cent, determined by the semi-micro determination of water.

Assay

Determine the number of viable units in the reconstituted vaccine by viable count on solid medium or using a suitable validated biochemical method for the vaccine under examination. The number is within the range stated on the label. Determine the number of viable units in the comparison vaccine in parallel.

Labelling. The label states (1) the minimum and maximum number of viable units per ml in the reconstituted vaccine; (2) that the vaccine must be protected from direct sunlight;

(3) that the vaccine is to be used immediately after broaching the container; (4) the age group for which the vaccine is intended; (5) the dose for each age group; (6) follow instructions as mentioned in the product insert/leaflet.

Diphtheria Antitoxin

Diphtheria Antitoxin is a preparation containing the specific antitoxic globulins or their derivatives obtained by purification of hyperimmune serum or plasma of healthy horses or other suitable animals and having the specific activity of neutralising the toxin formed by *Corynebacterium diphtheriae*. The liquid preparation may contain a suitable antimicrobial preservative.

Diphtheria Antitoxin has a potency of not less than 1000 Units per ml when obtained from horse serum and not less than 500 Units per ml when obtained from other animals.

Description. A clear, colourless or pale yellow liquid or a freeze-dried, cream-coloured powder or pellet.

Identification

Specifically neutralises and renders the toxin formed by *C. diphtheriae* harmless to susceptible animals or by any other suitable *in-vitro* test.

Tests

Potency. Carry out the biological assay of diphtheria antitoxin described below.

Biological Assay of Diphtheria Antitoxin

The potency of diphtheria antitoxin is determined by comparing the dose necessary to protect guinea-pigs or rabbits against the erythrogenic effects of a fixed dose of the Standard Preparation of diphtheria antitoxin necessary to give the same protection. For this purpose, a suitable preparation of diphtheria toxin is required to be used as a test toxin. The test dose of the toxin is determined in relation to the Standard Preparation. The potency of the preparation under examination is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation

The Standard Preparation is the 1st International Standard for Diphtheria antitoxin, equine, established in 1934, consisting of the dried hyperimmune horse serum and glycerin, or another suitable preparation the potency of which has been determined in relation to the International Standard.

Suggested Method

Test toxin. Prepare diphtheria toxin by filtering through bacteria-proof filter the medium in which a toxigenic strain of *C. diphtheriae* has grown. Store at a temperature of 2° and 8°.

Selection of test toxin. In selecting a toxin for use as the test toxin determine the following.

Lr/100 dose — This is the smallest quantity of the toxin which, when mixed with 0.01 Unit of antitoxin and injected intracutaneously into guinea-pigs or rabbits causes a characteristic reaction at the site of the injection within 48 hours.

Minimal reacting dose — This is the smallest quantity of toxin which, when injected intracutaneously into guinea-pigs or rabbits, causes a characteristic reaction at the site of injection within 48 hours.

A suitable toxin is one which contains at least 200 minimal reacting doses in the Lr/100 dose. The test toxin is allowed to stand for some months before being used for the assay of samples of antitoxin. During this time its toxicity declines and the Lr/100 dose may be slightly increased. When experiment shows that the Lr/100 dose is constant, the test toxin is ready for use and may be used for a long period. Determine the minimal reacting dose and the Lr/100 dose at frequent intervals. Store the test toxin in the dark at a temperature between 0° and 5°. Maintain its sterility by the addition of *toluene* or other antimicrobial preservative which does not cause a rapid decline in specific toxicity.

Determination of test dose of toxin (*Lr/100 dose*). Prepare a solution of the Standard Preparation with *saline solution* such that 1 ml contains 0.1 Unit. Prepare mixtures such that 2.0 ml of each mixture contains 1.0 ml of the dilution of the Standard Preparation (0.1 Unit) and one of a series of graded volumes of the test toxin. Dilute each mixture with *saline solution* to the same final volume (2.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 15 to 60 minutes and inject intracutaneously 0.2 ml of each mixture at suitably spaced sites into the shaven or depilated flanks of two animals. Observe the animals for 48 hours.

The test dose (Lr/100) of the toxin is the amount present in 0.2 ml of that mixture which causes at the site of injection a small, characteristic reaction in the skin of the guinea-pig or rabbit. Mixtures containing larger amounts of toxin cause larger reaction and necrosis and mixtures containing smaller amount of toxin cause no reaction.

Determination of potency of the antitoxin. Dilute the test toxin with *saline solution* so that 1.0 ml contains 10 times the test dose. Prepare mixtures such that 2.0 ml of each mixture contains 1.0 ml of the dilution of the toxin and one of a series of graded volumes of the preparation under examination. Prepare further mixtures such that 2.0 ml of each contains 1.0 ml of the solution of the test toxin and 0.1 Unit of antitoxin. Dilute each mixture with *saline solution* to the same final volume (2.0 ml). Allow the mixtures to stand at room temperature, protected from

light, for 15 to 60 minutes. Inject a dose of 0.2 ml of each mixture into the animals under the conditions described in the determination of the Lr/100 dose of the toxin.

The mixture of the preparation under examination that contains 0.01 Unit of antitoxin in 0.2 ml is the mixture that produces the same degree of local reaction as that produced by the injection into the same animals of the mixture of the Standard Preparation that contains in 0.2 ml the test dose (Lr/100) of the toxin and 0.01 Unit of antitoxin.

When at least four distinct tests are carried out by this method, the limits of error have been estimated to be between 90 per cent and 111 per cent.

Other tests. Complies with the tests stated under Antisera.

Storage. As stated under Antisera.

Labelling. The label states (1) the number of Units per ml; (2) the species of animal from which the preparation has been made; (3) the recommended dose (if space is inadequate, it may be stated in the instruction leaflet); (4) the name and proportion of any added preservative; (5) that the preparation, if liquid, should not be allowed to freeze; (6) that the preparation, if dried, should be used immediately after reconstitution in the stated quantity of the diluent supplied by the manufacturer.

Diphtheria and Tetanus Vaccine (Adsorbed)

Diphtheria and Tetanus Vaccine (Adsorbed) is a preparation of diphtheria formol toxoid and tetanus formol toxoid adsorbed on mineral carrier. The formol toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

The specification for individual component used in formulation is referred in the text of individual monograph.

Production

General provisions

Bulk purified diphtheria and tetanus toxoids

The bulk purified diphtheria and tetanus toxoids are prepared as described in the monographs on Diphtheria vaccine (adsorbed) and Tetanus vaccine (adsorbed) and comply with the requirements prescribed therein.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Absence of toxin and irreversibility of toxoid

Inject subcutaneously into each of 5 guinea-pigs at least 500 Lf of the non-incubated bulk purified toxoid in a volume of 1 ml, using the same buffer solution as for the final vaccine, without adsorbent. Animals that die shall be autopsied and examined for symptoms of diphtheria intoxication (red adrenals). The bulk purified toxoid shall pass the test if no guinea-pig shows symptoms of specific intoxication within six weeks of injection and if at least 80 per cent of the animals survive the test period. The guinea-pigs shall not have been used previously for experimental purposes.

Alternatively, a cell-culture test system may be used; in this case, the sensitivity of the test shall have been demonstrated to be not less than that of the guinea-pig test, and the test procedures shall be approved by the National Regulatory Authority.

Each bulk purified toxoid shall be tested to ensure that reversion to toxicity cannot take place on storage. The bulk purified toxoid shall be diluted in order to obtain the same concentration and chemical environment as that present in the final bulk vaccine, except for the presence of adjuvant.

To determine whether reversion has occurred, diluted toxoids that have been stored at 37° for six weeks shall be tested. The test employed shall be approved by the National Regulatory Authority and should be sufficiently sensitive to detect very small amounts of toxin. No toxicity shall be detected.

Intradermal tests in guinea-pigs and cell-culture tests both are considered to be suitable.

Antigenic purity

Not less than 1500 Lf per mg of protein nitrogen for diphtheria toxoid and not less than 1000 Lf/mg of protein nitrogen for tetanus toxoid.

FINAL BULK VACICNE

The final bulk vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto mineral carrier such as hydrated aluminium phosphate, aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Antimicrobial preservatives of the phenolic type must not be used.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Identification

A. Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. The clear supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

B. The clear supernatant obtained in test A reacts with a suitable tetanus antitoxin and yields a precipitate.

pH (2.4.24). 6.0 to 7.0.

Specific toxicity. Use 5 normal, healthy guinea-pigs weighing between 250 and 350 g which have been maintained for at least 1 week on a uniform, unrestricted diet, and have not been previously treated with any material that will interfere with the test. Weigh the animals separately and record their weights. Inject subcutaneously into each animal 5 times the dose stated on the label. Weigh all the animals at weekly intervals for 6 weeks. None of the animals shows any symptoms of diphtheria or tetanus toxaemia or dies from diphtheria within 42 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight, repeat the test. If an animal dies or loses weight in the second test, the vaccine fails the test.

Assay

Diphtheria toxoid

Complies with the test as stated under Diphtheria Vaccine (Adsorbed).

Tetanus toxoid

Complies with the test as stated under Tetanus Vaccine (Adsorbed).

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Free formaldehyde (2.3.20). Maximum 0.2 g/l

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is filled and stored aseptically into sterile containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity, free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

A. Diphtheria toxoid is identified by a suitable immuno-chemical method (2.2.14).

Dissolve in the vaccine under examination by adding sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant liquid is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate or visible floccules.

B. Tetanus toxoid is identified by a suitable immuno-chemical method (2.2.14).

The clear supernatant liquid obtained during test A reacts with a suitable tetanus antitoxin, giving a precipitate or visible floccules.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity

Aluminium (2.3.9). Not more than 1.25 mg per single human dose when hydrated aluminium phosphate or aluminium hydroxide is used as the adsorbent.

pH (2.4.24). The pH of the vaccine is within the range approved for the product (6.0 to 7.0).

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the quantity stated on the label.

Assay

Diphtheria component

Carry out one of the described methods for the assay of Diphtheria Vaccine (Adsorbed).

Viz a) Intradermal challenge method, b) Lethal challenge method, c) Antibody induction method, d) Validated serological assay in guinea pigs or mice as approved by National Regulatory Authority.

Tetanus component

Carry out one of the described methods for the assay of Tetanus Vaccine (Adsorbed) Viz a) Antibody induction method; b) Challenge method in guinea pigs/mice; c) Validated serological assay in guinea pigs or mice as approved by National Regulatory Authority.

Labelling. The label states (1) the human dose; (2) the minimum Lf units per single human dose or the minimum International Units per single human dose if potency test done by challenge method; (3) the name and the amount of the adsorbent and preservative; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen.

Diphtheria and Tetanus Vaccine (Adsorbed) for Adults and Adolescents

Diphtheria and Tetanus Vaccine (Adsorbed) for Adults and Adolescents is a preparation of diphtheria formol toxoid and tetanus formol toxoid adsorbed on a mineral carrier. The formol toxoids are prepared from the toxins produced by the growth

of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively.

Production

General provisions

Bulk purified diphtheria and tetanus toxoids

The bulk purified diphtheria and tetanus toxoids are prepared as described in the monographs on *Diphtheria vaccine (adsorbed)* and *Tetanus vaccine (adsorbed)* and comply with the requirements prescribed therein.

FINAL BULK VACCINE

The vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Identification

A. Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. The clear supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

B. The clear supernatant obtained in test A reacts with a suitable tetanus antitoxin and yields a precipitate.

pH (2.4.24). 6.0 to 7.0.

Specific toxicity

Inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. Weigh the animals separately and record their weights. Inject subcutaneously into each animal 5 times the dose stated on the label. Weigh all the animals at weekly intervals for 6 weeks. None of the animals shows any symptoms of diphtheria or tetanus toxaemia or dies from diphtheria within 42 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight, repeat the test. If an animal dies or loses weight in the second test, the vaccine fails the test.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate. If a satisfactory result is not obtained with a vaccine adsorbed on aluminium hydroxide, carry out the test as follows.

Centrifuge 15 ml of the vaccine under examination and suspend the residue in 5 ml of a freshly prepared mixture of 1 volume of a 56 g/l solution of *sodium edetate* and 49 volumes of a 90 g/l solution of *disodium hydrogen phosphate*. Maintain at 37° for not less than 6 hours and centrifuge. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. The clear supernatant obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

pH (2.4.24). 6.0 to 7.0.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Assay

Diphtheria component

Carry out the prescribed method for assay of Diphtheria Vaccine by lethal challenge method described in the assay of Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 2 IU per single human dose.

Tetanus component

Carry out one of the prescribed methods for the assay as stated under Tetanus Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 20 IU per single human dose.

Labelling. The label states (1) the human dose; (2) the minimum number of International Units of each component per single human dose, if potency determined by challenge method; (3) the name and the amount of the adsorbent and preservative; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen.

Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed)

Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed) is a preparation of diphtheria formol toxoid, tetanus formol toxoid adsorbed on mineral carrier and a suspension of killed *Bordetella pertussis* organisms. The formal toxoids are prepared from the toxins produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively. The *Bordetella pertussis* suspension is prepared by growth of suitable strains in an appropriate medium, under controlled conditions.

The specification for individual component used in formulation is referred in the text of individual monograph.

Production

General Provisions

The production method must be validated to demonstrate that the product if tested, would comply with the tests for safety as described under monographs of Diphtheria Vaccine, Tetanus Vaccine (Adsorbed) and Pertussis Vaccine.

The bulk purified diphtheria and tetanus toxoids and inactivated *B. pertussis* suspension are prepared as described in the monograph on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed) and Pertussis Vaccine respectively and comply with the respective requirements.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of suitable quantities of bulk purified diphtheria toxoid and tetanus toxoid onto hydrated aluminium phosphate or aluminium hydroxide and admixture of an appropriate quantity of a suspension of inactivated *B. pertussis*. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IOU per single human dose. If two or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. Suitable antimicrobial preservatives may be added to the bulk vaccine. Antimicrobial preservatives particularly those of phenolic type which affect the antigenic activity must not be used.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not more than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is filled and stored aseptically into sterile containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity of diphtheria, tetanus and pertussis components, free formaldehyde, antimicrobial preservative and the Assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

A. Diphtheria toxoid is identified by a suitable immunochemical method (2.2.14).

Dissolve in the vaccine under examination by adding sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant is obtained; reserve the precipitate for identification test C. The clear supernatant reacts with a suitable diphtheria antitoxin, giving a precipitate.

B. Tetanus toxoid is identified by a suitable immunochemical method (2.2.14).

The clear supernatant obtained during identification test A reacts with a suitable tetanus antitoxin, giving a precipitate.

C. The pertussis component is identified by agglutination of the bacteria from the resuspended centrifugation residue (see

identification test A; other suitable methods for separating the bacteria from the adsorbent may also be used) by antisera specific to *B. pertussis* or by the assay of the pertussis component.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Each final lot shall be tested for abnormal toxicity by injecting intraperitoneally one human dose, but not more than 0.25 ml into each of the five mice weighing between 17 to 22 g and at least one human dose but not more than 1.0 ml into each of the two guinea pigs weighing between 250 and 350 g. The preparation passes the test if none of the animals dies or shows signs of ill health in 7 days following the injection. If one of the animal dies or shows the signs of ill health, repeat the test. The preparation passes the test if none of the animals in the second group dies or shows signs of ill health in the time interval specified.

Specific toxicity

Diphtheria and tetanus components. Inject subcutaneously five times the single human dose stated on the label into each of five healthy guinea-pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. The animals should be weighted every week and observations be made. None of the animals shows any symptoms of diphtheria or tetanus toxaemia or dies from diphtheria within 42 days or loses weight at the end of the test. If more than one animal dies from non-specific cause or loses weight, repeat the test. If an animal dies or loses weight in the second test, the vaccine fails the test.

Pertussis component. Use not less than 10 healthy mice each weighing between 14 and 16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 hours before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 0.9 per cent sterile solution of *sodium chloride*, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the mice groups immediately before the injection and 72 hours and 7 days after the injection. The vaccine complies with the test if: (a) at the end of 72 hours the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60 per cent of that per control mouse; and (c) not more than 5 per cent of vaccinated mice should die during the test. The test may be repeated and the results of the tests combined.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose, when hydrated aluminium phosphate or aluminium hydroxide is used as the adsorbent.

pH (2.4.24). 6.0 to 7.0.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not more than115.0 per cent of the intended amount.

Assay

Diphtheria component

Carry out one of the methods for the assay as stated under Diphtheria Vaccine (Adsorbed).

Tetanus component

Carry out one of the methods for the assay as stated under Tetanus Vaccine (Adsorbed).

If test is carried out in Guinea pigs, the lower confidence limit (P=0.95) of estimated potency is not less than 40 IU/Single human dose; if the test is carried in mice, the lower confidence limit (P=0.95) of estimated potency is not less than 60 IU/ Single human dose.

Pertussis component

Carry out the assay as stated under Pertussis Vaccine.

Labelling. The label states (1) in case done by challenge method the minimum number of International Units; if units (as applicable for each component) per single human dose; (2) In case done by antibody induction method the minimum number of International Units per single human dose of pertussis component and minimum number of Lf of diphtheria toxoid and tetanus toxoid; (3) the name and the amount of the adsorbent and preservative; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen; (6) for vaccine contained in single-dose containers where the space is too small to accommodate the full name of the vaccine, the abbreviation 'DTP' may be used in the label and the container provided that the same code is also stated in the label on the package.

Diphtheria, Tetanus, Pertussis (Whole Cell), Hepatitis B (rDNA) and Haemophilus Type b Conjugate Vaccine (Adsorbed)

Diphtheria, Tetanus, Pertussis (Whole cell), Hepatitis B (rDNA) and Haemophilus Type b Conjugate Vaccine (Adsorbed) is a combined vaccine composed of diphtheria formol toxoid

containing not less than 1,500 Lf, (2.2.16) per mg of protein nitrogen, purified tetanus formol toxoid containing not less than 1,000 Lf, (2.2.16), per mg of protein nitrogen, hepatitis B surface antigen and haemophilus type b conjugated to suitable protein with a mineral adsorbent to which a suspension of killed *Bordetella pertussis* has been added. Mineral adsorbent is a suspension of hydrated aluminium hydroxide, aluminium phosphate or calcium phosphate, in saline solution or other appropriate solution isotonic with blood.

The formol toxoids are prepared from the toxin produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively, in suitable media. The toxins are converted to toxoids by treatment with formaldehyde solution by methods which avoid reversibility of the toxoids.

Hepatitis B surface antigen is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

The polysaccharide, polyribosyl ribitol phosphate, PRP is a linear copolymer composed of repeated units of 3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell dependent B-cell immune response to the polysaccharide.

The product may be presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before or during use.

The final product contains a suitable antimicrobial preservative. The antigenic properties of the vaccine are adversely affected by the presence of certain antimicrobial preservatives particularly those of the phenolic type and some of the quaternary ammonium type and must not be used.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

If the vaccine is presented with the haemophilus component in a separate vial, as part of consistency studies the assays of the diphtheria, tetanus, pertussis and hepatitis B are carried out on a suitable number of batches of vaccine reconstituted as for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

The production method is validated to demonstrate that the product, if tested, would comply with the following test for specific toxicity of the diphtheria and tetanus component: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal shows signs of or dies in the second test, the vaccine does not comply with the test.

The stability of the final lot and the relevant intermediates is evaluated using one or more indicator tests. For the haemophilus component, such tests may include determination of molecular size, determination of free PRP in the conjugate and kinetics of depolymerisation. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilized by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine, Hepatitis B Vaccine (rDNA) and Haemophilus Type b Conjugate Vaccine.

FINAL BULK VACCINE

Vaccine with all components in the same container

The final bulk is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid, bulk purified hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, admixture of an appropriate quantity of a suspension of inactivated *B. pertussis* component and admixture of a suitable quantity of PRP conjugate; the resulting mixture is approximately isotonic with blood. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to an

opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. Suitable antimicrobial preservatives may be added.

Vaccine with the haemophilus component in a separate container

The final bulk is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid, bulk purified hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, admixture of an appropriate quantity of a suspension of inactivated *B. pertussis* component and admixture of a suitable quantity of PRP conjugate; the resulting mixture is approximately isotonic with blood. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. The final bulk is filled separately. Suitable antimicrobial preservatives may be added. The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabilizer may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended content.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity of diphtheria toxoid, tetanus toxoid and pertussis component and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot. If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Free PRP

Unbound PRP is determined after removal of the conjugate, for example by anion exchange, size exclusion or hydrophobic chromatography (2.4.16), ultrafiltration or other validated methods. The amount of free PRP is not greater than that approved for the particular product.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

pH (2.4.24). 6.0 to 7.0.

Description. Whitish turbid liquid in which the mineral carrier tends to settle down slowly on keeping.

Identification

Tests A, B, C, D and E may be omitted if test F is carried out. Test F may be omitted if tests A, B, C, D and E are carried out.

A. *Diphtheria toxoid.* Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. Reserve the residue for test C. The clear supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

B. *Tetanus toxoid.* Tetanus toxoid is identified by suitable immunochemical method. The clear supernatant obtained as described in identification test A reacts with a suitable tetanus antitoxin to give a positive reaction, when tested by a suitable validated immunological method.

C. *Pertussis component.* To a suspension of the residue obtained in test A in *saline solution* add a suitable *Bordetella pertussis* antiserum; *agglutination* indicates presence of pertussis component.

D. *Hepatitis B surface antigen.* The suspension of the residue obtained in test A gives a positive reactions when tested by suitable *in-vitro* assay.

E. *PRP.* The suspension of the residue obtained in the test A gives a positive reaction when tested by a suitable immunochemical method for PRP.

F. The vaccine confers an active immunity in mice and bguinea-pigs when administered as directed in the test for Assay.

Tests

If the product is presented with the haemophilus component in a separate container; the tests for specific toxicity of diphtheria toxoid, tetanus toxoid and pertussis component, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus, pertussis and hepatitis B components; the tests for PRP content, water (where applicable), sterility

and pyrogens are carried out on the container with the haemophilus component.

If the haemophilus component is freeze-dried, some tests may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component under test.

PRP. Not less than 80.0 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.7.1), or phosphorus (2.7.1), by an immunochemical method (2.2.14) or by anion exchange liquid chromatography with pulsed amperometric detection.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and is not greater than 115 per cent of the quantity stated on the label.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Each final lot shall be tested for abnormal toxicity by injecting intraperitoneally 0.25 ml of the vaccine into each of five mice weighing between 17 and 22 g and at least one human dose but not more than 1.0 ml into each of two guinea pigs weighing between 250 and 350 g. The preparation passes the test if none of the animals dies or shows signs of ill health in seven days following the injection. If one of the animals dies or shows signs of ill health, repeat the test. The preparation passes the test if none of the animals in the second group dies or shows signs of ill health in the time interval specified.

Pyrogens (2.2.8). This test is carried out for *Haemophilus influenzae* type b vaccine only if *Haemophilus influenzae* type b vaccine is presented as separate lyophilized vial. The vaccine complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to: 1 mg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria toxoid as carrier; 0.1 mg of PRP for a vaccine with tetanus toxoid as carrier protein; 0.025 mg of PRP for vaccine with OMP as carrier.

Specific toxicity

Diphtheria and tetanus components

Complies with the test as stated under Diphtheria and Tetanus Vaccine (Adsorbed).

Pertussis component

Complies with the test as stated under Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed).

Assay

Diphtheria toxoid (adsorbed)

Complies with the test as stated under Diphtheria and Tetanus Vaccine (Adsorbed).

Tetanus toxoid (adsorbed)

Complies with the test as stated under assay of Tetanus Vaccine (Adsorbed).

Pertussis vaccine

Complies with the test as stated under Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed).

Hepatitis B surface antigen (adsorbed)

Complies with the test as stated under Hepatitis B Vaccine (Adsorbed).

Storage. When stored under the prescribed conditions the vaccine may be expected to retain potency for not less than 2 years from the date on which the potency test for the pertussis component was started.

Labelling. The label states (1) the human dose; (2) Diphtheria and Tetanus components; (a) in case done by challenge method, the minimum number of International Units (as applicable for each component) per single human dose; (b) in case done by antibody induction, the minimum Lf units per single human dose; (3) pertussis component – IU or IOU per single human dose; (4) hepatitis B component - µg HBsAg per single human dose; (5) haemophilus conjugate component - mg PRP per single human dose; (6) the type and nominal amount of carrier protein per single human dose; (7) the name and amount of adsorbent and added preservative; (8) that the vaccine must be shaken before use; (9) that the vaccine is not to be frozen.

Diphtheria, Tetanus, Pertussis (Whole Cell) and Hepatitis B (rDNA) Vaccine (Adsorbed)

Diphtheria, Tetanus, Pertussis and Hepatitis B (rDNA) Vaccine (Adsorbed) is a combined vaccine composed of diphtheria formol toxoid containing not less than 1,500 Lf (2.2.16), per mg of protein nitrogen, purified tetanus formol toxoid containing not less than 1,000 Lf (2.2.16), per mg of protein nitrogen and hepatitis B surface antigen with a mineral adsorbent to which a suspension of killed *Bordetella pertussis* has been added. Mineral adsorbent is a suspension of hydrated aluminium hydroxide, aluminium phosphate or calcium phosphate in saline solution or other appropriate solution isotonic with blood.

The formol toxoids are prepared from the toxin produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively, in suitable media. The toxins are converted to toxoids by treatment with formaldehyde solution by methods, which avoid reversibility of the toxoids.

Hepatitis B surface antigen is a component protein of hepatitis B virus; the antigen is obtained by recombinant DNA technology.

The final product contains a suitable antimicrobial preservative. The antigenic properties of the vaccine are adversely affected by the presence of certain antimicrobial preservatives particularly those of the phenolic type and some of the quaternary ammonium type and must not be used.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the following test for specific toxicity of the diphtheria and tetanus component: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxaemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal shows signs of or dies in the second test, the vaccine does not comply with the test.

The stability of the final lot and the relevant intermediates is evaluated using one or more indicator tests. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine and Hepatitis B Vaccine (rDNA).

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, tetanus toxoid, pertussis whole cell suspension and hepatitis B surface antigen onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate. Suitable antimicrobial preservatives may be added. Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended content.

pH (2.4.24). 6.0 to 7.0.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity of diphtheria toxoid, tetanus toxoid and pertussis component and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot. If an *in vivo* assay is used for the hepatitis B component, provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

Description. Whitish turbid liquid in which the mineral carrier tends to settle down slowly on keeping.

Identification

Tests A, B, C and D may be omitted if test E is carried out. Test E may be omitted if tests A, B C and D are carried out.

A. *Diphtheria toxoid.* Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. Reserve the residue for test C. The clear

supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

B. *Tetanus toxoid.* The clear supernatant obtained in test A reacts with a suitable tetanus antitoxin and yields a precipitate.

C. *Pertussis component.* To a suspension of the residue obtained in test A in *saline solution* add a suitable *B. pertussis* antiserum; agglutination indicates presence of pertussis component.

D. *Hepatitis B surface antigen.* The suspension of the residue obtained in test A gives a positive reactions when tested by suitable *in-vitro* assay.

E. The vaccine confers an active immunity in mice and guinea-pigs when administered as directed under Assay.

Tests

pH (2.4.24). 6.0 to 7.0.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Each final lot shall be tested for abnormal toxicity by injecting intraperitoneally one human dose but not more than 0.25 ml into each of five mice weighing between 17 and 22 g and at least one human dose but not more than 1.0 ml into each of two guinea pigs weighing between 250 and 350 g. The preparation passes the test if none of the animals dies or shows signs of ill health in seven days following the injection. If one of the animals dies or shows signs of ill health, repeat the test. The preparation passes the test if none of the animals in the second group dies or shows signs of ill health in the time interval specified.

Specific toxicity

Diphtheria and tetanus components

Complies with the test as stated under Diphtheria and Tetanus Vaccine (Adsorbed).

Pertussis component

Complies with the test as stated under Pertussis Vaccine (Adsorbed).

Assay

Diphtheria toxoid (adsorbed)

Complies with the test as stated under Diphtheria Vaccine (Adsorbed).

Tetanus toxoid (adsorbed)

Complies with the test as stated under assay for Tetanus Vaccine (Adsorbed).

Pertussis vaccine

Complies with the test as stated under Pertussis Vaccine (Adsorbed).

Hepatitis B surface antigen (adsorbed)

Complies with the test as stated under Hepatitis B Vaccine (Adsorbed).

Storage. When stored under the prescribed conditions the vaccine may be expected to retain potency for not less than 2 years from the date on which the potency test for the *pertussis component* was started.

Labelling. The label states (1) the human dose (ml); (2) diphtheria and tetanus components, (a) in case done by challenge method, the minimum number of International Units (as applicable for each component) per single human dose and (b) in case done by antibody induction, the minimum Lf units per single human dose; (3) pertussis component - IU or IOU per single human dose; (4) hepatitis B component -µg HBsAg per single human dose; (5) the name and amount of adsorbent and added preservative; (6) that the vaccine must be shaken before use; (7) that the vaccine is not to be frozen.

Diphtheria, Tetanus, Pertussis (Whole Cell) and Haemophilus Type b Conjugate Vaccine (Adsorbed)

Diphtheria, Tetanus, Pertussis and Haemophilus type b Conjugate Vaccine (Adsorbed) is a combined vaccine composed of diphtheria formol toxoid containing not less than 1,500 Lf, (2.2.16) per mg of protein nitrogen, purified tetanus formol toxoid containing not less than 1,000 Lf, (2.2.16), per mg of protein nitrogen, and Haemophilus type b conjugated to suitable protein with a mineral adsorbent to which a suspension of killed *Bordetella pertussis* has been added. The mineral adsorbent is a suspension of hydrated aluminium hydroxide, aluminium phosphate or calcium phosphate, in saline solution or other appropriate solution isotonic with blood.

The formol toxoids are prepared from the toxin produced by the growth of *Corynebacterium diphtheriae* and *Clostridium tetani*, respectively in suitable media. The toxins are converted to toxoids by treatment with formaldehyde solution by methods which avoid reversibility of the toxoids.

The polysaccharide, polyribosyl ribitol phosphate, PRP is a linear copolymer composed of repeated units of

3-β-D-ribofuranosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size and derived from a suitable strain of *Haemophilus influenzae* type b. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell dependent B-cell immune response to the polysaccharide.

The product may be presented with the haemophilus component in a separate container, the contents of which are mixed with the other components immediately before or during use.

The final product contains a suitable antimicrobial preservative. The antigenic properties of the vaccine are adversely affected by the presence of certain antimicrobial preservatives particularly those of the phenolic type and some of the quaternary ammonium type must not be used.

Production

General provisions

The production method shall have been shown to yield consistently the vaccines comparable with the vaccine of proven clinical efficacy and safety in man.

If the vaccine is presented with the haemophilus component in a separate vial, as part of consistency studies, the assays of the diphtheria, tetanus and pertussis are carried out on a suitable number of batches of vaccine reconstituted for use. For subsequent routine control, the assays of these components may be carried out without mixing with the haemophilus component.

The production method is validated to demonstrate that the product, if tested, would comply with the following test for specific toxicity of the diphtheria and tetanus component: inject subcutaneously 5 times the single human dose stated on the label into each of 5 healthy guinea pigs, each weighing between 250 and 350 g, that have not previously been treated with any material that will interfere with the test. If within 42 days of the injection any of the animals shows signs of or dies from diphtheria, toxemia or tetanus, the vaccine does not comply with the test. If more than 1 animal dies from non-specific causes, repeat the test once; if more than 1 animal shows signs of or dies in the second test, the vaccine does not comply with the test.

The stability of the final lot and the relevant intermediates is evaluated using one or more indicator tests. For the haemophilus component, such tests may include determination of molecular size, determination of free PRP in the conjugate and kinetics of depolymerisation. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

Reference vaccine(s)

Provided valid assays can be performed, monocomponent reference vaccines may be used for the assays on the combined vaccine. If this is not possible because of interaction between the components of the combined vaccine or because of the difference in composition between monocomponent reference vaccine and the test vaccine, a batch of combined vaccine shown to be effective in clinical trials or a batch representative thereof is used as a reference vaccine. For the preparation of a representative batch, strict adherence to the production process used for the batch tested in clinical trials is necessary. The reference vaccine may be stabilized by a method that has been shown to have no effect on the assay procedure.

Production of the components

The production of the components complies with the requirements of the monographs on Diphtheria Vaccine (Adsorbed), Tetanus Vaccine (Adsorbed), Pertussis Vaccine (Whole Cell) and *Haemophilus influenzae* Type b Conjugate Vaccine.

FINAL BULK VACCINE

Vaccine with all components in the same container

The final bulk is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid, onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, admixture of an appropriate quantity of a suspension of inactivated *B. pertussis* component and admixture of a suitable quantity of PRP conjugate; the resulting mixture is approximately isotonic with blood. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. Suitable antimicrobial preservatives may be added.

Vaccine with the haemophilus component in a separate container

The final bulk is prepared by adsorption, separately or together, of suitable quantities of bulk purified diphtheria toxoid, bulk purified tetanus toxoid onto a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate, admixture of an appropriate quantity of a suspension of inactivated *B. pertussis* component and admixture of a suitable quantity of PRP conjugate; the resulting mixture is approximately isotonic with blood. The *B. pertussis* concentration of the final bulk vaccine does not exceed that corresponding to opacity of 20 IU per single human dose. If 2

or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units. The final bulk is filled separately. Suitable antimicrobial preservatives may be added. The final bulk of the haemophilus component is prepared by dilution of the bulk conjugate to the final concentration with a suitable diluent. A stabilizer may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 percent of the intended content.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Where the haemophilus component is in a separate container, the final bulk of the haemophilus component is freeze-dried. Only a final lot that is satisfactory with respect to the test for osmolality and with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests specific toxicity of diphtheria toxoid, tetanus toxoid and pertussis component and antimicrobial preservative and the assays for the diphtheria, tetanus and pertussis components have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. Provided the content of free formaldehyde has been determined on the bulk purified antigens or on the final bulk and it has been shown that the content in the final lot will not exceed 0.2 g/l, the test for free formaldehyde may be omitted on the final lot.

Free PRP. Unbound PRP is determined after removal of the conjugate, for example by anion exchange, size exclusion or hydrophobic chromatography (2.4.16), ultrafiltration or other validated methods. The amount of free PRP is not greater than approved for the particular product.

Osmolality (2.4.23). The osmolality of the vaccine is within the limits approved for the particular preparation.

pH (2.4.24). 6.0 to 7.0.

Description. Whitish turbid liquid in which the mineral carrier tends to settle down slowly on keeping.

Production

Identification

Tests A, B, C and D may be omitted if test E is carried out. Test E may be omitted if tests A, B, C and D are carried out.

A. *Diphtheria toxoid.* Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. Reserve the residue for test C. The clear supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

B. *Tetanus toxoid.* The clear supernatant obtained in test A reacts with a suitable tetanus antitoxin and yields a precipitate.

C. *Pertussis component.* To a suspension of the residue obtained in test A in *saline solution* add a suitable *Bordetella pertussis* antiserum; agglutination indicates presence of pertussis component.

D. *PRP.* The suspension of the residue obtained in test A gives a positive reaction when tested by a suitable immunochemical method for PRP.

E. The vaccine confers an active immunity in mice and guinea pigs when administered as directed in the test for Potency.

Tests

If the product is presented with the haemophilus component in a separate container; the tests for specific toxicity of diphtheria toxoid, tetanus toxoid and pertussis component, aluminium, free formaldehyde, antimicrobial preservative and sterility are carried out on the container with the diphtheria, tetanus and pertussis components; the tests for PRP content, water (where applicable), sterility and pyrogens are carried out on the container with the haemophilus component.

If the haemophilus component is freeze-dried, some tests may be carried out on the freeze-dried product rather than on the bulk conjugate where the freeze-drying process may affect the component under test.

PRP. Not less than 80.0 per cent of the amount of PRP stated on the label. PRP is determined either by assay of ribose (2.7.1) or phosphorus (2.7.1), by an immunochemical method (2.2.14) or by anion exchange liquid chromatography with pulsed amperometric detection.

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Each final lot shall be tested for abnormal toxicity by injecting intraperitoneally a maximum of one human dose, but not more than 0.25 ml into each of the

five mice weighing between 17 to 22 g and at least one human dose but not more than 1.0 ml into each of the two guinea pigs weighing between 250 and 350 g. The preparation passes the test if none of the animals dies or shows signs of ill health in 7 days following the injection . If one of the animal dies or shows the signs of ill health, repeat the test. The preparation passes the test if none of the animals in the second group dies or shows signs of ill health in the time interval specified.

Pyrogens (2.2.8). This test is carried out for *Haemophilus influenzae* type b vaccine only if *Haemophilus influenzae* type b vaccine is presented as separate lyophilized vial. The vaccine complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to: 1 mg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria toxoid as carrier; 0.1 mg of PRP for a vaccine with tetanus toxoid as carrier protein; 0.025 mg of PRP for vaccine with OMP as carrier.

Specific toxicity

Diphtheria and tetanus components

Complies with the test as stated under Diphtheria and Tetanus Vaccine (Adsorbed).

Pertussis component

Complies with the test as stated under Diphtheria, Tetanus and Pertussis Vaccine (Adsorbed).

Assay

Diphtheria toxoid (adsorbed)

Complies with the test as stated under Diphtheria Vaccine (Adsorbed).

Tetanus toxoid (adsorbed)

Complies with the test as stated under assay of Tetanus Vaccine (Adsorbed).

Pertussis vaccine

Complies with the test as stated under Pertussis Vaccine (Adsorbed).

Storage. When stored under the prescribed conditions the vaccine may be expected to retain potency for not less than 2 years from the date on which the potency test for the *pertussis component* was started.

Labelling. The label states (1) the human dose (ml); (2) Diphtheria and Tetanus components (a) in case done by challenge method the minimum number of international units (as applicable for each component) per single human dose; (b) in case done by antibody induction method, the minimum Lf units per single human dose; (3) pertussis component – IU or IOU per single human dose; (4) haemophilus conjugate component - mg PRP per single human dose; (5) the type and nominal amount of carrier protein per single human dose; (6) the name and amount of adsorbent and added preservative; (7) that the vaccine must be shaken before use; (9) that the vaccine is not to be frozen.

Diphtheria Vaccine (Adsorbed)

Diphtheria Vaccine (Adsorbed) is a preparation of diphtheria formol toxoid with a mineral adsorbent. The formol toxoid is prepared from the toxin produced by the growth of *Corynebacterium diphtheriae*.

Production

General provisions

The maximum number of Lf units per single human dose of diphtheria vaccine (adsorbed) is 30.

Bulk purified toxoid

For the production of diphtheria toxin, from which toxoid is prepared, seed cultures are managed in a defined seed-lot system in which toxinogenicity is conserved and, where necessary, restored by deliberate reselection. A highly toxinogenic strain of *Corynebacterium diphtheriae* with known origin and history is grown in a suitable liquid medium. At the end of cultivation, the purity of each culture is tested and contaminated cultures are discarded. Toxin-containing culture medium is separated aseptically from the bacterial mass as soon as possible. The toxin content (Lf per ml) is checked to monitor consistency of production. Single harvests may be pooled to prepare the bulk purified toxoid. The toxin is purified to remove components likely to cause adverse reactions in humans. The purified toxin is detoxified with formaldehyde by a method that avoids destruction of the immunogenic potency of the toxoid and reversion of the toxoid to toxin, particularly on exposure to heat. Alternatively, purification may be carried out after detoxification.

Only bulk purified toxoid that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Absence of toxin and irreversibility of toxoid

Inject subcutaneously into each of 5 guinea-pigs at least 500 Lf of the *non-incubated bulk purified toxoid* in a volume of 1 ml, using the same buffer solution as for the final vaccine, without adsorbent. Animals that die shall be autopsied and examined for symptoms of diphtheria intoxication (red adrenals). The bulk purified toxoid shall pass the test if no

guinea-pig shows symptoms of specific intoxication within six weeks of injection and if at least 80 per cent of the animals survive the test period. The guinea-pigs shall not have been used previously for experimental purposes.

Alternatively, a cell-culture test system may be used; in this case, the sensitivity of the test shall have been demonstrated to be not less than that of the guinea-pig test, and the test procedures shall be approved by the National Regulatory Authority.

Each bulk purified toxoid shall be tested to ensure that reversion to toxicity cannot take place on storage. The bulk purified toxoid shall be diluted in order to obtain the same concentration and chemical environment as those present in the final bulk vaccine, except for the presence of adjuvant.

To determine whether reversion has occurred, diluted toxoids that have been stored at 37° for six weeks shall be tested. The test employed shall be approved by the National Regulatory Authority and should be sufficiently sensitive to detect very small amounts of toxin. No toxicity shall be detected.

Intradermal tests in guinea-pigs and cell-culture tests both are considered to be suitable.

Cell culture method

Using the same buffer solution as for the final vaccine, without adsorbent, prepare a solution of bulk purified toxoid at 100 Lf per ml. Divide the solution into 2 equal parts. Maintain 1 part at 5° ± 3° and the other at 37° for 6 weeks. Carry out a test in Vero cells for active diphtheria toxin using 50 µl per well of both samples. The sample should not contain antimicrobial preservatives and detoxifying agents should be determined to be below the concentration toxic to Vero cells. Non-specific toxicity may be eliminated by dialysis.

Use *freshly trypsinised Vero cells* at a suitable concentration, for example 2.5×10^5 per ml and a *reference diphtheria toxin* diluted in 100 Lf per ml *diphtheria toxoid*. A suitable *reference diphtheria toxin* will contain either not less than 100 LD_{50}/ml or 67 to 133 lr/100 in 1 Lf and 25,000 to 50,000 minimal reacting doses for guinea-pig skin in 1 Lf (*diphtheria toxin RP* is suitable for use as the reference toxin). Dilute the toxin in 100 Lf/ml *diphtheria toxoid* to a suitable concentration, for example 2×10^{-4} Lf per ml. Prepare serial twofold dilutions of the diluted diphtheria toxin and use undiluted test samples (50 µl per well). Distribute them in the wells of a sterile tissue culture plate containing a medium suitable for Vero cells. To ascertain that any cytotoxic effect noted is specific to diphtheria toxin, prepare in parallel dilutions where the toxin is neutralised by a suitable concentration of *diphtheria antitoxin*, for example 100 IU/ml. Include control wells without toxoid or toxin and with non-toxic toxoid at 100 Lf per ml on each plate to verify normal cell growth. Add cell suspension to each well, seal the plates and incubate at 37° for 5 to 6 days. Cytotoxic effect is judged to be present where there is complete metabolic inhibition of the Vero cells, indicated by the pH indicator of the medium. Confirm cytopathic effect by microscopic examination or suitable staining such as *MTT dye*. The test is invalid if 5×10^{-5} Lf per ml of reference diphtheria toxin in 100 Lf per ml toxoid has no cytotoxic effect on Vero cells or if the cytotoxic effect of this amount of toxin is not neutralised in the wells containing *diphtheria antitoxin*. The bulk purified toxoid complies with the test if no toxicity neutralisable by antitoxin is found in either sample.

Antigenic purity. Not less than 1,500 Lf per mg of protein nitrogen.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Identification

Dissolve sufficient *sodium citrate* in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. The clear supernatant reacts with a suitable diphtheria antitoxin and yields a precipitate.

pH (2.4.24). 6.0 to 7.0.

Specific toxicity. Use 5 normal, healthy guinea-pigs weighing between 250 and 350 g, which have been maintained for at least 1 week on a uniform, unrestricted diet, and have not been previously treated with any material that will interfere with the test. Weigh the animals separately and record their weights. Inject subcutaneously into each animal 5 times the dose stated on the label. Weigh all the animals at weekly intervals for 6 weeks. None of the animals show signs of or dies from diphtheria toxaemia within 42 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight, repeat the test. If an animal dies or loses weight in the second test, the vaccine fails the test.

Potency. Determine by any of the methods of biological assay of Adsorbed Diphtheria Vaccine described.

Biological assay of adsorbed diphtheria vaccine

(a) Intradermal challenge method

The potency of adsorbed diphtheria vaccine is determined by comparing the dose necessary to protect guinea-pigs against the erythrogenic effects of a range of intradermal injections of diphtheria toxin with the dose of the Standard preparation of adsorbed diphtheria toxoid necessary to give the same protection. For this comparison, the Standard preparation of adsorbed diphtheria toxoid and a suitable preparation of diphtheria toxin, for use as a challenge toxin, are required.

Standard preparation

The Standard preparation is International standard of Diphtheria toxoid, adsorbed, or another suitable preparation the potency of which has been determined in relation to the International Standard.

Suggested method

Test animals. Use white guinea-pigs, weighing between 250 and 350 g, from the same stock. Distribute the guinea-pigs into no fewer than six equal groups; use groups containing a number of animals sufficient to obtain results that fulfill the requirements for a valid Assay prescribed below. The guinea-pigs are all of the same sex or the males and females are distributed equally among the groups. If the challenge toxin to be used has not been shown to be stable or has not been adequately standardized, include five guinea-pigs as unvaccinated controls.

Selection of the challenge toxin. Select a preparation of diphtheria toxin containing 67 to 133 Limes reactionis/100 (Lr/100) in Limes flocculationis (Lf) and 25,000 to 50,000 minimal reacting doses for guinea-pig skin in 1 Lf. If the challenge toxin preparation has been shown to be stable, it is not necessary to verify the activity for every assay.

Preparation of the challenge toxin solutions. Immediately prior to use, dilute the challenge toxin with a suitable diluent to obtain a challenge toxin solution containing about 512×10^{-4} Lf in 0.2 ml. Dilute a portion of this challenge toxin solution to give a series of five 4-fold dilutions.

Determination of potency of the vaccine. Prepare in *saline solution* dilutions of the vaccine under examination and of the Standard preparation such that, for each, the dilutions form a series differing by not more than 2.5 fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1.0 ml volumes into guinea-pigs, result in an intradermal score of approximately three when the animals are challenged. Allocate the dilutions, one to each of the groups of guinea-pigs, and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the group to which that dilution is allocated. After 28 days shave both flanks of each guinea-pig and inject each animal intradermally with 0.2 ml of the challenge toxin solution and with 0.2 ml of each of the five dilutions thereof in such a way as to minimize interference between adjacent sites. If necessary, inject the unvaccinated control guinea-pigs with dilutions containing 8×10^{-5}, 4×10^{-5}, 2×10^{-5}, 1×10^{-5} and 5×10^{-6} Lf of the challenge toxin. Examine all the injection sites 48 hours after injection of the challenge toxin and record the incidence of specific diphtheria erythema. Record also the number of sites free from such reactions as the intradermal challenge score. Tabulate the intradermal challenge scores for all the animals receiving the same dilution of vaccine and use those data with a suitable transformation, such as $(\text{score})^2$ or arcsin $[(\text{score}/6)^2]$, to obtain an estimate of the relative potency for each of the test preparations by parallel-line quantitative analysis.

The test is not valid unless (a) for both the preparation under examination and the Standard preparation, the mean score obtained at the lowest dose level is more than three; (b) if applicable, the toxin dilution that contains 4×10^{-5} Lf gives a positive erythema in at least 80.0 per cent of the control guinea-pigs and the dilution that contains 2×10^{-5} Lf gives no reaction in at least 80 per cent of the guinea-pigs (if these criteria are not met a different toxin has to be selected); (c) the fiducial limits of the assay fall between 50.0 and 200.0 per cent of the estimated potency; (d) the statistical analysis shows no deviation from linearity and parallelism. The test may be repeated but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

The lower fiducial limit of error of the estimated potency is not less than 30 Units per dose.

(b) Lethal challenge method

Test animals. Use healthy, white or light-coloured guinea-pigs from the same stock, weighing between 250 and 350 g. Distribute them into six groups of sixteen; and four groups of four. The guinea-pigs should all be of the same sex or the males and females should be distributed equally between the six groups of sixteen.

Challenge toxin. Select a preparation of diphtheria toxin containing not less than 100 LD_{50} in 1.0 ml.

Preparation of the challenge toxin solutions. Immediately prior to use, prepare from the challenge toxin by dilution in *phosphate buffered saline pH 7.4*, or *normal saline* a challenge toxin containing approximately 100 LD_{50} in 1.0 ml. Dilute portions of this challenge toxin solution to $2LD_{50}$, 1 LD_{50} and ½ LD_{50} in the same solution

Determination of potency of the vaccine. Prepare in *saline solution* three dilutions of the vaccine under examination and three dilutions of the Standard preparation such that for each, the dilutions form a series differing by not more than 2.5 fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1.0 ml volumes into guinea-

pigs, protect approximately 50 per cent of the animals from the lethal effects of the subcutaneous injection of the quantity of diphtheria toxin prescribed for this test. Allocate the six dilutions, one to each of the six groups of sixteen guinea-pigs, and inject subcutaneously 1.0 ml of each dilution into each guinea-pig in the groups to which that dilution is allocated. After 28 days inject subcutaneously into each animal in the six groups of sixteen, 1.0 ml of the challenge toxin solution. Allocate the challenge toxin solution and the three dilutions made from it, one to each of the four groups of four guinea-pigs and inject subcutaneously 1.0 ml of each toxin solution into each guinea-pig in the group to which that solution is allocated. Examine the guinea pigs twice in a day, remove dead animals and kill the animals showing definite signs of diphtheria. Count the number of surviving animals 5 days later and calculate the potency of the vaccine under examination relative to the potency of the Standard preparation on the basis of the number of animals that survive in each of the six groups of sixteen, using appropriate statistical methods.

The test is not valid unless (a) for the vaccine under examination and the Standard preparation the 50.0 per cent protective doses lie between the largest and smallest doses of the preparations given to the guinea-pigs; (b) the survivors among the four groups of guinea-pigs injected with the challenge toxin and its dilutions indicate that the challenge was approximately 100 LD_{50} and; (c) statistical analysis shows parallelism, linearity and a significant slope of the dose-response lines. The test may be repeated any number of times but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

Estimated potency should not be less than 30 IU per single human dose. If the lower limit of 95.0 per cent confidence interval of estimated potency is less than 30 IU per single human dose then the limits of the 95.0 per cent confidence interval should be within 50 to 200 of the estimated potency.

(c) Antibody induction method

Inject subcutaneously on each of two occasions separated by an interval of not more than 4 weeks, one-fiftieth of the stated human dose diluted to 1 ml with *saline solution*, into each of 10 normal, healthy guinea-pigs weighing between 250 and 350 g. Not earlier than 2 weeks and not later than 3 weeks after the second injection, collect the serum from each animal and determine the antitoxin content of the serum of each animal, as described under Diphtheria Antitoxin or any other method approved by National Regulatory Authority. The geometric mean of the antitoxin contents shall be not less than 2.0 Units per ml with reference to the Diphtheria antitoxin standard.

(d) Any other validated serological assay in guinea pigs or mice as approved by National Regulatory Authority

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under, Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity, free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

Diphtheria toxoid is identified by a suitable immunochemical method. The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent w/v solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable diphtheria antitoxin, giving a precipitate.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the absorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

pH (2.4.24). 6.0 to 7.0.

Assay

Carry out one of the prescribed methods for the assay of Diphtheria Vaccine (Adsorbed).

The lower confidence limit (P = 0.95) of the estimated potency is not less than 30 IU per single human dose.

Labelling. The label states (1) the human dose; (2) the minimum Lf units per single human dose or the minimum International Units per single human dose if potency test done by challenge method; (3) the name and the amount of the adsorbent and preservative; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen.

Gas-Gangrene Antitoxin (Oedematiens)

Gas-gangrene Antitoxin (Novyi); Anti-gas-gangrene (Oedematiens) Serum

Gas-gangrene Antitoxin (Oedematiens) is a preparation containing the specific antitoxic globulins obtained by purification of hyperimmune serum of horses or other suitable animals and having the specific activity of neutralising the alpha toxin formed by *Clostridium oedematiens (C. novyi)*.

Gas-gangrene Antitoxin (Oedematiens) reconstituted where necessary as stated on the label complies with the following tests.

Gas-Gangrene Antitoxin (Oedematiens) has a potency of not less than 3750 Units per ml.

Description. A clear colourless or pale yellow liquid free from suspended particles or a freeze-dried, cream coloured powder or pellet for reconstitution with the diluent supplied by the manufacturer.

Identification

Specifically neutralises and renders the alpha toxin formed by *Cl. oedematiens* harmless to susceptible animals or may be identified by any other suitable *in-vitro* test.

Tests

Potency. Carry out the biological assay of gas-gangrene antitoxin (oedematiens).

Biological Assay of Gas-Gangrene Antitoxin (Oedematiens)

The potency of the gas-gangrene antitoxin (oedematiens) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of gas-gangrene toxin (oedematiens) with the dose of the Standard Preparation of gas-gangrene antitoxin (*C. oedematiens*) necessary to give the same protection. For this purpose the Standard Preparation of gas-gangrene toxin (*C. oedematiens*) and a suitable preparation of gas-gangrene toxin (oedematien),for use as a test toxin, are required. The test dose of the toxin is determined in the relation to the Standard Preparation and the potency of the preparation under examination is then determined in relation to the Standard preparation using the test toxin.

Standard Preparation

The Standard Preparation is a dried hyperimmune horse serum or another suitable preparation of gas-gangrene antitoxin (oedematiens), the potency of which has been determined in relation to the International Standard. The Unit is the specific neutralising activity for gas-gangrene toxin (oedematiens) contained in such an amount of the Standard Preparation as the Ministry of Health and Family Welfare, Government of India may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

Test animals. Use healthy mice having body weights such that the difference between the lightest and heaviest is not more than 5 g.

Test toxin. Prepare gas-gangrene toxin (oedematiens) by growing *C. oedematiens* in a liquid culture medium for about 5 days, filtering aseptically and precipitating with *ammonium sulphate*. The resulting precipitate, which contains the toxin, is collected, dried over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, powdered and kept dry.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities.

L+ dose — This is the smallest quantity of the toxin which, when mixed with 1 Unit of antitoxin and injected intramuscularly into mice, causes the death of the animals within 72 hours.

LD_{50} — This is the smallest quantity of toxin which, when injected intramuscularly into mice, causes the death of half the animals within 72 hours.

A suitable toxin is one which has an L+ dose in 0.5 mg or less and contains not less than 25 LD_{50} in an L+ dose.

Determination of test dose of toxin *(L+ dose)*. Prepare a solution of the Standard Preparation with *saline solution* such that 1.0 ml contains 12.5 Units. Weigh accurately a quantity of the dried toxin and dissolve in *saline solution* so that 1.0 ml contains a precise known amount such as 10 mg. Prepare mixtures so that 2.0 ml of each mixture contains 0.8 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the test toxin. Dilute each mixture with *saline solution* to the same final volume (2.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and, using six mice for each mixture, inject intramuscularly into each mouse a dose of 0.2 ml of each mixture. Observe the mice thereafter for 72 hours.

The test dose of toxin is the amount present in 0.2 ml of that mixture which contains the smallest amount of toxin sufficient to cause the death within 72 hours of all six mice injected with it.

Determination of potency of the antitoxin. Dilute the test toxin with *saline solution* so that 1.0 ml contains 12.5 times the test dose. Prepare mixtures such that 2.0 ml of each mixture contains 0.8 ml of the dilution of the toxin and one of a series of graded volumes of the preparation under examination. Prepare further mixtures such that 2.0 ml of each contains 0.8 ml of the dilution of the toxin and one of a series of graded volumes, of the Standard Preparation diluted with *saline solution* so that the central graded volume contains 10 Units. Dilute each mixture with *saline solution* to the same final volume (2.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject intramuscularly a dose of 0.2 ml of each mixture into each of six mice under the conditions described for determination of the test dose of toxin. Observe the mice for 72 hours. If none of the mice is killed, 0.2 ml of the mixture contains more than 1 Unit of antitoxin. If all the mice are killed, 0.2 ml of the mixture contains less than 1 Unit of antitoxin.

The mixture that contains the largest volume of the preparation under examination that fails to protect the mice from death contains 10 Units. The test is not valid unless all the mice injected with mixtures containing 0.8 ml or less of the solution of the Standard Preparation die and all those injected with mixtures containing more survive.

Other tests. Complies with the tests stated under Antisera.

Storage. Store protected from light at a temperature between 2° to 8°. Liquid preparations should not be allowed to freeze.

Labelling. The label states (1) the number of Units per ml, where appropriate; (2) the animal species from which the preparation has been made; (3) the recommended dose; (4) the name and concentration of any antimicrobial preservative added; (5) that in case of a liquid preparation, it should not to be allowed to freeze.

Gas-Gangrene Antitoxin (Perfringens)

Anti-gas-gangrene (Perfringens) Serum

Gas-gangrene Antitoxin (Perfringens) is a preparation containing the specific antitoxic globulins obtained by purification of hyperimmune serum of horses or other suitable animals and having the specific activity of neutralising the alpha toxin formed by *Clostridium perfringens*.

Gas-gangrene Antitoxin (Perfringens) reconstituted where necessary as stated on the label complies with the following tests.

Gas-Gangrene Antitoxin (Perfringens) has a potency of not less than 1500 Units per ml.

Description. A clear, colourless or pale yellow liquid free from suspended particles or a freeze- dried, cream coloured powder or pellet for reconstitution with the diluent supplied by the manufacturer.

Identification

Specifically neutralises and renders the alpha toxin formed by *C. perfringens* harmless to susceptible animals or may be identified by any other suitable *in-vitro* test.

Tests

Potency. Carry out the biological assay of gas-gangrene antitoxin (perfringens).

Biological Assay of Gas-Gangrene Antitoxin (Perfringens)

The potency of the gas-gangrene antitoxin (perfringens) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of fixed dose of the Standard Preparation of gas-gangrene toxin (perfringens, type A) with the dose of the Standard Preparation of gas-gangrene antitoxin (perfringens) (*C. perfringens* alpha antitoxin) necessary to give the same protection. For this purpose the Standard Preparation of gas-gangrene antitoxin (*C. perfringens* alpha antitoxin) and a suitable preparation of gas-gangrene toxin (perfringens, type A),for use as a test toxin, are required. The test dose of the toxin is determined in the relation to the Standard Preparation and the potency of the preparation under examination is then determined in relation to the Standard preparation using the test toxin.

Standard Preparation

The Standard Preparation is a dried 1:3 dilution of hyperimmune horse serum or another suitable preparation of gas-gangrene antitoxin (perfringens), the potency of which has been determined in relation to the International Standard. The Unit is the specific neutralising activity for gas-gangrene toxin (perfringens) contained in such an amount of the Standard Preparation as the Ministry of Health and Family Welfare, Government of India may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

Test animals. Use healthy mice having body weights such that the difference between the lightest and heaviest is not more than 5 g.

Test toxin. Prepare gas-gangrene toxin (perfringens) by growing *C. perfringens*, type A, in a liquid culture medium for about 5 days, filtering aseptically and precipitating with

ammonium sulphate. The resulting precipitate, which contains the toxin, is collected, dried over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, powdered and kept dry.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities.

L+ dose — This is the smallest quantity of the toxin which, when mixed with 1 Unit of antitoxin and injected intravenously into mice, causes the death of the animals within 48 hours.

LD_{50} — This is the smallest quantity of toxin which, when injected intravenously into mice, causes the death of half the animals within 48 hours.

A suitable toxin is one which has an L+ dose in 4 mg or less, and contains not less than 20 LD_{50} in an L+ dose.

Determination of test dose of toxin *(L+dose)*. Prepare a solution of the Standard Preparation with *saline solution* such that 1.0 ml contains 5 Units. Weigh accurately a quantity of the dried toxin and dissolve it in *saline solution* so that 1.0 ml contains a precise known amount such as 10 mg. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the toxin. Dilute each mixture with *saline solution* to the same final volume (5.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and, using six mice for each mixture, inject intravenously into each mouse a dose of 0.5 ml of each mixture. Observe the mice thereafter for 48 hours.

The test dose of toxin is the amount present in 0.5 ml of that mixture which contains the smallest amount of toxin sufficient to cause the death within 48 hours of all six mice injected with it.

Determination of potency of the antitoxin. Dilute the test toxin with *saline solution* so that 1.0 ml contains five times the test dose. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the dilution of the toxin and one of a series of graded volumes of the preparation under examination. Prepare further mixtures such that 5.0 ml of each contains 2.0 ml of the dilution of the toxin and one of a series of graded volumes of the Standard Preparation diluted with *saline solution* so that the central graded volume contains 10 Units. Dilute each mixture with *saline solution* to the same final volume (5.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject intravenously a dose of 0.5 ml of each mixture into each of six mice under the conditions described for determination of the test dose of toxin. Observe the mice for 48 hours. If none of the mice is killed, 0.5 ml of the mixture contains more than 1 Unit of antitoxin. If all mice are killed, 0.5 ml of the mixture contains less than 1 Unit of antitoxin.

The mixture that contains the largest volume of the preparation under examination that fails to protect the mice from death contains 10 Units. The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the Standard Preparation die and all those injected with mixtures containing more survive.

Other tests. Complies with the tests stated under Antisera.

Storage. Store protected from light at a temperature between 2° to 8°. Liquid preparations should not be allowed to freeze.

Labelling. The label states (1) the number of Units per ml, where appropriate; (2) the animal species from which the preparation has been made; (3) the recommended dose; (4) the name and concentration of any antimicrobial preservative added; (5) that in case of a liquid preparation, it should not be allowed to freeze.

Gas-Gangrene Antitoxin (Septicum)

Anti-gas-gangrene (Septicum) Serum

Gas-gangrene Antitoxin (Septicum) is a preparation containing the specific antitoxic globulins obtained by purification of hyperimmune serum of horses or other suitable animals and having the specific activity of neutralising the alpha toxin formed by *Clostridium septicum*.

Gas-gangrene Antitoxin (Septicum) reconstituted where necessary as stated on the label complies with the following tests.

Gas-gangrene Antitoxin (Septicum) has a potency of not less than 1500 Units per ml.

Description. A clear, colourless or pale yellow liquid free from suspended particles or a freeze-dried, cream coloured powder or pellet for reconstitution with the diluent supplied by the manufacturer.

Identification

Specifically neutralises and renders the alpha toxin formed by *C. septicum* harmless to susceptible animals or may be identified by any other suitable *in-vitro* test.

Tests

Potency. Carry out the biological assay of gas-gangrene antitoxin (septicum).

Biological Assay of Gas-Gangrene Antitoxin (Septicum)

The potency of the gas-gangrene antitoxin (septicum) is determined by comparing the dose necessary to protect mice or other suitable animals against the lethal effects of gas-gangrene toxin (septicum) with the dose of the Standard Preparation of gas-gangrene antitoxin (*C. septicum*) necessary to give the same protection. For this purpose the

Standard Preparation of gas-gangrene antitoxin (*C. septicum*) and a suitable preparation of gas-gangrene toxin (septicum), for use as a test toxin, are required. The test dose of the toxin is determined in the relation to the Standard Preparation and the potency of the preparation under examination is then determined in relation to the Standard preparation using the test toxin.

Standard Preparation

The Standard Preparation is a dried 1:3 dilution of hyperimmune horse serum of gas-gangrene antitoxin (septicum) in *phosphate-buffered saline* or another suitable preparation the potency of which has been determined in relation to the International Standard. The Unit is the specific neutralising activity for gas-gangrene toxin (septicum) contained in such an amount of the Standard Preparation as the Ministry of Health and Family Welfare, Government of India may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Suggested Method

Test animals. Use healthy mice having body weights such that the difference between the lightest and heaviest is not more than 5 g.

Test toxin. Prepare gas-gangrene toxin (septicum) by growing *C. septicum* (Vibrion septique) in a liquid culture medium for about 5 days, filtering aseptically and precipitating with *ammonium sulphate*. The resulting precipitate, which contains the toxin, is collected, dried over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, powdered and kept dry.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities.

L+ dose — This is the smallest quantity of toxin which, when mixed with 1 Unit of antitoxin and injected intravenously into mice, causes the death of the animals within 72 hours.

LD_{50} — This is the smallest quantity of the toxin which, when injected intravenously into mice, causes the death of half the animals within 72 hours.

A suitable toxin is one which has an L+ dose in 0.5 mg or less and contains not less than 25 LD_{50} in an L+ dose.

Determination of test dose of toxin *(L+dose)*. Prepare a solution of the Standard Preparation in *saline solution* such that 1.0 ml contains 5 Units. Weigh accurately a quantity of the dried toxin and dissolve in *saline solution* so that 1.0 ml contains a precise known amount such as 20 mg. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard Preparation (10 Units) and one of a series of graded volumes of the solution of the toxin. Dilute each mixture with *saline solution* to the same final volume (5.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and, using six mice for each mixture, inject intravenously into each mouse a dose of 0.5 ml of each mixture. Observe the mice thereafter for 72 hours.

The test dose of toxin is the amount present in 0.5 ml of that mixture which contains the smallest amount of toxin sufficient to cause the death within 72 hours of all six mice injected with it.

Determination of potency of the antitoxin. Dilute the test toxin with *saline solution* so that 1.0 ml contains five times the test dose. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the dilution of the toxin and one of a series of graded volumes of the preparation under examination. Prepare further mixtures such that 5.0 ml of each contains 2.0 ml of the dilution of the toxin and one of a series of graded volumes of the Standard Preparation diluted with *saline solution* so that the central graded volume contains 10 Units. Dilute each mixture with *saline solution* to the same final volume (5.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject intravenously a dose of 0.5 ml of each mixture into each of six mice under the conditions described for determination of the test dose of toxin. Observe the mice for 48 hours. If none of the mice is killed, 0.5 ml of the mixture contains more than 1 Unit of antitoxin. If all the mice are killed, 0.5 ml of the mixture contains less than 1 Unit of antitoxin.

The mixture that contains the largest volume of the preparation under examination that fails to protect the mice from death contains 10 Units. The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the Standard Preparation die and all those injected with mixtures containing more survive.

Other tests. Complies with the tests stated under Antisera.

Storage. Store protected from light at a temperature between 2° to 8°. Liquid preparations should not be allowed to freeze.

Labelling. The label states (1) the number of Units per ml, where appropriate; (2) the animal species from which the preparation has been made; (3) the recommended dose; (4) the name and concentration of any antimicrobial preservative added; (5) that in case of a liquid preparation, it should not to be allowed to freeze.

Mixed Gas-Gangrene Antitoxin

Mixed Gas-gangrene Antitoxin is prepared by mixing Gas-gangrene Antitoxin (Oedematiens), Gas-gangrene Anti-toxin (Perfringens) and Gas-gangrene Antitoxin (Septicum) in appropriate quantities.

Mixed Gas-gangrene Antitoxin has a potency of not less than 1000 Units per ml of Gas-gangrene Antitoxin (Oedematiens),

not less than 1000 Units per ml of Gas-gangrene Antitoxin (Perfringens) and not less than 500 Units per ml of Gas-gangrene Antitoxin (Septicum).

Description. An almost colourless or very faintly yellow liquid, free from turbidity.

Identification

Specifically neutralises and renders the alpha-toxins formed by *C. oedematiens*, *C. perfringens* and *C. septicum* harmless to susceptible animals.

Tests

Potency. Carry out the biological assay for each component as described in the relevant individual monographs.

Other tests. Complies with the tests stated under Antisera.

Storage. Store protected from light at a temperature 2° to 8°. It should not be allowed to freeze. Liquid preparations should not be allowed to freeze.

Labelling. The label states the number of Units of each component per ml.

Haemophilus Type b Conjugate Vaccine

Haemophilus Type b Conjugate Vaccine is a liquid or freeze-dried preparation of a polysaccharide, derived from a suitable strain of *Haemophilus influenzae* type b, covalently bound to a carrier protein. The polysaccharide, polyribosylribitol phosphate, referred to as PRP, is a linear copolymer composed of repeated units of 3-β-D-ribofuronosyl-(1→1)-ribitol-5-phosphate $[(C_{10}H_{19}O_{12}P)_n]$, with a defined molecular size. The carrier protein, when conjugated to PRP, is capable of inducing a T-cell-dependent B-cell immune response to the polysaccharide.

Production

General provisions

The production method shall have been shown to yield consistently *H. influenzae* type b conjugate vaccines of adequate safety and immunogenicity in humans. The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy of Vaccines. The stability of the final lot and relevant intermediates is evaluated using one or more indicator tests. Such tests may include determination of molecular size, determination of free PRP in the conjugate and the immunogenicity test on mice. Taking account of the results of the stability testing, release requirements are set for these indicator tests to ensure that the vaccine will be satisfactory at the end of the period of validity.

SEED LOT

The strain of *H. influenzae* type b used in preparing Haemophilus type b conjugate vaccine shall be identified by a record of its history, including the source from which it was obtained and the tests made to determine the characteristics of the strain. The strain shall have been shown to be capable of producing Type b polysaccharide.

The production of PRP and of the carrier protein is based on defined seed lot systems. Master seed lot and working seed lot shall be properly characterized and defined. Cultures derived from the working seed shall have the same characteristics as of the master seed lot. The sample of culture of single harvests taken before killing shall be tested for contamination by examination of Gram-stained smears and by inoculation on suitable media.

H. Influenzae Type b Polysaccharide (PRP)

H. influenzae Type b is grown in a liquid medium that does not contain high-molecular-weight polysaccharides; if any ingredient of the medium contains blood-group substances, the process shall be validated to demonstrate that after the purification step they are no longer detectable. The culture may be inactivated. PRP is separated from the culture liquid and purified by a suitable method. Volatile matter, including water, In the purified polysaccharide is determined by methods such as thermogravimetry, Karl Fischer or any other suitable method. All chemical analysis shall be based on the dry weight of the polysaccharide, in its salt form.

Only those pools of PRP that comply with the following requirements may be used in the preparation of the conjugate. The partially purified PRP shall be stored frozen at or below -20°.

Identification

The PRP is identified by an immunochemical method (2.2.14) or other suitable method (e.g. ^1H or ^{13}C NMR spectroscopy).

Molecular size. The percentage of PRP eluted before a given K_0 value or within a range of K_0 values, is determined by gel filtration or high performance size-exclusion chromatography (HPSEC) (2.4.16), either alone or in combination with light scattering and refractive index detectors (e.g. multiple angle laser light scattering i.e. MALLS) or any other suitable method. An acceptable value is established for the particular product and each batch of PRP must be shown to comply with this limit. Limits for currently approved products, using the

Table 1 - Specifications for different components of Haemophilus Type b Conjugate Vaccine.

Polysaccharide		Carrier Protein			Conjugation	
Type of PRP	Nominal amount per dose	Type	Purity	Nominal amount per dose	Coupling method	Procedure
Polysaccharide (size reduced) K_0 60.0 per cent: 0.6-0.7	25 µg	Diphtheria toxoid	>1500 Lf per mg of protein nitrogen	18 µg	Cyanogen bromide activation of PRP	Activated diphtheria toxoid (D-AH^+), cyanogen bromide activated PRP
Polysaccharide: PRP ≥ 50.0 per cent ≤K_0: 0.30	10 µg	Tetanus toxoid	>1500 Lf per mg of protein nitrogen	20 µg	Carbodiimide-mediated coupling	ADH-activated PRP (PRP-cov.-AH) + tetanus toxoid + EDAC
Polysaccharide (size reduced) Dp=15-35 or 10-35	10 µg	CRM 197 diphtheria protein	>90.0 per cent diphtheria protein	25µg	Reductive amination one-(step method) or *N*-hydroxy-succinimide activation	Direct coupling of PRP to CRM 197 (cyanoboro-hydride activated)
Polysaccharide (size-reduced) K_0 0.3 – 0.6	15 µg	Meningococcal group B outer membrane protein (OMP)	Outer membrane protein vesicles <8.0 per cent of lipopolysaccharide	125 or 250 µg	Thioether bond	PRP activation by CDI PRP-IM + BuA2 + BrAc = PRP-BuA2-BrAc + thioactivated OMP

Abbreviations:

ADH	=	adipic acid dihydrazide	Dp	=	degree of polymerization
BrAc	=	bromoacetyl chloride	EDAC	=	l-ethyl-3-(3-dimethylaminopropyl) carbodimide
BuA2	=	butane-1, 4-diamide	IM	=	imidazolium
CDI	=	carbonyldi-imidazole	Mw	=	weight-average molecular weight.

Table 2 - Requirements on bulk conjugate

Test	Protein Carrier			
Specifications	*Diphtheria toxoid*	*Tetanus toxoid*	*CRM 197*	*OMP*
Free Polysaccharide (PRP)	<37.0 per cent	<20.0 per cent	<25.0 per cent	<15.0 per cent
Unbound protein	<5.0 per cent, where applicable	<1.0 per cent, where applicable	<1.0 per cent or <2.0 per cent, depending on the coupling method	Not applicable
PRP to protein ratio	1.25-1.75	0.30-0.55	0.3-0.7	0.05-0.1
Molecular size (K_0): Cross-linked agarose for chromatography R	95.0 per cent <0.75	60.0 per cent <0.2	50.0 per cent 0.3-0.6	85.0 per cent < 0.25
Cross-linked agarose for chromatography R1	0.6-0.7	85.0 per cent <0.5		

indicated stationary phases, are shown for information in Tables 1 and 2. Where applicable, the molecular-size distribution is also determined after chemical modification of the polysaccharide.

A validated determination of the degree of polymerization or of the weight-average molecular weight and the dispersion of molecular masses may be used instead of the determination of molecular size distribution.

Ribose (2.7.1). Not less than 32.0 per cent, calculated with reference to the dried substance, as estimated by Bial reaction for pentose, using D-ribose as a standard or any other suitable assay.

Phosphorus (2.7.1). 6.8 per cent to 9.0 per cent, calculated with reference to the dried substance.

Protein (2.3.49). Not more than 1.0 per cent, calculated with reference to the dried substance. Use sufficient PRP to allow detection of 1 per cent of protein (e.g. a minimum of 1 mg of PRP).

Nucleic acid (2.7.1). Not more than 1.0 per cent, calculated with reference to the dried substance by spectroscopy or any other suitable method.

Bacterial endotoxins (2.2.3). Not more than 25 IU of endotoxin per microgram of PRP.

Residual reagents. Where applicable, tests are carried out to determine residues of reagents used during inactivation and purification. An acceptable value for each reagent is established for the particular product and each batch of PRP must be shown to comply with this limit. Where validation studies have demonstrated removal of a residual reagent, the test on PRP may be omitted.

Carrier protein

The carrier protein is chosen in a way so that when the PRP is conjugated it is able to induce a T-cell-dependent immune response. Currently approved carrier proteins and coupling methods are listed for information in Table 1. The carrier proteins are produced by culture of suitable microorganisms; the bacterial purity of the culture is verified; the culture may be inactivated; the carrier protein is purified by a suitable method.

Only a carrier protein that complies with the following requirements may be used in preparation of the conjugate.

Identification

The carrier protein is identified by a suitable immunochemical method (2.2.14).

Sterility (2.2.11). Carry out the test for sterility using for each medium 10 ml or the equivalent of one hundred doses, whichever is less.

Diphtheria toxoid. Diphtheria toxoid is produced as stated under Diphtheria Vaccine (Adsorbed) and complies with the requirements prescribed there for bulk purified toxoid.

Tetanus toxoid. Tetanus toxoid is produced as stated under Tetanus Vaccine (Adsorbed) and complies with the requirements prescribed there for bulk purified toxoid except that the antigenic purity is not less than 1500 Lf per mg of protein nitrogen.

Diphtheria protein CRM 197. Suitable tests are carried out, for validation or routinely, to demonstrate that the product is non-toxic. The protein obtained contains not less than 90.0 per cent of diphtheria CRM 197 protein, when prepared by liquid chromatography (2.4.14) or any other suitable method. The carrier protein shall be characterized by a suitable chemical or physicochemical method like SDS-PAGE, HPLC, isoelectric focusing, amino acid sequencing, circular dichroism, fluorescence spectroscopy, peptide mapping or mass spectroscopy, as appropriate.

OMP (Meningococcal group B outer membrane protein complex)

OMP complex of *Neisseria meningitidis* complies with the following requirements for lipopolysaccharide and pyrogens.

Lipopolysaccharide. Not more than 8.0 per cent of lipopolysaccharide, determined by a suitable method.

Pyrogens (2.2.8). Inject into each rabbit 0.25 µg of OMP per kg body weight, for determining the pyrogenic effect.

Bulk conjugate

PRP is chemically modified to enable conjugation; it is usually partly depolymerised either before or during this procedure. Reactive functional groups or spacers may be introduced into the carrier protein or PRP prior to conjugation. The conjugate is obtained by the covalent binding of PRP and carrier protein. Where applicable, unreacted but potentially reactogenic functional groups are made unreactive by means of capping agents; the conjugate is purified to remove reagents. Where validation studies have demonstrated removal of a residual reagent (eg. CN, Br etc.), the test on bulk conjugate may be omitted.

Only a bulk conjugate that complies with the following requirements may be used in preparation of the final bulk vaccine. For each test and for each particular product, limits of acceptance are established and each batch of conjugate must be shown to comply with these limits. Limits applied to currently approved products for some of these tests are listed for information in Table 2.

PRP. The PRP content is determined by assay of phosphorus (2.7.1) or by assay of ribose (2.7.1) or by an immunochemical method (2.2.14) or by any suitable method.

Protein (2.7.1). The protein content is determined by a suitable chemical method.

PRP to protein ratio. Determine the ratio by calculation.

Molecular size. Molecular-size distribution is determined by gel filtration or size-exclusion chromatography (2.4.16), using a gel matrix, appropriate to the expected size of the conjugate.

Free PRP. Unbound PRP is determined after removal of the conjugate, for example by size-exclusion or hydrophobic chromatography (2.4.16), ultra-filtration or other validated methods.

Free carrier protein. Free carrier protein is determined by a suitable method (which may include deriving the content by calculation from the results of other tests). The amount is within the limits approved for the particular product.

Unreacted functional groups. No unreacted functional groups are detectable in the bulk conjugate unless process validation has shown that unreacted functional groups detectable at this stage are removed during the subsequent manufacturing process (for example, owing to short half-life).

Residual reagents. Removal of residual reagents such as *cyanide*, EDAC (*ethyldimethylaminopropylcarbodimide*) and *phenol* is confirmed by suitable tests or by validation of the purification process.

Sterility (2.2.11). Carry out the tests for sterility using for each medium 10 ml or the equivalent of one hundred doses, whichever is less.

FINAL BULK VACCINE

An adjuvant, an antimicrobial preservative and a stabilizer may be added to the bulk conjugate before dilution to the final concentration with a suitable diluent.

Only a final bulk vaccine that complies with the following requirements may be used in preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final lots are filled in suitable containers, under stringent aseptic conditions.

Only a final lot that is satisfactory with respect to each of the requirements given under Identification, Tests and Assay may be released for use. Provided the test for antimicrobial preservative has been carried out on the final bulk vaccine, it may be omitted on the final lot.

Identification

The vaccine is identified by a suitable immunochemical method (2.2.14) for PRP.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a quantity of the vaccine equivalent to 1 µg of PRP for a vaccine with diphtheria toxoid or CRM 197 diphtheria protein as carrier; 0.1 µg of PRP for a vaccine with tetanus toxoid as carrier; 0.025 µg of PRP for a vaccine with OMP as carrier.

pH (2.4.24). The pH of the vaccine, reconstituted if necessary, is within the range approved for the product.

Aluminium (2.3.9). Not more than 1.25 mg per single human dose. When aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent,

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and is not greater than 115.0 per cent of that stated on the label.

Water (2.3.43). Not more than 3.0 per cent.

PRP. Not less than 80.0 per cent and not greater than 120.0 per cent of the amount of PRP stated on the label as determined by ribose assay (2.7.1) or by phosphorus assay (2.7.1) or by an immunochemical method (2.2.14) or by any other suitable method like colorimetery or by anion exchange liquid chromatography (2.4.14) with pulsed amperometric detection.

Free PRP. Unbound PRP is determined after removal of the conjugate for example by size-exclusion or hydrophobic chromatography (2.4.16), ultra filtration or other validated methods.

Labelling. The label states (1) the number of micrograms of PRP per human dose; (2) the type and nominal amount of carrier protein per single human dose; (3) for vaccine contained in single-dose containers where the space is too small to accommodate the full name of the vaccine the abbreviation 'Hib' may be used in the label on the container provided that the same code is also stated in the label on the package.

Hepatitis A (Inactivated) and Hepatitis B (rDNA) Vaccine (Adsorbed)

Hepatitis A (Inactivated) and Hepatitis B (rDNA) Vaccine (Adsorbed) is a suspension consisting of a suitable strain of hepatitis A virus, grown in cell cultures and inactivated by a

validated method, and of hepatitis B surface antigen (HBsAg), a component protein of hepatitis B virus obtained by recombinant DNA technology; the antigens are adsorbed on a mineral carrier such as aluminium hydroxide or hydrated aluminium phosphate.

Production

General provisions

The two components are prepared as described in the monographs on Hepatitis A Vaccine (Inactivated, Adsorbed) and Hepatitis B Vaccine (rDNA) and comply with the requirements prescribed therein. The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity for antisera and vaccines.

Reference preparation

The reference preparation is part of a representative batch shown to be at least as immunogenic in animals as a batch that, in clinical studies in young, healthy adults, produces not less than 95.0 per cent seroconversion, corresponding to a level of neutralizing antibody recognized to be protective, after a full-course primary immunization. For hepatitis A, an antibody level not less than 20 mIU/ml determined by enzyme-linked immunosorbent assay is recognized as being protective. For hepatitis B, antibody level not less than 10 mIU/ml against HBsAg is recognized as being protective.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated harvests of hepatitis A virus and one or more batches of purified antigen of Hepatitis B (rDNA).

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. If the assay of the hepatitis A and/or the hepatitis B component is carried out *in vivo*, then provided it has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Identification

The vaccine is shown to contain hepatitis A virus antigen and hepatitis B surface antigen by suitable immunochemical methods (2.2.14), using specific antibodies or by the mouse immunogenicity tests described under assay.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Less than 2 IU per human dose.

Assay

Hepatitis A component

Complies with the assay as stated under Inactivated Hepatitis A Vaccine (Adsorbed).

Hepatitis B component

Complies with the assay as stated under Hepatitis B Vaccine (rDNA).

Labelling. The label states (1) the amount of hepatitis A virus antigen and hepatitis B surface antigen per container; (2) the type of cells used for production of the vaccine; (3) the name and amount of the adsorbent used; (4) that the vaccine must be shaken before use; (5) that the vaccine must not be frozen.

Hepatitis B Vaccine (rDNA)

Hepatitis B Vaccine (rDNA) is a non-infectious preparation containing the purified major surface antigen of Hepatitis B virus (HBsAg). This preparation is white or almost white translucent liquid in which the mineral carrier tends to settle down slowly on keeping but is free from foreign particles/ floccules.

Production

General provisions

The antigen is manufactured by recombinant DNA technology by culturing genetically engineered yeast cells or other suitable

cell lines which carry the gene that codes for major surface antigen of the Hepatitis-B virus as approved by the competent authority. Several physico-chemical steps are employed to purify the Hepatitis-B surface antigen (HBsAg). The vaccine may contain the product of the S gene (major protein), a combination of the S gene and pre-S2 gene products (middle protein) or a combination of S gene, the pre-S2 gene, and pre-S1 gene products (large protein). The purity of the antigen is determined by comparison with a reference preparation using liquid chromatography or other suitable methods such as SDS-PAGE with any suitable staining method. The purified antigen is finally adsorbed on aluminium hydroxide or aluminium phosphate.

The method used for production of the vaccine must have been shown to yield a product consistently complying with the requirements for immunogenicity and safety. It must also have been shown to induce specific, protective antibodies in human beings.

The production method must be validated to demonstrate that the product if tested, would comply with the tests for safety and efficacy.

Reference preparation. A part of a batch shown to be at least as immunogenic as a batch that was used in clinical studies and approved by National Regulatory Authority and determined by any suitable method. For hepatitis B, antibody level not less than 10 mIU/ml against HBsAg is recognized as being protective..

Characterisation of the substance

Development studies are carried out to characterize the antigen. The complete protein, lipid and carbohydrate structure of the antigen is established. The morphological characteristics of the antigen particles are established by electron microscopy. The buoyant density of the antigen particles is determined by a physico-chemical method, for example gradient centrifugation. The antigenic epitopes are characterized. The protein fraction of the antigen is characterized in terms of the primary structure (for example, by determination of the amino-acid composition, by partial amino-acid sequence analysis).

PROPAGATION AND HARVEST

Identity, microbial purity, plasmid retention and consistency of yield are determined at suitable production stages. If mammalian cells are used, tests for extraneous agents and mycoplasmas are performed in accordance with tests for extraneous agents in viral vaccines for human use.

PURIFIED ANTIGEN

Only a purified antigen that complies with the following requirements may be used in the preparation of the final bulk.

Total protein (2.3.49). The total protein is determined by a validated method. The content is within the limits approved for the specific product.

Antigen content and identification. The quantity and specificity of HBsAg is determined in comparison with the International standard for HBsAg subtype ad or an in-house reference, by a suitable immunochemical method such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), immunoblot (preferably using a monoclonal antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio is within the limits approved for the specific product.

The molecular weight of the major band in a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions corresponds to the value expected from the gene sequence and possible glycosylation.

Antigenic purity. The purity of the antigen is determined by comparison with a reference preparation using liquid chromatography or other suitable methods such as SDS-PAGE with staining. A suitable method is sensitive enough to detect a potential contaminant at a concentration of 1.0 per cent of total protein. Not less than 95.0 per cent of the total protein consists of hepatitis B surface antigen.

Composition. The content of proteins, lipids, nucleic acids and carbohydrates is determined.

Host-cell and vector-derived DNA (2.2.15). If mammalian cells are used for production, not more than 10 pg of DNA in the quantity of purified antigen equivalent to a single human dose of vaccine.

Caesium. If a caesium salt is used during production, a test for residual caesium is carried out on the purified antigen. The content is within the limits approved for the specific product.

Sterility (2.2.11). The purified antigen complies with the test for sterility, carried out using 10 ml for each medium.

Additional tests on the purified antigen may be required depending on the production method used: for example, a test for residual animal serum where mammalian cells are used for production or tests for residual chemicals used during extraction and purification.

FINAL BULK VACCINE

An antimicrobial preservative and an adjuvant may be included in the vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, antimicrobial preservative content and the assay in animals, where applicable, have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot. The estimate of potency shall be expected in terms of µg/ml i.e not less than 20 µg/ml.

Identification

The assay or, where applicable, the electrophoretic profile, also serves to identify the vaccine.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbant.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115.0 per cent of that stated on the label.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject the equivalent of one human dose into each rabbit.

A validated test for bacterial endotoxins may be used instead of the test for pyrogens.

Assay. The vaccine complies with the assay of Hepatitis-B Vaccine (rDNA) described below.

Potency. The upper fiducial limit (P = 0.95) of the estimated relative potency is not less than 1.0. The estimate of potency shall be expressed in terms of µg/ml i.e, not less than 20 µg/ml.

Determine the potency either in animals (Method A) or by a validated *in vitro* procedure (Method B) described below:

Method A (Biological)

The potency of the vaccine under examination is determined in animals by comparing in given conditions its capacity to induce specific anti-HBsAg antibodies in mice or guinea-pigs with the same capacity as with the reference standard.

Inject intraperitoneally not less than three suitable dilutions of the vaccine under examination diluted with adjuvant used in the vaccine into groups of a suitable strain of mice, weighing between 15 and 20 g (about 5 weeks old), of either sex distributed randomly into several groups of mice. Healthy guinea pigs weighing between 300 and 350 g (about 7 weeks old) that have not been previously treated with any material that will interfere with the test will also be suitable for the test. Use animals of the same sex in the test. Inject similar groups of animals with the reference preparation of Hepatitis-B vaccine (r DNA). One group of control animals remains unvaccinated but is injected intraperitoneally with the same volume of the diluent alone. Anaesthetize and bleed the animals 28 to 42 days later, keeping the individual sera separate. Assay the individual sera for specific HBsAg antibody concentration by a suitable immunochemical method such as ELISA or RIA.

Calculate the result of the assay by standard statistical methods (5.7). From the distribution of reaction levels measured on all the sera in the unvaccinated (control group), the maximum reaction level that can be expected to occur in an unvaccinated animal for that particular assay is determined. Any response in the vaccinated animals that exceeds this level is by definition seroconversion. The percentage of animals showing seroconversion in each group is transformed (for example, probit transformation) and a parallel line model, using the log dose response curve, is applied to the data. The potency of the preparation under examination relative to the reference preparation is thus established. The test is not valid unless (a) for both the test and reference vaccine, the ED_{50} lies between the smallest and the largest doses given to animals ; (b) the statistical analysis shows no deviation from linearity or parallelism; (c) the fiducial limits of the estimated relative potency fall between 33.0 and 300.0 per cent of the estimated potency.

Method B *(In vitro)*

The potency of the vaccine under examination is determined by an *in vitro* method that has been validated against the biological test.

Enzyme Linked Immunosorbent Assay (ELISA) using monoclonal antibodies specific for protection inducing epitopes of HBsAg have been shown to be suitable. Adequate number of dilutions and replicates of the vaccine under examination and the reference standard are employed in the assay. The data obtained is analyzed by a parallel-line model and may be suitably transformed for statistical evaluation. Commercially available kits for measuring HBsAg *in vitro* may be used provided they are validated to produce equally precise and accurate results.

The test is not valid unless (a) the statistical analysis shows no deviation from linearity or parallelism; (b) the fiducial limits of the estimated relative potency fall between 80.0 and 125.0 per cent of the estimated potency.

The acceptance criteria are approved for a given reference preparation by the National Regulatory Authority in the light of the validation data.

Labelling. The label states (a) the amount of HBsAg per dose; (b) the type of cells used for production of the vaccine; (c) the

name and amount of the adjuvant; (d) that the vaccine must be shaken before use; (e) that the vaccine must not be frozen.

Inactivated Hepatitis A Vaccine (Adsorbed)

Hepatitis A Vaccine (Inactivated, Adsorbed) is a liquid preparation of a suitable strain of hepatitis A virus grown in cell cultures, inactivated by a validated method and adsorbed on a mineral carrier. The vaccine is an opalescent suspension.

The vaccine complies with the monograph on Vaccines.

Production

Production of the vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

Reference preparation. A part of a batch shown to be at least as immunogenic as a batch that, in clinical studies in young healthy adults, produced not less than 95.0 per cent seroconversion, corresponding to a level of neutralising antibody accepted to be protective, after a full-course of primary immunisation is used as a reference preparation. An antibody level of 20 mIU /ml determined by enzyme-linked immunosorbent assay is recognised as being protective.

Substrate for virus propagation

The virus is propagated in a human diploid cell line or in a continuous cell line approved by the competent authority.

SEED LOT

The strain of hepatitis A virus used to prepare the master seed lot shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Description. A clear, colourless or light coloured liquid.

Identification

Each master and working seed lot is identified as hepatitis A virus using specific antibodies.

Virus concentration. The virus concentration of each master and working seed lot is determined to monitor consistency of production.

Extraneous agents (2.7.3). The master and working seed lots comply with the requirements for seed lots for virus vaccines. In addition, if primary monkey cells have been used for isolation of the strain, measures are taken to ensure that the strain is not contaminated with simian viruses such as simian immunodeficiency virus and filoviruses.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator, such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. When the determination of the ratio of virus concentration to antigen content has been carried out on a suitable number of single harvests to demonstrate consistency, it may subsequently be omitted as a routine test.

Identification

The test for antigen content also serves to identify the single harvest.

Sterility (2.2.11). The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas (2.7.4). The single harvest complies with the test for mycoplasmas carried out using 1ml for each medium.

Control cells. The control cells of the production cell culture comply with a test for identity and the requirements for extraneous agents.

Antigen content. Determine the hepatitis A antigen content by a suitable immunochemical method (2.2.14) to monitor production consistency; the content is within the limits approved for the particular product.

Ratio of virus concentration to antigen content. The consistency of the ratio of the concentration of infectious virus, as determined by a suitable cell culture method, to antigen content is established by validation on a suitable number of single harvests.

PURIFICATION AND PURIFIED HARVEST

The harvest, which may be a pool of several single harvests, is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the level of host-cell DNA. Only a purified harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Virus concentration. The concentration of infective virus in the purified harvest is determined by a suitable cell culture method to monitor production consistency and as a starting point for monitoring the inactivation curve.

Antigen total protein ratio. Determine the hepatitis A virus antigen content by a suitable immunochemical method (2.2.14). Determine the total protein by a validated method. The ratio of hepatitis A virus antigen content to total protein content is within the limits approved for the particular product.

Bovine serum albumin. Not more than 50 ng in the equivalent of a single human dose, determined by a suitable immunochemical method (2.2.14). Where appropriate in view of the manufacturing process, other suitable protein markers may be used to demonstrate effective purification.

Residual host-cell DNA (2.2.15). If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method is not greater than 10 ng per single human dose.

Residual chemicals. If chemical substances are used during the purification process, tests for these substances are carried out on the purified harvest (or on the inactivated harvest), unless validation of the process has demonstrated total clearance. The concentration must not exceed the limits approved for the particular product.

INACTIVATION AND INACTIVATED HARVEST

Several purified harvests may be pooled before inactivation. In order to avoid interference with the inactivation process, virus aggregation must be prevented or aggregates must be removed immediately before and/or during the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating hepatitis A virus without destroying the antigenic and immunogenic activity; as part of the validation studies, an inactivation curve is plotted representing residual live virus concentration measured on at least three occasions (for example, on days 0, 1 and 2 of the inactivation process). If formaldehyde is used for inactivation, the presence of excess free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation. Carry out an amplification test for residual infectious hepatitis A virus by inoculating a quantity of the inactivated harvest equivalent to 5 per cent of the batch or if the harvest contains the equivalent of 30,000 doses or more, not less than 1,500 doses of vaccine into cell cultures of the same type as those used for production of the vaccine and incubating the cells for at least 28 days. Make two passages and at the end of incubation carry out a test of suitable sensitivity for residual infectious virus. No evidence of hepatitis A virus multiplication is found in the samples taken at the end of the inactivation process. Use infective virus inocula concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Sterility (2.2.11). The inactivated viral harvest complies with the test for sterility, carried out using 10 ml for each medium.

Bacterial endotoxins (2.2.3). Not more than 2 IU of endotoxin in the equivalent of a single human dose.

Antigen content. Determine the hepatitis A virus antigen content by a suitable immunochemical method (2.2.14).

Residual chemicals. As stated under Purification and Purified Harvest.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated harvests. Approved adjuvants, stabilisers and antimicrobial preservatives may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde (where applicable) and antimicrobial preservative content (where applicable) and the assay have been carried out on the final bulk vaccine with satisfactory results, these tests may be omitted on the final lot. If the assay is carried out using mice or other animals, then provided it has been carried with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Identification

The vaccine is shown to contain hepatitis A virus antigen by a suitable immunochemical method using specific antibodies or by the mouse immunogenicity test described under Assay.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if hydrated aluminium phosphate or aluminium hydroxide is used as the adsorbent.

Free formaldehyde (2.3.20). When formaldehyde has been used for inactivation, the vaccine complies with the test prescribed in Vaccines.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Assay

The vaccine complies with the assay of Hepatitis A vaccine.

The assay is carried out either *in vivo*, by comparing in given condition the capacity to induce specific antibodies in mice with the same capacity of a reference preparation or *in vitro* by an immunochemical determination of antigen content (2.2.14).

In vivo assay

The test on mice shown below is given as an example of a method that has been found suitable for a given vaccine; other validated methods may also be used.

Selection and distribution of the test animals. Healthy mice from the same stock, about 5 weeks old and from a strain shown to be suitable should be used in the test. Use animals of the same sex. Distribute the animals in at least seven equal groups of a number suitable for the requirements of the assay.

Determination of potency of the vaccine under examination. Using a 0.9 per cent w/v solution of *sodium chloride* containing the aluminium adjuvant used for the vaccine, prepare at least three dilutions of the vaccine under examination and matching dilutions of the reference preparation. Allocate the dilutions one to each of the groups of animals and inject subcutaneously not more than 0.5 ml of each dilution into each animal in the group to which that dilution is allocated. Maintain a group of unvaccinated controls, injected subcutaneously with the same volume of diluent. After 28 to 32 days, anaesthetise and bleed all animals, keeping the individual sera separate. Assay the individual sera for specific antibodies against hepatitis A virus by a suitable immunochemical method (2.2.14).

Calculations. Carry out the calculations by the usual statistical methods (5.7) for an assay with a quantal response.

From the distribution of reaction levels measured on all the sera in the unvaccinated group, determine the maximum reaction level that can be expected to occur in an unvaccinated animal for that particular assay. Any response in vaccinated animals that exceeds this level is by definition a seroconversion.

Make a suitable transformation of the percentage of animals showing seroconversion in each group (for example, a probit transformation) and analyse the data according to a parallel-line log dose-response model. Determine the potency of the test preparation relative to the reference preparation.

Validity conditions. The test is not valid unless (a) for both the test and the reference vaccine, the ED_{50} lies between the smallest and the largest doses given to the animals; (b) the statistical analysis shows no significant deviation from linearity or parallelism; (c) the fiducial limits of the estimated relative potency fall between 33.0 and 300.0 per cent of the estimated potency.

Potency. The upper fiducial limit (P = 0.95) of the estimated relative potency is not less than 1.0.

In vitro assay

Carry out an immunochemical determination of antigen content (2.2.14) with acceptance criteria validated against the *in vivo* test. The acceptance criteria are approved for a given reference preparation by the National Regulatory Authority in the light of the validation data.

Labelling. The label states (1) the biological origin of the cells and; (2) the adjuvant used for the preparation of the vaccine.

Inactivated Hepatitis B Vaccine

Inactivated Hepatitis B Vaccine is a non-infectious inactivated liquid preparation derived from the surface antigen of Hepatitis B virus (HbsAg). This preparation is white or almost white translucent liquid in which the mineral carrier tends to settle down slowly on keeping but is free from foreign particles / floccules.

Production

The antigen is harvested and purified from the plasma of human carriers of Hepatitis B virus. The surface antigen contains all the three antigen species (S, Pre-S1, Pre-S2). The individual donor plasma is shown by sensitive tests to be seronegative for HIV-I and HIV-2 and for HCV. The plasma pool is tested for freedom from adventitious viruses and blood borne transmissible pathogens by appropriate methods. The purified antigen is further inactivated by a validated method, usually with formalin or any other inactivating agent, to render the

hepatitis B virus harmless. The preparation is also tested for the residual HBV DNA using a sensitive test approved by the competent authority and the level is shown to be less than 1 pg HBV DNA per 50 doses.

The method used for production of the vaccine must have been shown to yield a product consistently complying with the requirements of immunogenicity, safety and stability. The production method must also be validated to demonstrate that the product, if tested, would comply with the tests for safety and efficacy.

Reference preparation. A part of a batch shown to be at least as immunogenic as a batch that produced in clinical studies in young healthy adults not less than 95.0 per cent seroconversion, corresponding to a level of neutralizing antibody accepted to be protective (HbsAg antibody titre not less than 10 mIU/ml after a full course of primary immunization determined by enzyme-linked immunosorbent assay (ELISA) is used as a reference preparation.

Characterisation of the substance

Development studies are carried out to characterize the antigen. The complete protein, lipid and carbohydrate structure of the antigen is established. The morphological characteristics of the antigen particles are established by electron microscopy. The buoyant density of the antigen particles is determined by a physico-chemical method (2.4.29), for example gradient centrifugation. The antigenic epitopes are characterized. The protein fraction of the antigen is characterized in terms of the primary structure (for example, by determination of the amino-acid composition, by partial amino-acid sequence analysis).

PROPAGATION AND HARVEST

Identity, microbial purity, plasmid retention and consistency of yield are determined at suitable production stages.

PURIFIED ANTIGEN

Only a purified antigen that complies with the following requirements may be used in the preparation of the final bulk.

Total protein (2.3.49). The total protein is determined by a validated method. The content is within the limits approved for the specific product.

Antigen content and identification. The quantity and specificity of HBsAg is determined in comparison with the International standard for HBsAg subtype *ad* or an in-house reference, by a suitable immunochemical method such as radio immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), immunoblot (preferably using a monoclonal antibody directed against a protective epitope) or single radial diffusion. The antigen/protein ratio is within the limits approved for the specific product.

The molecular weight of the major band in a sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions corresponds to the value expected from the gene sequence and possible glycosylation.

Antigenic purity. The purity of the antigen is determined by comparison with a reference preparation using liquid chromatography or other suitable methods such as SDS-PAGE with staining. A suitable method is sensitive enough to detect a potential contaminant at a concentration of 1.0 per cent of total protein. Not less than 95.0 per cent of the total protein consists of hepatitis B surface antigen.

Composition. The content of proteins, lipids, nucleic acids and carbohydrates is determined.

Sterility (2.2.11). The purified antigen complies with the test for sterility carried out using 10 ml for each medium.

Additional tests on the purified antigen may be required depending on the production method used.

FINAL BULK VACCINE

An antimicrobial preservative and an adjuvant may be included in the vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical or physico-chemical method. The amount is not less than the 85.0 per cent and not greater than 115.0 per cent of that stated on the label.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, antimicrobial preservative content and the assay in animals, where applicable, have been carried out on the final bulk vaccine with satisfactory results, they may be omitted on the final lot.

Identification

The assay or, where applicable, the electrophoretic profile, also serves to identify the vaccine.

Tests

Aluminium (2.3.9). When hydrated aluminium phosphate or aluminium hydroxide is used as the adsorbent, the vaccine complies with the test prescribed in the monograph on Vaccines.

Test for inactivating agent. The concentration of any inactivating agent remaining in the final vaccine shall be determined by methods approved by the competent authority. The concentration shall not exceed a specified upper limit.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject the equivalent of one human dose into each rabbit.

A validated test for bacterial endotoxins (2.2.3) may be used instead of the test for pyrogens.

Assay

The upper fiducial limit (P = 0.95) of the estimated relative potency is not less than 1.0.

Determine the potency by method A (Biological) as described under Hepatitis-B vaccine (rDNA).

Labelling. The label states (1) the amount of HBsAg per dose; (2) the name and amount of inactivating agent; (3) the name and amount of the adjuvant; (4) that the vaccine must be shaken before use; (5) that the vaccine must not be frozen.

Inactivated Influenza Vaccine (Split Virion)

Influenza Vaccine (Split Virion, Inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the two types grown individually in eggs derived from specific pathogen free flock or cell cultures, inactivated and treated so that the integrity of the virus particles has been disrupted without diminishing the antigenic properties of the haemagglutinin and neuraminidase antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

Production

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Choice of vaccine strain

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends new strains corresponding to prevailing epidemiological evidence.

The origin and passage history of virus strains shall be approved by the National Regulatory Authority.

Substrate for virus propagation

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens or in suitable cell cultures, such as chick-embryo fibroblasts or chick kidney cells obtained from chicken flocks free from specified pathogens. For production, the virus of each strain is grown in the allantoic cavity of eggs derived from specific pathogen free flocks.

SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than fifteen passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents one passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

Mycoplasmas (2.7.4). Carry out the test for mycoplasmas using 10 ml.

PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production, penicillin or streptomycin is used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on three consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at a temperature of 5 ± 3°. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of

formaldehyde at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent v/v at any time during inactivation.

Before or after the inactivation procedure, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method and the virus particles are disrupted into component subunits by the use of approved procedures. For each new strain, a validation test is carried out to show that the monovalent bulk consists predominantly of disrupted virus particles.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen. Determine the content of haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it. Carry out the test at 20° to 25°.

For some vaccines, the physical form of the haemagglutinin particles prevents quantitative determination by immunodiffusion after inactivation of the virus. For these vaccines, a determination of haemagglutinin antigen is made on the monovalent pooled harvest before inactivation. The production process is validated to demonstrate suitable conservation of haemagglutinin antigen and a suitable tracer is used for formulation, for example, protein content.

Neuraminidase antigen. The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods (2.2.14) on the first three monovalent pooled harvests from each working seed lot.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

Viral inactivation. Carry out as described below under Tests.

Chemicals used for disruption. Tests are carried out on the monovalent pooled harvest for the chemicals used for disruption, the limits being approved by the National Regulatory Authority.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for viral inactivation has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Description. The vaccine is a slightly opalescent liquid.

Identification

The assay serves to confirm the antigenic specificity of the vaccine.

Tests

Viral inactivation. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryoes survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Total protein (2.3.49). Not more than six times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

Ovalbumin. Not more than 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bacterial endotoxins (2.2.3). Not more than 100 IU of endotoxin per human dose.

Assay

Determine the content of haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with an appropriate haemagglutinin antigen reference preparation. Carry out the test at 20 to 25°. The confidence interval (P = 0.95) of the assay is not greater than 80.0 per cent to 125.0 per cent of the estimated content. The lower confidence limit (P = 0.95) of the estimate of haemagglutinin antigen content is not less than 80.0 per cent of the amount stated on the label for each strain.

For some vaccines, quantitative determination of haemagglutinin antigen with respect to available reference preparations is not possible. An immunological identification of the haemagglutinin antigen and a semi-quantitative determination are carried out instead by suitable methods.

Labelling. The label complies with the requirements stated under Vaccines and also states (a) that the vaccine has been prepared on eggs; (b) the strain or strains of influenza virus used to prepare the vaccine; (c) the method of inactivation; (d) the haemagglutinin content in µg per virus strain per dose; (e) the season during which the vaccine is intended to protect.

Inactivated Influenza Vaccine (Surface Antigen)

Influenza Vaccine (Surface Antigen, Inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the two types grown individually in eggs derived from specific pathogen free flocks or cell cultures, inactivated and treated so that the preparation consists predominantly of haemagglutinin and neuraminidase antigens, without diminishing the antigenic properties of these antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

Production

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Choice of vaccine strain

The World Health Organisation reviews the world epidemiological situation annually and if necessary recommends new strains corresponding to prevailing epidemiological evidence.

The origin and passage history of virus strains shall be approved by the competent authority.

Substrate for virus propagation

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens or in suitable cell cultures, such as chick-embryo fibroblasts or chick kidney cells obtained from chicken flocks free from specified pathogens. For production, the virus of each strain is grown in the allantoic cavity of eggs derived from SPF flocks.

SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than fifteen passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents one passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

Mycoplasmas (2.7.4). Carry out the test for mycoplasmas using 10 ml.

PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production penicillin or streptomycin is used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on three consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at a temperature of 5±3°. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of formaldehyde at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent v/v at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method. Virus particles are disrupted into component subunits by approved procedures and further purified so that the monovalent bulk consists mainly of haemagglutinin and neuraminidase antigens.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen. Determine the content of haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it. Carry out the test at 20° to 25°.

Neuraminidase antigen. The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods (2.2.14) on the first three monovalent pooled harvests from each working seed lot.

Sterility (2.2.11). Carry out the test for sterility, using 10 ml for each medium.

Viral inactivation. Carry out the test described below under Tests.

Purity. The purity of the monovalent pooled harvest is examined by polyacrylamide gel electrophoresis or by other approved techniques. Mainly haemagglutinin and neuraminidase antigens shall be present.

Chemicals used for disruption and purification. Tests are carried out on the monovalent pooled harvest for the chemicals used for disruption and purification, the limits being approved by the competent authority.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for viral inactivation has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Description. The vaccine is a clear liquid.

Identification

The assay serves to confirm the antigenic specificity of the vaccine.

Tests

Viral inactivation. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Total protein (2.3.49). Not more than 40 µg of protein other than haemagglutinin per virus strain per human dose and not more than a total of 120 µg of protein other than haemagglutinin per human dose.

Ovalbumin. Not more than 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Free formaldehyde (2.3.20). Complies with the test for free formaldehyde as stated under General Requirements for Vaccines for Human Use.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Not more than 100 IU of endotoxin per human dose.

Assay

Determine the content haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with an appropriate haemagglutinin antigen reference preparation. Carry out the test at 20° to 25°. The confidence interval (P = 0.95) of the assay is not greater than 80.0 per cent to 125.0 per cent of the estimated content. The lower confidence limit (P = 0.95) of the estimate of haemagglutinin antigen content is not less than 80.0 per cent of the amount stated on the label for each strain.

Labelling. The label states (1) that the vaccine has been prepared on eggs; (2) the strain or strains of influenza virus used to prepare the vaccine; (3) the method of inactivation; (4) the haemagglutinin content in micrograms per virus strain per dose; (5) the season during which the vaccine is intended to protect.

Inactivated Influenza Vaccine (Whole Virion)

Influenza Vaccine (Whole Virion, Inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the two types grown individually in eggs derived from specific pathogen free flocks or cell culture and inactivated in such a manner that their antigenic properties are retained. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

Production

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Choice of vaccine strain

The World Health Organisation reviews the world epidemiological situation annually and, if necessary, recommends new strains corresponding to prevailing epidemiological evidence.

The origin and passage history of virus strains shall be approved by the competent authority.

Substrate for virus propagation

Influenza virus seed to be used in the production of vaccine is propagated in fertilised eggs from chicken flocks free from specified pathogens or in suitable cell cultures, such as chick-embryo fibroblasts or chick kidney cells obtained from chicken flocks free from specified pathogens .For production, the virus of each strain is grown in the allantoic cavity of eggs derived from specific pathogen free flocks.

SEED LOT

The production of vaccine is based on a seed-lot system. Working seed lots represent not more than fifteen passages from the approved reassorted virus or the approved virus isolate. The final vaccine represents one passage from the working seed lot. The haemagglutinin and neuraminidase antigens of each seed lot are identified as originating from the correct strain of influenza virus by suitable methods.

Only a working virus seed lot that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

Mycoplasmas (2.7.4). Carry out the test for mycoplasmas using 10 ml.

PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on three consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at a temperature of 5±3°. If formaldehyde solution is used, the concentration does not exceed 0.2 g/l of formaldehyde at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent v/v at any time during inactivation.

Before or after the inactivation process, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen. Determine the content of haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it. Carry out the test at 20° to 25°.

Neuraminidase antigen. The presence and type of neuraminidase antigen are confirmed by suitable enzymatic or immunological methods (2.2.14) on the first three monovalent pooled harvests from each working seed lot.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

Viral inactivation. Carry out the test described below under Tests.

FINAL BULK VACCINE

Appropriate quantities of the monovalent pooled harvests are blended to make the final bulk vaccine.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Tests and Assay may be released for use. Provided that the test for viral inactivation has been performed with satisfactory results on each monovalent pooled harvest and that the tests for free formaldehyde, ovalbumin and total protein have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Description. The vaccine is a slightly opalescent liquid.

Identification

The assay serves to confirm the antigenic specificity of the vaccine.

Tests

Viral inactivation. Inoculate 0.2 ml of the vaccine into the allantoic cavity of each of ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryos survive. Harvest 0.5 ml of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 ml of the pooled fluid into a further ten fertilised eggs and incubate at 33° to 37° for 3 days. The test is not valid unless at least eight of the ten embryos survive. Harvest about 0.1 ml of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage in eggs and test for haemagglutination; no haemagglutination occurs.

Total protein (2.3.49). Not more than six times the total haemagglutinin content of the vaccine as determined in the assay, but in any case, not more than 100 µg of protein per virus strain per human dose and not more than a total of 300 µg of protein per human dose.

Ovalbumin. Not more than 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and is not greater than 115.0 per cent of the quantity stated on the label.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Not more than 100 IU of endotoxin per human dose.

Assay

Determine the content of haemagglutinin antigen by an immunodiffusion test (2.2.14), by comparison with an appropriate haemagglutinin antigen reference preparation. Carry out the test at 20° to 25°. The confidence interval (P = 0.95) of the assay is not greater than 80.0 per cent to 125.0 per cent of the estimated content. The lower confidence limit (P = 0.95) of the estimate of haemagglutinin antigen content is not less than 80.0 per cent of the amount stated on the label for each strain.

Labelling. The label states (1) that the vaccine has been prepared on eggs; (2) the strain or strains of influenza virus used to prepare the vaccine; (3) the method of inactivation; (4) the haemagglutinin content in micrograms per virus strain per dose; (5) the season during which the vaccine is intended to protect.

Japanese Encephalitis Vaccine (Human)

Japanese Encephalitis Vaccine for human use is a liquid or freeze dried preparation of Japanese encephalitis virus grown in approved substrate and inactivated by a validated method.

Production

General provisions

The vaccine is produced on the basis of virus seed lot system The production method shall have been shown to yield consistently the vaccines that comply with the tests for immunogenicity, safety and stability. The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Substrate for virus propagation

The virus is propagated in an approved cell substrate like a Vero cell line.

SEED LOT

The strain of Japanese encephalitis virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

The National Regulatory Authority shall determine the acceptable number of passages from the master virus seed lot to produce working virus seed lots.

Only a working seed lot that complies with the following tests may be used for virus propagation.

Identification

Each working seed lot is identified as Japanese encephalitis virus using specific antibodies by an approved method.

Virus concentration. The virus concentration of each working seed lot is determined by a cell culture method using immunofluorescence or any other approved method.

Extraneous agents (2.7.3). The working seed lot complies with the tests for the virus seed lots.

PROPAGATION AND HARVEST

a) Mouse brain vaccine

The vaccine is prepared by using a seed-lot system. An approved strain of virus is grown by inoculating intracerebrally into healthy mice. Virus harvests are pooled, concentrated and inactivated by addition of formalin or any other suitable inactivating agent. It may contain a suitable preservative. The vaccine may be issued in single or multidose containers.

b) Cell culture vaccine

All processing of the cell bank and subsequent cell cultures are done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum; the media may contain human albumin. Serum proteins, if present are reduced to an acceptable level by suitable method of purification. Serum and trypsin used in the preparation of cell suspension and media are shown to be free from infectious extraneous agents. The cell culture media may contain a pH indicator such as phenol red. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). The virus suspension is harvested on one or more occasions during incubation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Virus harvests that comply with the tests given under Identification and Virus concentration are pooled in the preparation of the inactivated viral harvest.

Control cells. The control cells of the production cell culture from which the single harvest is derived should comply with the test for identification and with the tests for extraneous agents (2.7.3).

Purification and inactivation

The virus harvest may be concentrated and/or purified by suitable methods; the virus harvest is inactivated by a validated method at a fixed, well defined stage of the process which may be before, during or after any concentration or purification. The method shall have been shown to be capable of inactivating Japanese encephalitis virus without destruction of the immunogenic activity. If formalin is used, the concentration shall at no time exceed 1:2000.

Only an inactivated viral suspension that complies with the following tests may be used in the preparation of the final bulk vaccine.

Inactivation. Inactivation is confirmed by carrying out an amplification test for residual infectious Japanese encephalitis virus. Inoculate a quantity of inactivated viral suspension equivalent to not less than 25 doses into cell cultures of the same type as those used for production of the vaccine. Make a passage after 7 days. Maintain the cultures for a further period of 14 days and then examine the cell cultures for Japanese encephalitis virus using an immunofluorescence test. No Japanese encephalitis virus is detected. Alternatively, 5 ml of each culture fluid is pooled on day 14 and 21 and 0.03 ml is inoculated intracerebrally into each of the 10 mice weighing between 12 and 15 g. The mice are observed for 14 days for symptoms caused by Japanese encephalitis virus, and mice showing symptoms of Japanese encephalitis virus are sacrificed and virus presence is confirmed by immunofluorescence test. No Japanese encephalitis virus shall be detected.

Residual host-cell DNA (2.2.15). The content of residual host-cell DNA, determined using a suitable method, should not be greater than 10 ng per single human dose if cells are used in the production.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated viral suspensions. An approved stabilizer may be added to maintain the activity of the product. Thiomersal can be used as preservative.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Formaldehyde (2.3.20). Not more than 0.01 per cent w/v.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then sealed so as to prevent contamination.

Only a final lot that complies with each of the tests given under Identification, Tests and Assay may be released for use. Provided that the test for inactivation has been carried out with satisfactory results on the inactivated virus suspension and the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

Identification

The vaccine is shown to contain Japanese encephalitis virus antigen by a suitable immunochemical method using specific antibodies, alternatively, the Assay also serves to identify the vaccine.

Tests

Complies with the test for Inactivation under final Purification and Inactivation.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Less than 25 IU per single human dose.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin (for cell culture vaccine). Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Residual host-cell DNA (for continuous cell line vaccines) (2.2.15). Not more than 10 ng per single human dose.

Free formaldehyde (2.3.20). Not more than 0.01 per cent w/v.

Antimicrobial preservative. Determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85 per cent and is not greater than 115.0 per cent of that stated on the label.

Biological assay

Potency of Japanese encephalitis virus vaccine is determined by titrating the neutralizing antibodies produced in the immunized mice by plaque reduction method or serum neutralization test (SNT) using appropriate cell culture.

Standard preparation

The Standard preparation is a freeze-dried Japanese encephalitis virus vaccine the potency of which has been determined in relation to the Japanese encephalitis reference vaccine obtained from the National Institute of Health, Tokyo, Japan.

Suggested method

Preparation of challenge virus suspension

The approved challenge virus strain is stored in freeze-dried form or in liquid form stored below -70°. Prepare a working pool of the challenge virus strain by inoculating intracerebrally 0.03 ml of 100 fold dilution of the standard strain in Hanks' balanced salt solution containing 5 per cent calf serum into a suitable number of 2-day old suckling mice. Sacrifice the animals after they show characteristic symptoms of encephalitis and become moribund. Harvest their brains aseptically, wash them in chilled sterile saline solution to remove blood clots. Homogenize the brains with Hanks' balanced salt solution containing 5 per cent calf serum to make a 10 per cent emulsion. Centrifuge the emulsion at 2000 g for 30 minutes. Dilute the supernatant with Hanks' balanced salt solution containing 5 per cent calf serum so as to contain about 200 Plaque-Forming Units (PFU) of the virus per 0.4 ml.

Determination of potency

Prepare appropriate dilutions of the vaccine under examination and of the Standard Preparation in a suitable medium. Inject intraperitoneally in two doses of 0.5 ml each at 7-day interval into at least 20 mice of 4 weeks of age. Bleed each mouse 7 days after the second injection, pool the separated serum from each group and inactivate the sera by heating at 56° for 30 minutes. The inactivated sera may be stored at -20°, if necessary.

Dilute the sera appropriately, e.g. 1:40. 1:160, 1:640 etc., mix with an equal volume of the challenge virus suspension and incubate at 37° for 90 minutes for neutralization. Inoculate the mixture into cell cultures and overlay the infected cells with 1 per cent agar. After incubation for an appropriate time (about 48 hours), stain the cells and count the number of plaques formed on the cultures to obtain the plaque reduction rates for the vaccine under examination and the Standard preparation. Calculate the neutralizing antibody titres for each group using standard statistical methods (5.7). The test is not valid unless (a) the mean number of plaques obtained with the Standard preparation is between 100 and 150 per dish and (b) the potency of the vaccine under examination is not less than that of the Standard preparation.

Labelling. The label states (1) the biological origin of the cells used for the preparation of the vaccine; (2) the strain of virus used.

Measles and Rubella Vaccine (Live)

Measles and Rubella Vaccine (Live) is a freeze-dried preparation of suitable attenuated strains of measles virus and rubella virus grown in suitable cell cultures.

The vaccine is reconstituted immediately before use to give a clear liquid that may be coloured owing to the presence of a pH indicator.

Production

General provisions

The two components are prepared as described in the monographs on Measles vaccine (live) and Rubella vaccine (live) and comply with the tests prescribed therein.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

FINAL BULK VACCINE

Virus harvests for each component are pooled and clarified to remove cells. A suitable stabilizer may be added and the pooled harvests diluted as appropriate. Suitable quantities of the pooled harvest for each component are mixed.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

For each component, a minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the tests for minimum virus concentration of each component for release, with the following test for thermal stability and with each of the tests given below under Identification and Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 7 days. Determine the virus concentration as described under Assay in parallel for the vaccine held at 37° for 7 days and for vaccine stored at 2° to 8°. For each component, the virus concentration of the heated vaccine is not more than 1.0 $\log_{10}$ lower than that of the unheated vaccine.

Identification

When the vaccine reconstituted as stated on the label is mixed with antibodies specific for measles virus and rubella virus, it is no longer able to infect cell cultures susceptible to these viruses. When the vaccine reconstituted as stated on the label is mixed with quantities of specific antibodies sufficient to neutralize any one viral components, the second viral component infects susceptible cell cultures.

Tests

Water (2.3.43). Not more than 3.0 per cent, determined by Karl Fischer, semi-micro determination of water or by any suitable validated method.

Sterility (2.2.11). The reconstituted vaccine complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Assay

A. Mix the vaccine with a sufficient quantity of antibodies specific for rubella virus.Titrate the vaccine for infective measles virus at least in triplicate, using at least eight cell cultures for each dilution 0.5 $\log_{10}$ step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated measles virus concentration is not less than that stated on the label; the minimum measles virus concentration stated on the label is not less than $1x10^3$ $CCID_{50}$ per single human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration are greater than ± 0.3.

Measles vaccine (Live) RS is suitable for use as a reference preparation.

B. Titrate the vaccine for infective rubella virus at least in triplicate, using at least eight cell cultures for each 0.5 $\log_{10}$ dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated rubella virus concentration is not less than that stated on the label; the minimum rubella virus concentration stated on the label are not less than $1x10^3$ $CCID_{50}$ per single human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration are greater than ± 0.3.

Rubella vaccine (Live) RS is suitable for use as a reference preparation.

Labelling. The label states (1) the strains of virus used in the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) the minimum virus

concentration for each component of the vaccine; (4) the time within which the vaccine must be used after reconstitution; (5) that the vaccine must not be given to a pregnant woman and that a woman should not become pregnant within two months after having the vaccine.

Measles Vaccine (Live)

Measles Vaccine (Live) is a freeze-dried preparation of a suitable attenuated strain of measles virus. The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

Production

General provisions

The production of vaccine is based on a virus seed-lot system and, if the virus is propagated in human diploid cells, a cell-bank system. Unless otherwise justified and authorized, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to abnormal toxicity and efficacy; even with authorized exceptions, the number of passages beyond the level used for clinical studies shall not exceed five.

The production method is validated to demonstrate that the product, if tested, would comply with the tests for abnormal toxicity and efficacy.

Substrate for virus propagation

The virus is propagated in human diploid cells or in cultures of chick embryo cells derived from a chicken flock free from specified pathogens.

SEED LOT

The strain of measles virus used in the production of measles vaccine shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at temperatures below -20° if freeze-dried, or below -60° if not freeze-dried.

Only a seed lot that complies with the following tests may be used for virus propagation.

Identification

The master and working seed lots are identified as measles virus by serum neutralization in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined to monitor consistency of production.

Extraneous agents (2.7.3). The working seed lot complies with the tests for seed lots.

Neurovirulence (2.7.5). The master/working seed lot complies with the test for neurovirulence of live virus vaccines. *Macaca* and *Cercopithecus* monkeys susceptible to measles virus are suitable for the test.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell culture is set aside as uninfected cell cultures (control cells). The viral suspensions are harvested at a time appropriate to the strain of virus being used.

Only a single harvest that complies with the following tests may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as measles virus by serum neutralisation in cell culture, using specific antibody.

Virus concentration. The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (2.7.3). Complies with the test for extraneous agents.

Control cells. If human diploid cells are used for production, the control cells comply with the test for identification and extraneous agents.

FINAL BULK VACCINE

Virus harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabilizer may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). The final bulk vaccine complies with the test for sterility carried out using 10 ml for each medium.

FINAL LOT

A minimum virus concentration for release of the product is established so as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the tests for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 7 days. Determine the virus concentration as described under Assay in parallel for the vaccine held at 37° for 7 days and for vaccine stored at 2° to 8°. The virus concentration of the heated vaccine is not more than 1.0 $\log_{10}$ lower than that of the unheated vaccine.

Identification

When the vaccine reconstituted as stated on the label is mixed with specific measles antibodies, it is no longer able to infect susceptible cell cultures.

Tests

Water (2.3.43). Not more than 3.0 per cent, determined by Karl Fischer, semi-micro determination of water or by any suitable validated method.

Sterility (2.2.11). The reconstituted vaccine complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Assay

Titrate the vaccine for infective virus at least in triplicate, using at least five cell cultures for each 0.5 $\log_{10}$ dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated virus concentration is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 1×10^3 $CCID_{50}$ per human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration is greater than ± 0.3.

Measles vaccine (Live) RS is suitable for use as a reference preparation.

Labelling. The label states (1) the strain of virus used for the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) the minimum virus concentration; (4) the time within which the vaccine must be used after reconstitution.

Measles, Mumps and Rubella Vaccine (Live)

Measles, Mumps and Rubella Vaccine (Live) is a freeze-dried preparation of suitable attenuated strains of measles virus, mumps virus and rubella virus grown in suitable cell cultures.

The vaccine is reconstituted immediately before use to give a clear liquid that may be coloured owing to the presence of a pH indicator.

Production

General provisions

The three components are prepared as described in the monographs on Measles Vaccine (Live), Mumps Vaccine (Live) and Rubella Vaccine (Live) and comply with the tests prescribed therein.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

FINAL BULK VACCINE

Virus harvests for each component are pooled and clarified to remove cells. A suitable stabilizer may be added and the pooled harvests diluted as appropriate. Suitable quantities of the pooled harvest for each component are mixed.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out the test for sterility using 10 ml for each medium.

FINAL LOT

For each component, a minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the tests for minimum virus concentration of each component for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 7 days. Determine the

virus concentration as described under Assay in parallel for the vaccine held at 37° for 7 days and for vaccine stored at 2° to 8°. The virus concentration of the heated vaccine is not more than 1.0 $\log_{10}$ lower than that of the unheated vaccine.

Identification

When the vaccine reconstituted as stated on the label is mixed with antibodies specific for measles virus, mumps virus and rubella virus, it is no longer able to infect cell cultures susceptible to these viruses. When the vaccine reconstituted as stated on the label is mixed with quantities of specific antibodies sufficient to neutralize any two viral components, the third viral component infects susceptible cell cultures.

Tests

Water (2.3.43). Not more than 3.0 per cent, determined by Karl Fischer, semi-micro determination of water or by any suitable validated method.

Sterility (2.2.11). The reconstituted vaccine complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Assay

A. Mix the vaccine with a sufficient quantity of antibodies specific for mumps virus and rubella virus.Titrate the vaccine for infective measles virus at least in triplicate, using at least eight cell cultures for each dilution 0.5 $\log_{10}$ step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated measles virus concentration is not less than that stated on the label; the minimum measles virus concentration stated on the label is not less than $1x10^3$ $CCID_{50}$ per single human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration are greater than ± 0.3.

Measles vaccine (Live) RS is suitable for use as a reference preparation.

B. Mix the vaccine with a sufficient quantity of antibodies specific for measles virus and rubella virus. Titrate the vaccine for infective mumps virus at least in triplicate, using at least eight cell cultures for each dilution 0.5 $\log_{10}$ step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated mumps virus concentration is not less than that stated on the label; the minimum mumps virus concentration stated on the label is not less than 5×10^3 $CCID_{50}$ per single human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration are greater than ± 0.3.

Mumps vaccine (Live) RS is suitable for use as a reference preparation.

C. Mix the vaccine with a sufficient quantity of antibodies specific for mumps virus and measles virus. Titrate the vaccine for infective rubella virus at least in triplicate, using at least eight cell cultures for each dilution 0.5 $\log_{10}$ step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated rubella virus concentration is not less than that stated on the label; the minimum rubella virus concentration stated on the label is not less than $1x10^3$ $CCID_{50}$ per single human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration are greater than ± 0.3.

Rubella vaccine (Live) RS is suitable for use as a reference preparation.

Labelling. The label states (1) the strains of virus used in the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) the minimum virus concentration for each component of the vaccine; (4) the time within which the vaccine must be used after reconstitution; (5) that the vaccine must not be given to a pregnant woman and that a woman should not become pregnant within two months after having the vaccine.

Meningococcal Polysaccharide Vaccine

Meningococcal Polysaccharide Vaccine is a freeze-dried preparation of one or more purified capsular polysaccharides obtained from one or more suitable strains of *Neisseria meningitidis* group A, group C, group Y and group W135 that are capable of consistently producing polysaccharides known to be safe and effective in man.

N. meningitidis group A polysaccharide consists of partly O-acetylated repeating units of N-acetylmannosamine, linked with 1α→6 phosphodiester bonds.

N. meningitidis group C polysaccharide consists of partly O-acetylated repeating units of sialic acid, linked with 2α→9 glycosidic bonds.

N. meningitidis group Y polysaccharide consists of partly O-acetylated alternating units of sialic acid and D-glucose, linked with 2α→6 and 1α→4 glycosidic bonds.

N. meningitidis group W135 polysaccharide consists of partly O-acetylated alternating units of sialic acid and D-galactose, linked with 2α→6 and 1α→4 glycosidic bonds.

Production

General provisions

Production of the meningococcal polysaccharides is based on a well defined seed-lot system. The method of production shall have been shown to yield consistently meningococcal polysaccharide vaccines of satisfactory immunogenicity and safety for man.

The production method is validated to demonstrate that the product, if tested, would comply with the test of abnormal toxicity for antisera and vaccines.

SEED LOT

The strains of *N. meningitidis* used for the master seed lots shall be identified by historical records that include information on their origin and by their biochemical, serological, physicochemical or molecular characteristics. Cultures from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot. The strains have the following characteristics:

a) Colonies obtained from a culture are round, uniform in shape and smooth with a mucous, opalescent, greyish appearance.
b) Gram staining reveals characteristic Gram-negative diplococci in 'coffee-bean' arrangement.
c) The oxidase test is positive.
d) The culture utilizes glucose and maltose.
e) Suspensions of the culture agglutinate with specific antisera of known titre.

PROPAGATION AND HARVEST

The working seed lots are cultured on solid media that do not contain blood-group substances or ingredients of mammalian origin. The inoculum may undergo one or more subcultures in liquid medium before being used for inoculating the final medium. The liquid media used and the final medium are semisynthetic and free from substances precipitated by *cetrimonium bromide (hexadecyltrimethylammonium bromide)* and do not contain blood-group substances or high-molecular-mass polysaccharides. The bacterial purity of the culture is verified by microscopic examination of Gram-stained smears and by inoculation into appropriate media. The cultures are centrifuged and the polysaccharides precipitated from the supernatant by addition of *cetrimonium bromide*. The precipitate obtained is harvested and may be stored at or below -20° awaiting further purification.

PURIFIED POLYSACCHARIDES

The polysaccharides are purified, after dissociation of the complex of *polysaccharide and cetrimonium bromide*, using suitable procedures to remove successively nucleic acids, proteins and lipopolysaccharides. The purification step consists of *ethanol* precipitation of the *polysaccharides* or purification with *chloroform* and *n-butanol* or by cold *phenol* treatment, which are then dried and stored at or below -20°. The loss on drying is determined by thermogravimetry, Karl Fischer or any other suitable method and the value is used to calculate the results of the other chemical tests with reference to the dried substance.

Only purified polysaccharides that tested comply with the following requirements may be used in the preparation of the final bulk vaccine.

Protein (2.7.1). Not more than 10 mg of protein per gram of purified polysaccharide for group A and C organisms and less than 50 mg of protein per gram of polysaccharide for group Y and W135 calculated using bovine plasma albumin as a reference or other methods approved by National Regulatory Authority.

Nucleic acids (2.7.1). Not more than 10 mg of nucleic acids per gram of purified polysaccharide, calculated with reference to the dried substance.

O-Acetyl groups (2.7.1). Not less than 2 mmol of O-acetyl groups per gram of purified polysaccharide for group A, not less than 1.5 mmol per gram of polysaccharide for group C, not less than 0.3 mmol per gram of polysaccharide for groups Y and W135, all calculated with reference to the dried substance.

Phosphorus (2.7.1). Not less than 80 mg of phosphorus per gram of group A purified polysaccharide, calculated with reference to the dried substance.

Sialic acid (2.7.1). Not less than 800 mg of sialic acid per gram of group C polysaccharide and not less than 560 mg of sialic acid per gram of purified polysaccharide for groups Y and W135, all calculated with reference to the dried substance. Use the following reference solutions:

Group C polysaccharide. A 150 mg/l solution of *N-acetylneuraminic acid.*

Group Y polysaccharide. A solution containing 95 mg/l of *N-acetylneuraminic acid* and 55 mg/l of *glucose.*

Group W135 polysaccharide. A solution containing 95 mg/l of *N-acetylneuraminic acid* and 55 mg/l of *galactose.*

Calcium. If a calcium salt is used during purification, determination of calcium is carried out on the purified polysaccharide by a suitable method; the content is within the limits approved for the product.

Molecular size. Examine by gel filtration or high performance size-exclusion chromatography (HPSEC) (2.4.16), using agarose for chromatography or cross-linked agarose for chromatography either alone or in combination with light scattering and refractive index detector (e.g. multiple angle

LASER light scattering, MALLS) or any other suitable method. Use a column 0.9 m x 15 mm equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0 to 7.5. Apply to the column about 2.5 mg of polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method.

At least 65.0 per cent of group A polysaccharide, 75.0 per cent of group C polysaccharide, 80.0 per cent of group Y polysaccharide and 80.0 per cent of group W135 polysaccharide is eluted before a distribution coefficient (K_0) of 0.50 is reached. In addition, the percentages eluted before this distribution coefficient are within the limits approved for the particular product.

Identification and serological specificity

The identity and serological specificity are determined by a suitable immunochemical method (2.2.14). Identity and purity of each polysaccharide shall be confirmed; it shall be shown that there is not more than 1.0 per cent m/m of group-heterologous *N. meningitidis* polysaccharide.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject each of the rabbit with 1ml per kg body weight of solution containing

a) 0.025 µg of polysaccharide for a monovalent vaccine,

b) 0.050 µg of polysaccharide for a bivalent vaccine,

c) 0.075 µg of polysaccharide for a trivalent vaccine,

d) 0.100 µg of polysaccharide for a tetravalent vaccine.

FINAL BULK VACCINE

One or more purified polysaccharides of one or more *N. meningitidis* groups are dissolved in a suitable solvent that may contain a stabilizer.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are then closed so as to avoid contamination. Only a final lot that is satisfactory with respect to each of the requirements prescribed below under Identification, Tests and Assay may be released for use.

Identification

Carry out an identification test for each polysaccharide present in the vaccine by a suitable immunochemical method (2.2.14).

Tests

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject each of the rabbit with 1ml per kg body weight of solution containing

a) 0.025 µg of polysaccharide for a monovalent vaccine,

b) 0.050 µg of polysaccharide for a bivalent vaccine,

c) 0.075 µg of polysaccharide for a trivalent vaccine,

d) 0.100 µg of polysaccharide for a tetravalent vaccine.

Water (2.3.43). Not more than 3.0 per cent, of moisture content by thermogravimetery, Karl Fischer or any other suitable method.

Molecular size. Examine by gel filtration or size-exclusion chromatography (2.4.16). Use a column about 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol/kg and a pH of 7.0 to 7.5. Apply to the column about 2.5 mg of each polysaccharide in a volume of about 1.5 ml and elute at about 20 ml/h. Collect fractions of about 2.5 ml and determine the content of polysaccharide by a suitable method.

Use cross-linked agarose for chromatography and apply a suitable immunochemical method (2.2.14) to establish the elution pattern of the different polysaccharide(s). The vaccine complies with the test if:

a) 65.0 per cent of Group A polysaccharide is eluted before K_0 of 0.50,

b) 75.0 per cent of Group C polysaccharide is eluted before K_0 of 0.50,

c) 80.0 per cent of Group Y & W135 polysaccharide is eluted before K_0 of 0.50.

For a tetravalent vaccine (group A + group C + group Y and group W135), use cross linked agarose for chromatography R1 and apply a suitable immunochemical method (2.2.14) to establish the elution pattern of the different polysaccharides. The vaccine complies with the test if K_0 for the principal peak is

a) not greater than 0.70 for group A and group C polysaccharide,

b) not greater than 0.57 for group Y polysaccharide,

c) not greater than 0.68 for group W135 polysaccharide.

Assay

Carry out an assay as stated under each polysaccharide present in the vaccine.

For a divalent vaccine (group A + group C), use measurement of phosphorus (2.7.1) to determine the content of polysaccharide A and measurement of sialic acid (2.7.1) to determine the content of polysaccharide C. To determine sialic acid, use as reference solution a 150 mg/l solution of *N-acetylneuraminic acid.*

For a tetravalent vaccine (group A + group C + group Y + group W135) a suitable immunochemical method (2.2.14) is used with a reference preparation of purified polysaccharide for each group.

The vaccine contains not less than 70.0 per cent and not more than 130.0 per cent of the quantity of each polysaccharide stated on the label.

Labelling. The label states (1) the group or groups of polysaccharides (A, C, Y or W135) present in the vaccine; (2) the number of µg of polysaccharide per human dose.

Mumps Vaccine (Live)

Mumps Vaccine (Live) is a freeze-dried preparation of a suitable attenuated strain of mumps virus. The vaccine is reconstituted immediately before use to give a clear liquid that may be coloured owing to the presence of a pH indicator.

Production

General provisions

The production of vaccine is based on a virus seed-lot system and, if the virus is propagated in human diploid cells, a cell-bank system. The production method shall have been shown to yield consistently live mumps vaccines of adequate immunogenicity and safety in man.

Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy even with authorized exceptions, the number of passages beyond the level used for clinical studies shall not exceed five.

The production method is validated to demonstrate that the product, if tested, would comply with the tests for safety and efficacy.

Substrate for virus propagation

The virus is propagated in human diploid cells or in primary cultures of chick embryo cells derived from a chicken flock free from specified pathogens.

SEED LOT

The strain of mumps virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. To avoid unnecessary use of monkeys in the test for neurovirulence, Virus seed lots are prepared in large quantities and stored at temperatures below -20° if freeze-dried, or below -60° if not freeze-dried.

Only a seed lot that complies with the following tests may be used for virus propagation.

Identification

The master and working seed lots are identified as mumps virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined to ensure consistency of production.

Extraneous agents (2.7.3). The working seed lot complies with the tests for seed lots.

Neurovirulence (2.7.5). The master/working seed lot complies with the test for neurovirulence of live virus vaccines. *Macaca* and *Cercopithecus* monkeys are suitable for the test.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Suitable animal (but not human) serum may be used in the growth media. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell culture is set aside as uninfected cell culture (control cells). The viral suspensions are harvested at a time appropriate to the strain of virus being used.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as mumps virus by serum neutralization in cell culture, using specific antibodies.

Virus concentration. The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Sterility (2.2.11). Single harvest complies with sterility test should be processed further.

Control cells. The control cells comply with a test for extraneous agents (2.7.3).

FINAL BULK VACCINE

Single harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). The final bulk vaccine complies with the test for sterility, carried out using 10 ml for each medium.

FINAL LOT

A minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the tests for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 7 days. Determine the virus concentration as described under Assay in parallel for the vaccine held at 37° for 7 days and for vaccine stored at 2° to 8°. The virus concentration of the vaccine exposed to 37° for 7 days is not more than 1.0 $\log_{10}$ lower than that of the unheated vaccine.

Identification

When the vaccine is reconstituted as stated on the label is mixed with specific mumps antibodies, it is no longer able to infect susceptible cell cultures.

Tests

Water (2.3.43). Not more than 3.0 per cent, determined by Karl Fischer, semi-micro determination of water or by any suitable validated method.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Assay

Titrate the vaccine for infective virus at least in triplicate, using at least five cell cultures for each 0.5 $\log_{10}$ dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated virus concentration is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 5 x 10^3 $CCID_{50}$ per human dose. The assay is not valid if the confidence limits (P = 0.95) of the logarithm of the virus concentration is greater than ± 0.3.

Mumps vaccine (Live) RS is suitable for use as a reference preparation.

Labelling. The label states (1) the strain of virus used for the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) the minimum virus concentration and; (4) the time within which the vaccine must be used after reconstitution.

Pertussis Vaccine

Pertussis Vaccine is a sterile saline suspension of inactivated whole cells of one or more strains of *Bordetella pertussis*.

Production

General provisions

Inactivated *B. pertussis* suspension

Production is based on a seed-lot system. One or more strains of *B. pertussis* with known origin and history are used. Strains, culture medium and cultivation method are chosen in such a way that agglutinogens 1, 2 and 3 are present in the final vaccine. Each strain is grown for 24 to 72 hours in a liquid medium or on a solid medium; the liquid medium used in the final cultivation stage does not contain blood or blood products. Human blood or blood products are not used in any culture media. The bacteria are harvested, washed to remove substances derived from the medium and suspended in a 0.9 per cent w/v solution of *sodium chloride* or other *suitable isotonic solution*. The opacity of the suspension is determined not later than 2 weeks after harvest by comparison with the *reference preparation* of Opacity and used as the basis of calculation for subsequent stages in vaccine preparation. Single harvests are not used for the final bulk vaccine unless they have been shown to contain *B. pertussis* cells with the same characteristics with regard to growth and agglutinogens, as the parent strain and to be free from contaminating bacteria and fungi. The bacteria are killed and detoxified in controlled conditions by means of a suitable chemical agent or by heating or by a combination of these methods. Freedom from live *B. pertussis* is tested using a suitable culture medium. The suspension is maintained at 5 ± 3° for a suitable period to diminish its toxicity.

FINAL BULK VACCINE

Suitable quantities of the inactivated single harvests are pooled to prepare the final bulk vaccine. Suitable antimicrobial preservatives may be added. The bacterial concentration of the final bulk vaccine does not exceed that corresponding to an opacity of 20 IU per single human dose. If 2 or more strains of *B. pertussis* are used, the composition of consecutive lots of the final bulk vaccine shall be consistent with respect to the proportion of each strain as measured in opacity units.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The amount is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for specific toxicity, free formaldehyde and antimicrobial preservative and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

Identify pertussis vaccine by agglutination of the bacteria in the vaccine by antisera specific to *B. pertussis*.

Tests

Specific toxicity

Use not less than 10 healthy mice each weighing between 14 to 16 g for the vaccine group and for the saline control. Use mice of the same sex or distribute males and females equally between the groups. Allow the animals access to food and water for at least 2 hours before injection and during the test. Inject each mouse of the vaccine group intraperitoneally with 0.5 ml, containing a quantity of the vaccine equivalent to not less than half the single human dose. Inject each mouse of the control group with 0.5 ml of a 0.9 per cent w/v sterile solution of *sodium chloride*, preferably containing the same amount of antimicrobial preservative as that injected with the vaccine. Weigh the groups of mice immediately before the injection, 72 hours and 7 days after the injection. The vaccine complies with the test if (a) at the end of 72 h the total mass of the group of vaccinated mice is not less than that preceding the injection; (b) at the end of 7 days the average increase in mass per vaccinated mouse is not less than 60.0 per cent of that per control mouse; and (c) not more than 5.0 per cent of the vaccinated mice die during the test. The test may be repeated and the results of the tests combined.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not more than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Assay

Carry out the assay of Pertussis Vaccine as described below :

Biological assay of pertussis vaccine

The potency of PertussisVaccine is determined by comparison of the dose necessary to protect mice against the effects of a lethal dose of *B. pertussis* challenge culture, administered intracerebrally, with the dose of a reference preparation, calibrated in International Units, required to give the same level of protection. For this comparison, the Standard preparation of Pertussis vaccine & a suitable strain of *B. pertussis* (e.g.18323, to be used as challenge strain), are required.

Reference preparation

The reference preparation is an International standard of Pertussis vaccine, consisting of a freeze dried vaccine or another suitable preparation, calibrated in comparison to International standard, from time to time. The International Unit is the activity contained in a stated amount of the International standard, which consists of a quantity of dried pertussis vaccine. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Use healthy mice of a suitable strain, weighing between 13 and 16 g from the same stock. Distribute the mice randomly, in six to eight groups of not less than 16 and not more than 24 and four groups of 10. The mice should all be of the same sex or the males and females should be distributed equally between the groups. Half of the groups of 16 to 24 should receive the reference preparation and the other half should receive the vaccine under examination. The four groups of 10 each should be used for the LD_{50} titration of challenge suspension.

Use at least, three dilutions of the reference vaccine and similar dilutions of the vaccine under examination. In each case the dilutions are so selected that the dilution protecting 50 per cent of the mice (ED_{50}) is as near as possible to middle of the dilution range. For example, suggested dilutions are (1/8, 1/40

and 1/200 of the human dose of the vaccine under examination) and (0.5 IU, 0.1 IU and 0.02 IU or any other suitable standardized dilutions, of the reference preparation), each dose being contained in a volume, not exceeding 0.5 ml. For each dilution use 16 to 24 mice and inject intraperitoneally, into each mouse one dose of the dilution.

Select a suitable strain of *B. pertussis* (e.g. 18323), capable of causing the death of mice within 14 days of intracerebral injection. Make two subcultures after reviving the strain on a suitable medium (e.g. B.G. medium) and suspend the harvested growth in a solution containing 1 per cent w/v of *casein hydrolysate* (e.g. casamino acid) and 0.85 per cent w/v of *sodium chloride* and having a pH of 7.0 to 7.2 or in another suitable solution. Determine the opacity of the suspension by using 5th International reference preparation for opacity (10 OU) and/or spectrophotometrically. Alternatively, aliquots of challenge suspension frozen in liquid nitrogen with a suitable preservative like 10 per cent DMSO may be used, to avoid heterogenicity. After 14 to 17 days of immunization, inject intracerebrally, a dose of 0.02 to 0.03 ml of the challenge dilution randomly, into each immunized mouse. The challenge should contain, approximately 1,00,000 organisms and 100 to 1000 LD_{50} per dose, in a volume of not more than 0.03 ml. In the same way, inject 4 groups of 10 control mice each, for LD_{50} titration of challenge preparation, prepared by a series of dilutions, from the dilution selected for challenge. The challenge should be completed within 2 to 2.5 hours of preparation. Exclude any mouse from consideration, that dies within 3 days of challenge. Count the number of mice surviving in each of the groups, after 14 days. On the basis of the numbers of animals surviving in each of the groups of 16 to 24 mice, calculate the potency of vaccine under examination, against the potency of reference preparation. Seed a suitable highest dilution of the challenge suspension, into each of two B.G medium plates, before and after challenge. Incubate the plates at 37° for 48 to 72 hours and calculate the number of colony forming units (CFUs).

Calculate the potency of the vaccine by Probit analysis and LD_{50} of the challenge suspension by Reed and Munch Method.

The test is not valid unless: a) for both the vaccine under examination and the reference preparation, the ED_{50} (protective dose), lies between the largest and the smallest doses given to the mice; b) the number of animals, which die in the four groups of 10 injected with the challenge suspension and its dilutions indicate that the challenge dose contains 100 to 1000 LD_{50} and 1 LD_{50} contains not more than 300 colony forming units; c) and the statistical analysis shows no deviation from linearity or parallelism, in terms of significance of the scope of dose response curve; d) the vaccine passes the requirements for potency, if the test results of a statistically valid assay show that the estimated potency of the vaccine is not less than 4.0 IU per single human dose and the lower fiducial limit (P = 0.95) of estimated potency is not less than 2.0 IU.

The test may be repeated once, but when more than one test is performed the results of all valid tests must be combined, in the estimate of potency.

Labelling. The label states (1) the minimum number of International Units per single human dose; (2) that the vaccine must be shaken before use; (3) that the vaccine is not to be frozen.

Plague Vaccine

Formolised Plague Vaccine

Plague Vaccine is a sterile suspension of killed plague bacilli, *Yersinia pestis*, of the 195/P strain grown in a suitable enriched medium such as acid hydrolysate of casein, harvested and killed by the addition of formaldehyde. It may contain a suitable preservative.

Plague Vaccine contains in each ml of human dose not less than 250 mouse median effective immunising doses (250 ED_{50}).

Description. A turbid, golden yellow or brownish liquid with or without flakes or clumps.

Tests

pH (2.4.24). 6.8 to 7.4.

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity but injecting into each mouse 1.0 ml and into each guinea-pig 3.0 ml.

Potency. Carry out the biological assay of plague vaccine described below.

Biological Assay of Plague Vaccine

The potency of plague vaccine is estimated by determining the dose necessary to protect mice against a lethal dose of a virulent strain of *Yersinia pestis*.

Test animals. Use white mice, 6 to 7 weeks old, each weighing between 20 and 28 g and of a strain susceptible to plague infection. The selected strain of mice should be such that an infective dose of 6 to 12 organisms of a virulent strain of *Y. pestis* per mouse given subcutaneously produces a mortality of not less than 80 per cent of the animals used. The animals should be healthy and free from intercurrent infection with organisms such as *Salmonella*.

Suggested Method

Selection of suitable virulent strain. A freeze-dried virulent culture of *Y. pestis*, established to be suitable for challenge, is

revived by subculturing 0.5 ml in 9.5 ml of *nutrient broth* contained in another test-tube and incubating at 28° for exactly 48 hours. Such a culture should contain 300 to 600 million organisms per ml. Make 10-fold dilutions in *nutrient broth* and test for virulence. Use a 10^{-7} dilution containing 6 to 12 organisms in 0.2 ml as the test infective dose per animal. Those strains which produce a mortality of 80 per cent among the animals tested with this dose are considered to be virulent.

Standard challenge dose. Freshly reconstitute the freeze-dried culture and dilute with *nutrient broth* to a strength such that 0.2 ml contains 60 to 120 organisms.

Measurement of protective power. Prepare a series of five graded doses of the preparation under examination arranged in such a manner that the 50 per cent protective dose (ED_{50}) lies about the middle of the selected series. For each dose a batch of 16 mice is used. Inject subcutaneously the selected dose in two equal parts with an interval of 7 days between them. Seven days after the second half of the dose, inject subcutaneously into each group of mice the standard challenge dose. At the same time inject into 10 control mice, 8 to 9 weeks old and weighing between 28 and 30 g, the standard challenge dose.

Observe the animals for 15 days and record the number of deaths in each group. Carry out a post-mortem on the dead animals and look for signs of plague in them. If plague organisms are not seen, such deaths are excluded from the calculation. The test is not valid unless the number of such deaths is not more than 1. At the end of the period of observation kill all the surviving animals and examine for signs of plague. Calculate the median effective immunising dose, ED_{50}, by standard statistical methods. The vaccine passes the test if it has an ED_{50} of 0.004 ml or less per mouse.

Storage. Store at a temperature between 2° and 8°. The vaccine should not be allowed to freeze. When stored under the prescribed conditions the vaccine is expected to retain its potency for not less than 3 years from the date of initiation of the potency test.

Labelling. The label states (1) ED_{50} unitage per dose; (2) the name and proportion of any preservative added; (3) that the contents should be shaken well before use; (4) that the vaccine should not be allowed to freeze.

Pneumococcal Polysaccharide Vaccine

Pneumococcal Polysaccharide Vaccine consists of a mixture of equal parts of purified capsular polysaccharide of various serotype antigens prepared from suitable pathogenic strains of *Streptococcus pneumoniae* in different desired combinations whose capsules have been shown to be made up of polysaccharides that are capable of inducing satisfactory levels of specific antibodies in man. It contains upto 23 immunochemically different capsular polysaccharides listed in the Table 1.

Production

General provisions

Production of the vaccine is based on a well defined seed-lot system for each type. The production method shall have been shown to yield consistently pneumococcal polysaccharide vaccines of acceptable immunogenicity and safety in man.

The production method is validated to demonstrate that the product, if tested, would comply with the tests for abnormal toxicity of vaccines for human use, modified as follows for the test on guinea-pig; inject 10 human dose into each guinea-pig and observe for 12 days.

Monovalent bulk polysaccharides

The bacteria are grown in a suitable liquid medium that does not contain blood-group substances or high-molecular-mass polysaccharides. The bacterial purity of the culture is verified and the culture is inactivated with *phenol*. Impurities are removed by such techniques as fractional precipitation, enzymatic digestion and ultrafiltration. The polysaccharide is obtained by fractional precipitation, washed, and dried in a vacuum to a residual moisture content shown to be favourable to the stability of the polysaccharide. The residual moisture content is determined by drying under reduced pressure over *diphosphorus pentoxide* or by thermogravimetric analysis and the value obtained is used to calculate the results of the tests shown below with reference to the dried substance. The monovalent bulk polysaccharide is stored at a suitable temperature in conditions that avoid the uptake of moisture.

Only a monovalent bulk polysaccharide that complies with the following requirements may be used in the preparation of the final bulk vaccine. Percentage contents of components, determined by the methods prescribed below, are shown in the Table 1.

The purified polysaccharides comply with the following tests as applicable:

Protein (2.7.1). Comply with the test for protein.

Nucleic acids (2.7.1). Comply with the test for nucleic acids.

Total nitrogen (2.3.30). Comply with the test for total nitrogen.

Phosphorus (2.7.1). Comply with the test for phosphorus.

Uronic acids (2.7.1). Comply with the test for uronic acids.

Hexosamine (2.7.1). Comply with the test for hexosamine.

Methylpentoses (2.7.1). Comply with the test for methylpentoses.

Table 1- Specifications on monovalent bulk polysaccharides (per cent contents):

Molecular Type*	Proteins	Nucleic acids	Total	Phosphorus Nitrogen	Molecular size K_0 CL-4B**	CL-2B***	Uronic acids	Hexo-samines	Methyl-pentoses	O-acetyl Groups
1	≤2	≤2	3.5-6	0-1.5	≤0.15		≥45			≥1.8
2	≤2	≤2	0-1	0-1.0	≤0.15		≥15		≥38	
3	≤5	≤2	0-1	0-1.0	≤0.15		≥40			
4	≤3	≤2	4-6	0-1.5	≤0.15			≥40		
5	≤7.5	≤2	2.5-6.0	≤2		≤0.60	≥12	≥20		
6B	≤2	≤2	0-2	2.5-5.0		≤0.50			≥15	
7F	≤5	≤2	1.5-4.0	0-1.0	≤0.20				≥13	
8	≤2	≤2	0-1	0-1.0	≤0.15		≥25			
9N	≤2	≤1	2.2-4	0-1.0	≤0.20		[3]20	≥28		
9V	≤2	≤2	0.5-3	0-1.0		≤0.45	[3]15	≥13		
10A	≤7	≤2	0.5-3.5	1.5-3.5		≤0.65		≥12		
11A	≤3	≤2	0-2.5	2.0-5.0		≤0.40				≥9
12F	≤3	≤2	3-5	0-1.0	≤0.25			≥25		
14	≤5	≤2	1.5-4	0-1.0	≤0.30			≥20		
15B	≤3	≤2	1-3	2.0-4.5		≤0.55		≥15		
17A or 17F	≤2	≤2	0-1.5	0-3.5		≤0.45			≥20	
18C	≤3	≤2	0-1	2.4-4.9	≤0.15				≥14	
19A	≤2	≤2	0.6-3.5	3.0-7.0	≤0.45			≥12	≥20	
19F	≤3	≤2	1.4-3.5	3.0-5.5	≤0.20			≥12.5	≥20	
20	≤2	≤2	0.5-2.5	1.5-4.0		≤0.60		≥12		
22F	≤2	≤2	0-2	0-1.0		≤0.55	≥15		≥25	
23F	≤2	≤2	0-1	3.0-4.5	≤0.15				≥37	
33F	≤2.5	≤2	0-2	0-1.0		≤0.50				

* The different types are indicated using the Danish nomenclature

** Cross linked agarose for chromatography R

*** Cross linked agarose for chromatography R1

O-Acetyl groups (2.7.1). Comply with the test for O-acetyl groups.

Sterility (2.2.11). Comply with the test for sterility.

Molecular size. Molecular size is determined by gel filtration or high performance size-exclusion chromatography (HPSEC) (2.4.16) using cross linked Agarose for chromatography R or chromatography Agarose for chromatograph R1, either alone or Multiple angle light laser scattering (MALLS) or any other suitable method.

Identification

Confirm the identity of the monovalent bulk polysaccharide by immunochemical method (2.2.14)(except for polysaccharides 7F, 14 and 33F).

Specificity

For establishing the specificity, no reaction should occur, when the antigens are tested against all the antisera specific for the other polysaccharides of the vaccine, including factor sera for distinguishing types within groups. The polysaccharides are tested at a concentration of 50 µg/ml using a method capable of detecting 0.5 µg/ml.

FINAL BULK VACCINE

The final bulk vaccine is obtained by aseptically mixing the different polysaccharide powders. The uniform mixture is aseptically dissolved in a suitable isotonic solution so that one human dose of 0.5 ml contains 25 µg of each polysaccharide. An antimicrobial preservative may be added.

The solution is sterilized by filtration through a bacteria-retentive filter.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed and filled aseptically into sterile containers (vials or ampoules). Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for phenol and for antimicrobial preservative have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. When consistency of production has been established on a suitable number of consecutive batches, the assay may be replaced by a qualitative test that identifies each polysaccharide, provided that an assay has been performed on each monovalent bulk polysaccharide used in the preparation of the final lot.

Identification

The assay also serves to identify the vaccine.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity with the following modifications.

Inject 10 human doses each in two guinea pigs weighing between 250 and 350 g by intraperitoneal route and observe for 12 days,

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject each of the rabbit with 1 ml of a dilution of the vaccine containing 2.5 µg/ml of each polysaccharide.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Phenol (2.3.36). Not more than 2.5 g/l.

pH (2.4.24). 4.5 to 7.4.

Assay

Determine the content of each polysaccharide by a suitable biochemical, physicochemical or immunochemical method (2.2.14), using antisera specific for each polysaccharide contained in the vaccine, including factor sera for types within groups, and purified polysaccharides of each type as standards.

The vaccine contains not less than 70.0 per cent and not more than 130.0 per cent of the quantity stated on the label for each polysaccharide. The confidence interval (P = 0.95) of the assay is not less than 80.0 and not more than 120.0 per cent of the estimated content.

Labelling. The label states (1) the number of µg of each polysaccharide per human dose; (2) the total amount of polysaccharide in the container.

Poliomyelitis Vaccine (Inactivated)

Poliomyelitis Vaccine (Inactivated) is a liquid preparation of suitable strains of human polioviruses 1, 2 and 3 grown in suitable cell cultures and inactivated by a validated method.

Production

General provisions

The production method should consistently yield vaccines of acceptable safety and immunogenicity in man.

Production of the vaccine is based on a virus seed-lot system. Cell lines are used according to a cell-bank system. If primary, secondary or tertiary monkey kidney cells are used, production complies with the requirements indicated below.

Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more passages from the master seed lot than was used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Substrate for virus propagation

The virus is propagated in a human diploid cell line (2.7.2), in a continuous cell line (2.7.2) or in primary, secondary or tertiary monkey kidney cells.

Primary, secondary or tertiary monkey kidney cells. The following special requirements for the substrate for virus propagation apply to primary, secondary or tertiary monkey kidney cells.

Monkeys used in the preparation of kidney cell cultures for production and control of the vaccine. The animals used are of a species approved by the competent authority, in good health and, unless otherwise justified and authorised, have not been previously employed for experimental purposes. Kidney cells used for vaccine production and control are

derived from monitored, closed colonies of monkeys bred in captivity, not from animals caught in the wild; a previously approved seed lot prepared using virus passaged in cells from wild monkeys may, subject to approval by the competent authority, be used for vaccine production if historical data on safety justify this.

Monitored, closed colonies of monkeys. The monkeys are kept in groups in cages. Freedom from extraneous agents is achieved by the use of animals maintained in closed colonies that are subject to continuous and systematic veterinary and laboratory monitoring for the presence of infectious agents. The supplier of animals is certified by the competent authority. Each monkey is tested serologically at regular intervals during a quarantine period of not less than 6 weeks imposed before entering the colony and then during its stay in the colony.

The monkeys used are shown to be tuberculin-negative and free from antibodies to simian virus 40 (SV40) and simian immunodeficiency virus. If *Macaca* spp. monkeys are used for production, the monkeys are also shown to be free from antibodies to herpesvirus B (*Cercopithecine herpesvirus 1*) infection. Human herpesvirus 1 has been used as an indicator for freedom from herpesvirus B antibodies on account of the danger of handling herpesvirus B (*Cercopithecine herpesvirus 1*).

Monkeys from which kidneys are to be removed are thoroughly examined, particularly for evidence of tuberculosis and herpesvirus B (*Cercopithecine herpesvirus 1*) infection. If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it is not to be used nor are any of the remaining monkeys of the group concerned unless it is evident that their use will not impair the safety of the product.

All the operations described in this section are conducted outside the area where the vaccine is produced.

Monkey cell cultures for vaccine production. Kidneys that show no pathological signs are used for preparing cell cultures. Each group of cell cultures derived from a single monkey forms a separate production cell culture giving rise to a separate single harvest.

The primary monkey kidney cell suspension complies with the test for mycobacteria ; disrupt the cells before carrying out the test.

If secondary or tertiary cells are used, it shall be demonstrated by suitable validation tests that cell cultures beyond the passage level used for production are free from tumorigenicity.

SEED LOT

Each of the three strains of poliovirus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Only a working seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as human poliovirus 1, 2 or 3 by virus neutralisation in cell culture using specific antibodies.

Virus concentration. The virus concentration of each working seed lot is determined to define the quantity of virus to be used for inoculation of production cell cultures.

Extraneous agents (2.7.3). The working seed lot complies with the requirements for seed lots for virus vaccines. In addition, if primary, secondary or tertiary monkey kidney cells have been used for isolation of the strain, measures are taken to ensure that the strain is not contaminated with simian viruses such as simian immunodeficiency virus, simian virus 40, filoviruses and herpesvirus B (*Cercopithecine herpesvirus 1*). A working seed lot produced in primary, secondary or tertiary monkey kidney cells complies with the requirements given below under Virus Propagation and Harvest for single harvests produced in such cells.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells or viruses are being handled. Approved animal serum (but not human serum) may be used in the cell culture media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells); where continuous cell lines in a fermenter are used for production, 200×10^6 cells are set aside to prepare control cells; where primary, secondary or tertiary monkey kidney cells are used for production, a cell sample equivalent to at least 500 ml of the cell suspension, at the concentration employed for vaccine production, is taken to prepare control cell cultures.

Only a single harvest that complies with the following requirements may be used in the preparation of the vaccine. The tests for Identification and Sterility may be carried out instead on the purified, pooled monovalent harvest. After demonstration of consistency of production at the stage of the single harvest, the test for virus concentration may be carried out instead on the purified, pooled monovalent harvest.

Control cells. The control cells of the production cell culture comply with a test for Identification (if a cell-bank system is

used for production) and with the requirements for extraneous agents, where primary, secondary or tertiary monkey kidney cells are used, the tests in cell cultures are carried out as shown below under Test in Rabbit Kidney Cell Cultures and Test in Cercopithecus Kidney Cell Cultures).

Test in rabbit kidney cell cultures. Test a sample of at least 10 ml of the pooled supernatant fluid from the control cultures for the absence of herpesvirus B (*Cercopithecine herpesvirus 1*) and other viruses in rabbit kidney cell cultures. The dilution of supernatant in the nutrient medium is not greater than 1:4 and the area of the cell layer is at least 3 cm^2 per ml of inoculum. Set aside one or more containers of each batch of cells with the same medium as non-inoculated control cells. Incubate the cultures at 37° and observe for at least 2 weeks. The test is not valid if more than 20 per cent of the control cells are discarded for non-specific, accidental reasons.

Test in Cercopithecus kidney cell cultures. Test a sample of at least 10 ml of the pooled supernatant fluid from the control cultures for the absence of SV40 virus and other extraneous agents by inoculation onto cell cultures prepared from the kidneys of cercopithecus monkeys, or other cells shown to be at least as sensitive for SV40, by the method described under *Test in Rabbit Kidney Cell Cultures*. The test is not valid if more than 20 per cent of the control cell cultures are discarded for non-specific, accidental reasons.

Identification

The single harvest is identified as containing human poliovirus 1, 2 or 3 by virus neutralisation in cell cultures using specific antibodies.

Virus concentration. The virus concentration of each single harvest is determined by titration of infectious virus in cell cultures.

Sterility (2.2.11). The single harvest complies with the test for sterility, carried out using 10 ml for each medium.

Mycoplasmas (2.7.4). The single harvest complies with the test for mycoplasmas, carried out using 10 ml.

Test in rabbit kidney cell cultures. Where primary, secondary or tertiary monkey kidney cells are used for production, test a sample of at least 10 ml of the single harvest for the absence of herpesvirus B (Cercopithecine herpesvirus 1) and other viruses in rabbit kidney cell cultures as described for the control cells.

Test in Cercopithecus kidney cell cultures. Where primary, secondary or tertiary monkey kidney cells are used for production, test a sample of at least 10 ml of the single harvest for the absence of SV40 virus and other extraneous agents. Neutralise the sample by a high-titre antiserum against the specific type of poliovirus. Test the sample in primary cercopithecus kidney cell cultures or cells that have been demonstrated to be at least as susceptible for SV40. Incubate the cultures at 37° and observe for 14 days. At the end of this period, make at least one subculture of fluid in the same cell culture system and observe both primary cultures and subcultures for an additional 14 days.

PURIFICATION AND PURIFIED MONOVALENT HARVEST

Several single harvest of the same type may be pooled and may be concentrated. The monovalent harvest or pooled monovalent harvest is purified by validated methods. If continuous cell lines are used for production, the purification process shall have been shown to reduce consistently the content of substrate-cell DNA to not more than 500 pg per single human dose.

Only a purified monovalent harvest that complies with the following requirements may be used for the preparation of the inactivated monovalent harvest.

Identification

The virus is identified by virus neutralisation in cell cultures using specific antibodies or by determination of D-antigen.

Virus concentration. The virus concentration is determined by titration of infectious virus.

Specific activity. The ratio of the virus concentration or the D-antigen content, determined by a suitable immunochemical method (2.2.14) to the total protein content (specific activity) of the purified monovalent harvest is within the limits approved for the particular product.

INACTIVATION AND INACTIVATED MONOVALENT HARVEST

Several purified monovalent harvests of the same type may be mixed before inactivation. To avoid failures in inactivation caused by the presence of virus aggregates, filtration is carried out before and during inactivation; inactivation is started within a suitable period, preferably not more than 24 h and in any case not more than 72 h, of the prior filtration. The virus suspension is inactivated by a validated method that has been shown to inactivate poliovirus without destruction of immunogenicity; during validation studies, an inactivation curve with at least four points (for example, time 0, 24, 48, and 96 h) is established showing the decrease in concentration of live virus with time. If formaldehyde is used for inactivation, the presence of an excess of formaldehyde at the end of the inactivation period is verified.

Only an inactivated monovalent harvest that complies with the following requirements may be used in the preparation of a trivalent pool of inactivated monovalent harvests or a final bulk vaccine.

Test for effective inactivation. After neutralisation of the formaldehyde with sodium bisulphite (where applicable), verify the absence of residual live poliovirus by inoculation on suitable cell cultures of two samples of each inactivated monovalent harvest, corresponding to at least 1500 human doses. Take one sample not later than three-quarters of the way through the inactivation period and the other at the end. Inoculate the samples in cell cultures such that the dilution of vaccine in the nutrient medium is not greater then ¼ and the area of the cell layer is at least 3 cm^2 per ml of inoculum. Set aside one or more containers with the same medium as non-inoculated control cells. Observe the cell cultures for at least 3 weeks. Make not fewer than two passages from each container, one at the end of the observation period and the other 1 week before; for the passages, use cell culture supernatant and inoculate as for the initial sample. Observe the subcultures for at least 2 weeks. No sign of poliovirus multiplication is present in the cell cultures. At the end of the observation period, test the susceptibility of the cell culture used by inoculation of live poliovirus of the same type as that present in the inactivated monovalent harvest.

Sterility (2.2.11). The inactivated monovalent harvest complies with the test for sterility, carried out using 10 ml for each medium.

D-antigen content. The content of D-antigen determined by a suitable immunochemical method (2.2.14) is within the limits approved for the particular preparation.

FINAL BULK VACCINE

The final bulk vaccine is prepared directly from the inactivated monovalent harvests of human polioviruses 1, 2 and 3 or from a trivalent pool of inactivated monovalent harvests. If a trivalent pool of inactivated monovalent harvests is used, a test for effective inactivation is carried out on this pool instead of on the final bulk vaccine. A stabiliser and an antimicrobial preservative may be added.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Inactivation. Before addition of any antimicrobial preservative, a sample of at least 1500 ml or, for a purified and concentrated vaccine, the equivalent of 1500 doses is tested for residual live poliovirus in cell cultures, as described for the inactivated monovalent harvest. If the final bulk vaccine is prepared from a trivalent pool of inactivated monovalent harvests, the test for inactivation is carried out on that pool rather than on the final bulk vaccine.

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde and antimicrobial preservative and the *in vivo* assay have been performed with satisfactory results on the final bulk vaccine, they may be omitted on the final lot. Provided that the test for bovine serum albumin has been performed with satisfactory results on the trivalent pool of inactivated monovalent harvests or on the final bulk vaccine, it may be omitted on the final lot.

Identification

The vaccine is shown to contain human polioviruses 1, 2 and 3 by a suitable immunochemical method such as the determination of D-antigen by enzyme-linked immunosorbent assay (ELISA).

Tests

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Protein content (2.3.49). Not more than 10 µg of protein nitrogen per human dose.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Not more than 5 IU per human dose.

Assay

D-antigen content. As a measure of consistency of production, determine the D-antigen content for human polioviruses 1, 2 and 3 by a suitable immunochemical method (2.2.14) using an appropriate reference preparation calibrated in D-antigen units. For each type, the content, expressed with reference to the amount of D-antigen stated on the label, is within the limits approved for the particular product.

***In vivo* test**. The capacity of the vaccine to induce the formation of neutralizing antibodies is determined *in-vivo* by one of the following methods:

Test in chicks or guinea-pigs. Prepare a suitable series of at least three dilutions of the vaccine under examination using a suitable *buffered saline solution.* Inject 0.5 ml of the dilutions

intramuscularly into groups of ten 3-week-old chickens or groups of ten guinea-pigs, each weighing between 250 and 350 g, using a separate group for each dilution of vaccine. Bleed the animals on the fifth or sixth day after the injection and separate the sera. Examine the sera for the presence of neutralising antibody, at a dilution of 1 in 4, to each of the human polioviruses 1, 2 and 3. Mix 100 $CCID_{50}$ of virus with the dilution of serum and incubate at 37° for 4 h 30 min to 6 h. Keep at 5 ± 3° for 12 to 18 h. Inoculate the mixtures into cell cultures for the detection of unneutralised virus and read the results up to 7 days after inoculation. For each group of animals, note the number of sera which have neutralising antibody and calculate the dilution of the vaccine giving an antibody response in 50.0 per cent of the animals. Carry out in parallel a control test using a suitable *reference preparation*.

The vaccine complies with the test if a dilution of 1 in 100 or more produces an antibody response for each of the three types of virus in 50.0 per cent of the animals.

Test in rats. A suitable i*n vivo* assay method consists of intramuscular injection into the hind limb(s) of not fewer than 3 dilutions of the vaccine under examination and a reference vaccine, using for each dilution a group of 10 specific pathogen-free rats of the suitable strain. Use of 4 dilutions is often necessary to obtain valid results for all 3 serotypes. The number of animals per group must be sufficient to obtain results that meet the validity criteria; groups of 10 rats are usually sufficient although valid results may be obtained with fewer animals per group. The weight of individual animal must not vary by more than 10.0 per cent from the group mean. An inoculum of 0.5 ml per rat is used. The dose range is chosen such that a dose response to all 3 poliovirus types is obtained. Bleed the animals after 20 to 22 days. Neutralising titres against all 3 polivirus types are measured separately using 100 CCID50 of the Sabin strains as challenge viruses. Vero or Hep2 as indicator cells, and neutralization conditions of 3 h at 35° to 37° followed by 18 h at 2° to 8°. Results are read microscopically following fixation and staining after 7 days of incubation at 35°. For a valid antibody assay, the titre of each challenge virus must be shown to be within the range of 10 to 1000 $CCID_{50}$ and the neutralizing antibody titre of a control serum must be within 2 twofold dilutions of the geometric mean titre of the serum. The potency is calculated by comparison of the preparation of responders for the vaccine under examination and the reference vaccine by the probit method or, after validation, using a parallel-line model. For the probit method it is necessary to establish a cut-off neutralising antibody titre for each poliovirus type to define a responder. Due to interlaboratory variation, it is not possible to define cut-off values that could be applied by all laboratories. Rather, the cut-off values are determined for each laboratory based on a minimum series of 3 tests with the reference vaccine. The mid-point on a log 2 scale of the minimum and maximum geometric mean titres of the series of 3 or more tests is used as the cut-off value. For each of the 3 poliovirus types, the potency of the vaccine is not significantly less than that of the *reference preparation*. The test is not valid unless (1) for both the test and reference vaccines the ED_{50} lies between the smallest and the largest doses given to the animals; (2) the statistical analysis shows no significant deviation from linearity or parallelism; (3) the fiducial limits of the estimated relative potency fall between 25.0 per cent and 400.0 per cent of the estimated potency.

Labelling. The label states (1) the types of poliovirus contained in the vaccine; (2) the nominal amount of virus of each type (1, 2 and 3), expressed in units of D-antigen per single human dose; (3) the cell substrate used to prepare the vaccine.

Poliomyelitis Vaccine, Live (Oral)

Oral Poliomyelitis Vaccine is a preparation of approved strains of live attenuated poliovirus type 1, 2 or 3 grown in *in vitro* cultures of approved cells, containing any one type or any combination of the three types of Sabin strains, prepared in a form suitable for oral administration. The vaccine is a clear liquid that may be coloured owing to the presence of a pH indicator.

Production

General provisions

The vaccine strains and the production method should consistently yield vaccines that are both immunogenic and safe in man.

The production of vaccine is based on a virus seed-lot system. Cell lines are used according to a cell-bank system. If primary monkey kidney cells are used, production complies with the requirements indicated below. Unless otherwise justified and authorised, the virus in the final vaccine shall not have undergone more than two passages from the master seed lot.

Substrate for virus propagation

The virus is propagated in human diploid cells (2.7.2) or in continuous cell lines (2.7.2) or in primary monkey kidney cells (including serially passaged cells from primary monkey kidney cells). Continuous cell lines are approved by the competent authority.

Primary monkey kidney cells. *The following special requirements for the substrate for virus propagation apply to primary monkey kidney cells.*

Monkeys used for preparation of kidney cell cultures and for testing of virus. If the vaccine is prepared in monkey kidney cell cultures, animals of a species approved by the competent

authority, in good health, and not previously employed for experimental purposes shall be used.

The monkeys shall be kept in well-constructed and adequately ventilated animal rooms in cages spaced as far apart as possible. Adequate precautions shall be taken to prevent cross-infection between cages. Not more than two monkeys shall be housed per cage and cage-mates shall not be interchanged. The monkeys shall be kept in the country of manufacture of the vaccine in quarantine groups for a period of not less than 6 weeks before use. A quarantine group is a colony of selected, healthy monkeys kept in one room, with separate feeding and cleaning facilities, and having no contact with other monkeys during the quarantine period. If at any time during the quarantine period the overall death rate of a shipment consisting of one or more groups reaches 5 per cent (excluding deaths from accidents or where the cause was specifically determined not to be an infectious disease), monkeys from that entire shipment shall continue in quarantine from that time for a minimum of 6 weeks. The groups shall be kept continuously in isolation, as in quarantine, even after completion of the quarantine period, until the monkeys are used. After the last monkey of a group has been taken, the room that housed the group shall be thoroughly cleaned and decontaminated before being used for a fresh group. If kidneys from near-term monkeys are used, the mother is quarantined for the term of pregnancy.

Monkeys from which kidneys are to be removed shall be anaesthetised and thoroughly examined, particularly for evidence of tuberculosis and cercopithecid herpesvirus 1 (B virus) infection.

If a monkey shows any pathological lesion relevant to the use of its kidneys in the preparation of a seed lot or vaccine, it shall not be used, nor shall any of the remaining monkeys of the quarantine group concerned be used unless it is evident that their use will not impair the safety of the product.

All the operations described in this section shall be conducted outside the areas where the vaccine is produced.

The monkeys used shall be shown to be free from antibodies to simian virus 40 (SV40) and simian immunodeficiency virus. If *Macaca* spp. are used for production, the monkeys shall also be shown to be free from antibodies to cercopithecid herpesvirus 1 (B virus). Human herpesvirus has been used as an indicator for freedom from B virus antibodies on account of the danger of handling cercopithecid herpesvirus 1 (B virus).

Monkey kidney cell cultures for vaccine production. Kidneys that show no pathological signs are used for preparing cell cultures. If the monkeys are from a colony maintained for vaccine production, serially passaged monkey kidney cell cultures from primary monkey kidney cells may be used for virus propagation, otherwise the monkey kidney cells are not propagated in series. Virus for the preparation of vaccine is grown by aseptic methods in such cultures. If animal serum is used in the propagation of the cells, the maintenance medium after virus inoculation shall contain no added serum.

Each group of cell cultures derived from a single monkey or from fetuses from no more than ten near-term monkeys is prepared and tested as an individual group.

SEED LOT

The strains of poliovirus used shall be identified by historical records that include information on the origin and subsequent manipulation of the strains.

Working seed lots are prepared by a single passage from a master seed lot and at an approved passage level from the original Sabin virus. Virus seed lots are prepared in large quantities and stored at a temperature below -60°.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as poliovirus of the given type, using specific antibodies.

Virus concentration. Determined by the method described below, the virus concentration is the basis for the quantity of virus used in the neurovirulence test.

Extraneous agents (2.7.3). If the working seed lot is produced in human diploid cells (2.7.2) or in continuous cell lines (2.7.2) it complies with the requirements for seed lots for virus vaccines. If the working seed lot is produced in primary monkey cells, it complies with the requirements given below under Propagation and Harvest and Monovalent Pooled Harvest and with the tests in adult mice, suckling mice and guinea-pigs given under Tests for extraneous agents in viral vaccines for human use.

Working seed lot shall be free from detectable DNA sequences from simian virus 40 (SV40).

Neurovirulence (2.7.6). Each master and working seed lot complies with the test for neurovirulence of poliomyelitis vaccine (oral) in monkeys. Furthermore, the seed lot shall cease to be used in vaccine production if the frequency of failure of the monovalent pooled harvests produced from it is greater than predicted statistically. This statistical prediction is calculated after each test on the basis of all the monovalent pooled harvests tested; it is equal to the probability of false rejection on the occasion of a first test (i.e. 1 per cent), the probability of false rejection on retest being negligible. If the test is carried out only by the manufacturer, the test slides are provided to the control authority for assessment.

Genetic markers. Each working seed lot is tested for its replicating properties at temperatures ranging from 36° to 40° as described under Monovalent Pooled Harvest.

PROPAGATION AND HARVEST

All processing of the cell-banks and subsequent cell-cultures is done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from live extraneous agents. The cell-culture medium may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 5 per cent and not more than 1000 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells); special requirements, given below, apply to control cells when the vaccine is produced in primary monkey cells The virus suspension is harvested not later than 4 days after virus inoculation. After inoculation of the production cell culture with the virus working seed lot, inoculated cells are maintained at a fixed temperature, shown to be suitable, within the range 33° to 35°; the temperature is maintained constant to ±0.5°; control cell cultures are maintained at 33° to 35° for the relevant incubation periods.

Only a single virus harvest that complies with the following requirements may be used in the preparation of the monovalent pooled harvest.

Virus concentration. The virus concentration of virus harvests is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (2.7.3). Complies with tests for extraneous agents.

Control cells. The control cells of the production cell culture from which the virus harvest is derived comply with a test for identity and with the requirements for extraneous agents or, where primary monkey cells are used, as shown below.

Primary monkey kidney cells. *The following special requirements apply to virus propagation and harvest in primary monkey kidney cells.*

Cell cultures. On the day of inoculation with virus seed, each cell culture is examined for degeneration caused by an infective agent. If, in this examination, evidence is found of the presence in a cell culture of any extraneous agent, the entire group of cultures concerned shall be rejected.

On the day of inoculation with the virus working seed lot, a sample of at least 30 ml of the pooled fluid removed from the cell cultures of the kidneys of each single monkey or from fetuses from not more than ten near-term monkeys is divided into two equal portions. One portion of the pooled fluid is tested in monkey kidney cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other portion of the pooled fluid is, where necessary, tested in monkey kidney cell cultures from another species so that tests on the pooled fluids are done in cell cultures from at least one species known to be sensitive to SV40. The pooled fluid is inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2 per ml of pooled fluid. At least one bottle of each kind of cell culture remains uninoculated to serve as a control. If the monkey species used for vaccine production is known to be sensitive to SV40, a test in a second species is not required. Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody, but the maintenance medium after inoculation of test material contains no added serum except as described below.

The cultures are incubated at a temperature of 35° to 37° and are observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks incubation, at least one subculture of fluid is made from each of these cultures in the same cell culture system. The subcultures are also observed for at least 2 weeks.

Serum may be added to the original culture at the time of subculturing, provided that the serum does not contain SV40 antibody.

Fluorescent-antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

A further sample of at least 10 ml of the pooled fluid is tested for cercopithecid herpesvirus 1 (B virus) and other viruses in rabbit kidney cell cultures. Serum used in the nutrient medium of these cultures shall have been shown to be free from inhibitors of B virus. Human herpesvirus has been used as an indicator for freedom from B virus inhibitors on account of the danger of handling cercopithecid herpesvirus 1 (B virus). The sample is inoculated into bottles of these cell cultures in such a way that the dilution of the pooled fluid in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2 per ml of pooled fluid. At least one bottle of the cell cultures remains uninoculated to serve as a control.

The cultures are incubated at a temperature of 35° to 37° and observed for at least 2 weeks.

A further sample of 10 ml of the pooled fluid removed from the cell cultures on the day of inoculation with the seed lot virus is tested for the presence of extraneous agents by inoculation into human cell cultures sensitive to measles virus.

The tests are not valid if more than 20 per cent of the culture vessels have been discarded for non-specific accidental reasons by the end of the respective test periods.

If, in these tests, evidence is found of the presence of an extraneous agent, the single harvest from the whole group of cell cultures concerned is rejected.

If the presence of *Cercopithecid herpesvirus 1* (B virus) is demonstrated, the manufacture of oral poliomyelitis vaccine shall be discontinued and the competent authority shall be informed. Manufacturing shall not be resumed until a thorough investigation has been completed and precautions have been taken against any reappearance of the infection, and then only with the approval of the competent authority.

If these tests are not done immediately, the samples of pooled cell-culture fluid shall be kept at a temperature of -60° or below, with the exception of the sample for the test for B virus, which may be held at 4°, provided that the test is done not more than 7 days after it has been taken.

Control cell cultures. On the day of inoculation with the virus working seed lot 25 per cent (but not more than 2500 ml) of the cell suspension obtained from the kidneys of each single monkey or from not more than ten near-term monkeys is taken to prepare uninoculated control cell cultures. These control cell cultures are incubated in the same conditions as the inoculated cultures for at least 2 weeks and are examined during this period for evidence of cytopathic changes. The tests are not valid if more than 20 per cent of the control cell cultures have been discarded for non-specific, accidental reasons. At the end of the observation period, the control cell cultures are examined for degeneration caused by an infectious agent. If this examination or any of the tests required in this section shows evidence of the presence in a control culture of any extraneous agent, the poliovirus grown in the corresponding inoculated cultures from the same group shall be rejected.

Tests for haemadsorbing viruses. At the time of harvest or within 4 days of inoculation of the production cultures with the virus working seed lot, a sample of 4 per cent of the control cell cultures is taken and tested for haemadsorbing viruses. At the end of the observation period, the remaining control cell cultures are similarly tested. The tests are made as described in *(2.7.3), Tests for extraneous agents in viral vaccines for human use.*

Tests for other extraneous agents. At the time of harvest, or within 7 days of the day of inoculation of the production cultures with the working seed lot, a sample of at least 20 ml of the pooled fluid from each group of control cultures is taken and tested in two kinds of monkey kidney cell culture, as described above.

At the end of the observation period for the original control cell cultures, similar samples of the pooled fluid are taken and the tests referred to in this section in the two kinds of monkey kidney cell culture and in the rabbit cell cultures are repeated, as described above under Cell cultures.

If the presence of *Cercopithecid herpesvirus 1* (B virus) is demonstrated, the production cell cultures shall not be used and the measures concerning vaccine production described above must be undertaken.

The fluids collected from the control cell cultures at the time of virus harvest and at the end of the observation period may be pooled before testing for extraneous agents. A sample of 2 per cent of the pooled fluid is tested in each of the cell culture systems specified.

Single harvests

Tests for neutralised single harvests in monkey kidney cell cultures. A sample of at least 10 ml of each single harvest is neutralised by a type-specific poliomyelitis antiserum prepared in animals other than monkeys. In preparing antisera for this purpose, the immunising antigens used shall be prepared in non-simian cells.

Half of the neutralised suspension (corresponding to at least 5 ml of single harvest) is tested in monkey kidney cell cultures prepared from the same species, but not the same animal, as that used for vaccine production. The other half of the neutralised suspension is tested, if necessary, in monkey kidney cell cultures from another species so that the tests on the neutralised suspension are done in cell cultures from at least one species known to be sensitive to SV40.

The neutralised suspensions are inoculated into bottles of these cell cultures in such a way that the dilution of the suspension in the nutrient medium does not exceed 1 in 4. The area of the cell sheet is at least 3 cm^2 per ml of neutralised suspension. At least one bottle of each type of cell culture remains uninoculated to serve as a control and is maintained by nutrient medium containing the same concentration of the specific antiserum used for neutralisation.

Animal serum may be used in the propagation of the cells, provided that it does not contain SV40 antibody, but the maintenance medium, after the inoculation of the test material, contains no added serum other than the poliovirus neutralising antiserum, except as described below.

The cultures are incubated at a temperature of 35° to 37° and observed for a total period of at least 4 weeks. During this observation period and after not less than 2 weeks incubation, at least one subculture of fluid is made from each of these cultures in the same cell-culture system. The subcultures are also observed for at least 2 weeks.

Serum may be added to the original cultures at the time of subculturing, provided that the serum does not contain SV40 antibody.

Additional tests are made for extraneous agents on a further sample of the neutralised single harvests by inoculation of 10 ml into human cell cultures sensitive to measles virus.

Fluorescent-antibody techniques may be useful for detecting SV40 virus and other viruses in the cells.

The tests are not valid if more than 20 per cent of the culture vessels have been discarded for non-specific accidental reasons by the end of the respective test periods.

If any cytopathic changes occur in any of the cultures, the causes of these change are investigated. If the cytopathic changes are shown to be due to unneutralised poliovirus, the test is repeated. If there is evidence of the presence of SV40 or other extraneous agents attributable to the single harvest, that single harvest is rejected.

MONOVALENT POOLED HARVEST

Monovalent pooled harvests are prepared by pooling a number of satisfactory single harvests of the same virus type. Monovalent pooled harvests from continuous cell lines may be purified. Each monovalent pooled harvest is filtered through a bacteria-retentive filter.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

Each monovalent pooled harvest is identified as poliovirus of the given type, using specific antiserum.

Virus concentration

The virus concentration is determined by the method described below and serves as the basis for calculating the dilutions for preparation of the final bulk, for the quantity of virus used in the neurovirulence test and to establish and monitor production consistency.

Genetic markers. A ratio of the replication capacities of the virus in the monovalent pooled harvest is obtained over a temperature range between 36° and 40° in comparison with the seed lot or a reference preparation for the marker tests and with appropriate rct/40- and rct/40+ strains of poliovirus of the same type. The incubation temperatures used in this test are controlled to within ±0.1°. The monovalent pooled harvest passes the test if, for both the virus in the harvest and the appropriate reference material, the titre determined at 36° is at least 5.0 log greater than that determined at 40°. If growth at 40° is so low that a valid comparison cannot be established, a temperature in the region of 39.0° to 39.5° is used, at which temperature the reduction in titre of the reference material must be in the range 3.0 to 5.0 log of its value at 36°; the acceptable minimum reduction is determined for each virus strain at a given temperature. If the titres obtained for one or more of the reference viruses are not concordant with the expected values, the test must be repeated.

Neurovirulence (2.7.6). Each monovalent pooled harvest complies with the test for neurovirulence of poliomyelitis vaccine (oral). If the test is carried out only by the manufacturer, the test slides are provided to the competent authority for assessment. The TgPVR21 transgenic mouse model provides a suitable alternative to the monkey neurovirulence test for neurovirulence testing of types 1, 2 or 3 vaccines once a laboratory qualifies as being competent to perform the test and the experience gained is to the satisfaction of the competent authority. The test is carried out using a standard operating procedure approved by the competent authority. A suitable procedure *(Neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus)* is available from WHO, Quality and Safety of Biologicals, Geneva.

Primary monkey kidney cells. *The following special requirements apply to monovalent pooled harvests derived from primary monkey kidney cells.*

Retroviruses. The monovalent pooled harvest is examined using a reverse transcriptase assay. No indication of the presence of retrovirus is found.

Test on rabbits. A sample of the monovalent pooled harvest is tested for cercopithecid herpesvirus 1 (B virus) and other viruses by injection of not less than 100 ml into not fewer than 10 healthy rabbits each weighing between 1.5 and 2.5 kg. Each rabbit receives not less than 10 ml and not more than 20 ml, of which 1 ml is given intradermally at multiple sites, and the remainder subcutaneously. The rabbits are observed for at least 3 weeks for death or signs of illness.

All rabbits that die after the first 24 h of the test and those showing signs of illness are examined by autopsy, and the brain and organs removed for detailed examination to establish the cause of death.

The test is not valid if more than 20.0 per cent of the inoculated rabbits show signs of intercurrent infection during the observation period. The monovalent pooled harvest passes the test if none of the rabbits shows evidence of infection with B virus or with other extraneous agents or lesions of any kind attributable to the bulk suspension.

If the presence of B virus is demonstrated, the measures concerning vaccine production described above under Cell cultures are taken.

Test on guinea pigs. Administer to not less than five guinea-pigs, each weighing between 350 and 450 g, 0.1 ml of the monovalent pooled harvest by intracerebral injection and 0.5 ml by intraperitoneal injection. Measure the rectal temperature of each animal on each working day for 6 weeks. At the end of the observation period carry out autopsy on each animal.

In addition, administer to not fewer than five guinea-pigs 0.5 ml by intraperitoneal injection and observe as described

above for 2 to 3 weeks. At the end of the observation period, carry out a passage from these animals to not fewer than five guinea pigs using blood and a suspension of liver or spleen tissue. Measure the rectal temperature of the latter guinea pigs for 2 to 3 weeks. Examine by autopsy all animals that, after the first day of the test, die or are killed because they show disease or show for three consecutive days a body temperature higher than 39°; carry out histological examination to detect infection with Marburg virus; in addition, inject a suspension of liver or spleen tissue or of blood intraperitoneally into not fewer than three guinea pigs. If any signs of infection with Marburg virus are noted, confirmatory serological tests are carried out on the blood of the affected animals. The monovalent pooled harvest complies with the test if not fewer than 80.0 per cent of the guinea pigs survive to the end of the observation period and remain in good health and no animal shows signs of infection with filoviruses virus.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more satisfactory monovalent pooled harvests and may contain more than one virus type. Suitable flavouring substances and stabilisers may be added.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility (2.2.11). Complies with the test for sterility.

FINAL LOT

Only a final lot that complies with the following requirement for thermal stability and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

Thermal stability. Expose samples of the final lot at 37° for 48 hours. Determine the total virus concentration as described under Assay in parallel for the heated vaccine and for unheated vaccine. The estimated difference between the total virus concentration of the unheated and heated vaccines is not greater than 0.5 $\log_{10}$ infectious virus units ($CCID_{50}$) per single human dose.

Identification

The vaccine is shown to contain poliovirus of each type stated on the label, using specific antibodies.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Assay

Titrate for infectious virus at least in triplicate using the method described below. Use an appropriate virus reference preparation to validate each assay. If the vaccine contains more than one poliovirus type, titrate each type separately, using appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralise each of the other types present.

For a trivalent vaccine, the estimated mean virus titres must be: not less than $1 \times 10^{6.0}$ infectious virus units ($CCID_{50}$) per single human dose for type 1; not less than $1 \times 10^{5.0}$ infectious virus units ($CCID_{50}$) for type 2; and not less than $1 \times 10^{5.8}$ infectious virus units ($CCID_{50}$) for type 3.

For monovalent or divalent vaccine, the minimum virus titres are decided by the competent authority.

Method. Groups of eight to twelve flat-bottomed wells in a microtitre plate are inoculated with 0.05 ml of each of the selected dilutions of virus followed by a suitable cell suspension of the Hep-2 (Cincinnati) line. The plates are incubated at a suitable temperature. Examine the cultures on days 7 to 9.

The assay is not valid if (a) the confidence interval (P = 0.95) of the logarithm of the virus concentration is greater than ±0.3; (b) the virus concentration of the reference preparation differs by more than 0.5 log $CCID_{50}$ from the assigned value.

Labelling. The label states (1) the types of poliovirus contained in the vaccine; (2) the minimum amount of virus of each type contained in one single human dose; (3) the cell substrate used for the preparation of the vaccine; (4) that the vaccine is not to be injected.

Rabies Antiserum

Antirabies Serum; Antirabic Serum

Rabies Antiserum is a preparation containing the specific globulin or its derivatives obtained by purification of hyperimmune serum or plasma of healthy horses or other suitable animals having the specific activity of neutralising the rabies virus. It may contain a suitable preservative.

Rabies Antiserum contains not less than 300 Units per ml.

Description. A clear, colourless or pale yellow liquid free from suspended particles or cream-coloured powder or pellet to be reconstituted with diluent supplied by the manufacturer.

Identification

Specifically neutralizes the standard strain of rabies virus rendering it harmless to susceptible animals or by any other suitable *in vitro* method.

Tests

Potency. Carry out the biological assay of rabies antiserum described below.

Biological Assay of Rabies Antiserum

The potency of rabies antiserum is determined by comparing the dose necessary to protect mice against a lethal intracerebral dose of rabies virus with the dose of the Standard Preparation of rabies antiserum necessary to give the same protection.

Standard Preparation

The standard preparation is a dried serum or other suitable preparation the potency of which has been determined in relation to the International Standard.

Suggested Method

Test animals. Use healthy mice of either sex from the same stock weighing between 10 and 14 g, but animals of the same sex should be used in any given test.

Test virus. Any suitable strain of rabies virus of known potency such as the CVS strain may be used.

Test dose of virus. The test dose of virus is such that each mouse receives by intracerebral injection between 20 and 1000 LD_{50} preferably about 100 LD_{50}. To determine the number of LD_{50} of virus used, mix the virus dilution used in the test with an equal quantity of a 2 per cent v/v solution of *heat-inactivated normal horse serum* in *water* and maintain the mixture at 37° for 1 hour. Prepare 10-fold dilutions in a 2 per cent v/v solution of *heat-inactivated normal horse serum* and inject them into mice. Carry out this test at the same time as the test for determining the potency of the rabies antiserum. The test is not valid unless the quantity of virus used lies between 20 and 1000 LD_{50}.

Determination of potency of the rabies antiserum. Prepare a series of six 2-fold dilutions of the Standard Preparation and of the preparation under examination with *water* containing 2 per cent v/v of *heat inactivated normal horse serum*. To each dilution add a quantity of a suspension of the test virus containing the test dose and keep the mixtures at 37° for 1 hour. Inject intracerebrally 0.03 ml of each mixture into 10 mice.

Observe the mice for 14 days after the injection. Mice dying before the fifth day after inoculation with the virus are eliminated from the test; all the mice dying between the fifth and fourteenth days after showing signs of rabies are considered to have died of rabies; mice living upto the fourteenth day but showing signs of rabies are also counted as having died from rabies. Calculate the result of the test by standard statistical methods. The preparation under examination passes the test if, in a single comparative assay, it is found to have 80 or more Units per ml. If it is found to have less than 80 Units per ml, two more similar assays may be carried out; the preparation passes the test if, in both these additional assays, it is found to have 80 or more Units per ml.

Other tests. Complies with the tests stated under Antisera.

Storage. As stated under Antisera.

Labelling. The label states (1) the number of Units per ml in case of liquid preparation; (2) the total volume in the container; (3) the name and volume of the diluent to be used for reconstituting the freeze-dried preparation; (4) the recommended dose; (5) the name and concentration of any added preservative; (6) the animal species in which the antiserum has been raised; (7) that the liquid preparation should not be allowed to freeze.

Rabies Vaccine, Human

Rabies Vaccine for Human use is a freeze-dried or liquid (adsorbed) preparation of a suitable approved, strain of fixed rabies virus grown in an approved cell culture/ embryos of duck/chicken and inactivated by a validated method.

The freeze-dried vaccine is reconstituted immediately before use as stated on the label to give a clear or slightly opalescent solution/suspension. It may be coloured owing to the presence of a pH indicator.

The vaccine complies with the General Requirements of Vaccines for Human Use.

Production

General provisions

The vaccine is produced on the basis of virus seed lot system and if a cell line is used for virus propagation, a cell-bank system shall be followed. The production method shall have been shown to yield consistently vaccines that comply with the requirements for immunogenicity, safety and stability. Unless otherwise justified and authorized, the virus in the final vaccine shall not have undergone more passages from the master seed lot than was used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Substrate for virus propagation

The virus is propagated in any suitable approved cell substrate like a human diploid cell line (2.7.2), a continuous cell line, or in duck embryos or in cultures of chicken embryos derived from a flock certified as free from specified pathogens(2.7.7).

SEED LOT

The strain of rabies virus used shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

Working seed lots are prepared by not more than five passages from the master seed lot.

Only a working seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each working seed lot is identified as rabies virus using specific antibodies by an approved method.

Virus concentration. The virus concentration of each working seed lot is determined by a cell culture method using immunofluoresence or any other approved method.

Extraneous agents (2.7.3). The working seed lot complies with the requirements for the virus seed lots.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures are done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum; the media may contain human albumin. Serum proteins, if present are reduced to an acceptable level by a suitable method of purification. Serum and trypsin used in the preparation of cell suspension and media are shown to be free from infectious extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. Not less than 500 ml of the cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). The virus suspension is harvested on one or more occasions during incubation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

When vaccine is prepared in embryonated eggs, the egg proteins are minimized by an appropriate method of purification. The eggs are inoculated with virus seed by the yolk sac route. The infected sterile living embryos are harvested, minced and emulsified in suitable diluent, and stabilizer with aseptic precautions. Emulsions are centrifuged, supernatants are collected and stored as raw virus harvest at a suitable temperature.

Viral harvests that comply with the following requirements are pooled in the preparation of the inactivated viral harvest.

Identification

The single harvest contains virus that is identified as rabies virus using specific antibodies by an approved method.

Virus concentration. Titrate for infective virus in cell cultures or by any other approved method. The titre is used to monitor consistency of production.

Control cells. The control cells of the production cell culture from which the single harvest is derived should comply with a test for identification and with the requirements for extraneous agents (2.7.3).

Control eggs. Control eggs shall be tested for freedom from haemagglutinating agents, and other extraneous agents.

PURIFICATION AND INACTIVATION

The virus harvests may be concentrated and/or purified by suitable methods; the virus harvest is inactivated by a validated method at a fixed, well defined stage of the process which may be before, during or after any concentration or purification. The method shall have been shown to be capable of inactivating rabies virus without destruction of the immunogenic activity. If betapropiolactone is used, the concentration shall at no time exceed 1:3500.

Cell culture vaccines

Only an inactivated viral suspension that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation. Inactivation is confirmed by carrying out an amplification test for residual infectious rabies virus, not more than 4 days after inactivation or on the sample frozen after inactivation and stored at -70°. Inoculate a quantity of inactivated viral suspension equivalent to not less than 25 vaccine doses into cell cultures of the same type as those used for production of the vaccine. Make a passage after 7 days. Maintain the cultures for a further period of 14 days and then examine the cell cultures for rabies virus using an immune fluorescence test. No rabies virus is detected. Alternatively, 5 ml of each culture fluid is pooled on days 14 and 21 and 0.03 ml is inoculated intracerebrally into each of the 10 mice weighing 12 to 15 g. The mice are observed for 14 days for symptoms caused by rabies virus, and mice showing symptoms of rabies are sacrificed and virus presence is confirmed by immunofluorescence test or tested for live virus in cell culture by immunofluorescence test or method of equal sensitivity. No rabies virus should be detected.

Residual host-cell DNA (2.2.15). If a continuous cell line is used for virus propagation, the content of residual host-cell DNA, determined using a suitable method, should not be greater than 10 ng per single human dose.

Embryonated egg vaccine

Only concentrated viral suspensions that comply with the test for sterility, antigen content and endotoxin requirements may be used for preparation of bulk for inactivation.

Inactivation is confirmed by carrying out Mice Inoculation Test for residual infectious rabies virus, not more than four days after inactivation or on the sample frozen 4 days after inactivation are stored at –70°. Inoculate intracerebrally 0.03

ml into each of 20 mice weighing between 12 and 15 g. The mice are observed for 14 days for symptoms caused by rabies virus and mice showing symptoms of rabies are sacrificed and virus presence is confirmed by an immunofluorescence test or tested for live virus in cell culture by immunofluorescence test or method of equal sensitivity. No rabies virus should be detected.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated viral suspensions. An approved stabilizer may be added to maintain the activity of the product during and after freeze-drying. Thiomersal can be used as preservative.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Antigen content. Determine the antigen content by a suitable approved *in vitro* or *in vivo* method. The content should be within the limits approved for the particular product.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers and can be freeze-dried in case of lyophilized products. The containers are then sealed so as to prevent contamination and the introduction of moisture.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for inactivation has been carried out with satisfactory results on the inactivated virus suspension and the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, these tests may be omitted on the final lot.

Identification

The vaccine is shown to contain rabies virus antigen by a suitable immunochemical method using specific antibodies, alternatively, the Assay also serves to identify the vaccine.

Tests

Inactivation. Inoculate a quantity equivalent to not less than 25 human doses of vaccine into cell cultures of the same type as those used for production of the vaccine. Make a passage after 7 days. Maintain the culture for a further 14 days and then examine the cell culture for rabies virus using an immunofluorescence test. No rabies virus is detected. Alternatively, inject 0.03ml of the vaccine intracerebrally into each of the 10 mice weighing between 12 and 15g. Neither symptoms of disease in the central nervous system nor death occurs in any of the animal within 14 days. If the inactivation test is already performed on inactivated virus used for final lot, it may be omitted from the test on final lot.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Less than 25 IU per single human dose.

Pyrogens (2.2.8). Complies with the test for pyrogens. Unless otherwise justified and authorized, inject into each rabbit a single human dose of the vaccine diluted to ten times its volume.

Water (2.3.43). Not more than 3.0 per cent determined by an approved method.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Accelerated degradation. The potency determined by method described under "Assay" of a sample of the preparation under examination after storage at 37° for 4 weeks is not less than 2.5 units per single human dose. This may not be mandatory for lot release, once the consistency of the product is approved by National Regulatory Authority.

Bovine serum albumin (for cell culture vaccine). Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Aluminium content (for gel absorbed vaccine) (2.3.9). Not more than 1.25 mg per single human dose.

Ovalbumin (for egg based vaccines). Not more than 1 µg of ovalbumin per human dose, determined by a suitable technique using a suitable reference preparation of ovalbumin.

Residual host-cell DNA (for continuous cell line vaccines) (2.2.15). Should not be greater than 10 ng per single human dose.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Assay

The Standard preparation is the international standard or another suitable preparation, the potency of which has been determined in relation to the International standard. The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against a lethal intracerebral dose of rabies vaccine necessary to provide the same protection. For this comparison, a reference preparation of rabies vaccine, calibrated in international units, and a suitable preparation of rabies virus for use as the challenge preparation are necessary.

The international unit is the activity contained in a stated quantity of the international standard. The equivalence in

international units of the international standard is stated by the World Health Organization.

The test described below uses a parallel-line model with at least three points for the vaccine under examination and the reference preparation.

Test animals. Use mice of a suitable strain, drawn from a uniform stock three to four weeks old, weighing between 11 and 15 g. Distribute the mice into six groups of at least 16 mice each and four groups of 10 mice each and must be of the same sex or the sexes must be equally distributed among the groups.

Throughout the test all mice that die before the fifth day after challenge are excluded from the test and all mice that die with signs of rabies between the fifth and fourteenth day after challenge are counted as failing to resist the challenge.

The strain of mice suitable for the test is such that when 0.03 ml containing 5 to 50 LD_{50} of the challenge virus suspension is injected intracerebrally per mouse there is 100 per cent mortality.

Standard challenge virus suspension. A working pool of the challenge virus strain is prepared by injecting intracerebrally 0.03ml of a 10 fold dilution of the CVS strain of rabies virus in 2 per cent v/v sterile inactivated normal horse serum in water for injection or another suitable diluent approved by the competent authority into a suitable number of test animals. The animals when moribund after showing characteristic signs of rabies are sacrificed and their brains harvested aseptically. They are then washed in chilled saline solution to remove blood clots. A 10 per cent suspension of the brains is prepared in a suitable diluent approved by the competent authority and thoroughly homogenised. After centrifuging lightly, the supernatant liquid is distributed into sterile vials and freeze dried. The sealed and freeze-dried supernatant liquid containing vials are stored at -20°. When stored under prescribed conditions the virus titre of the freeze-dried preparation may be expected to be maintained for not less than 3 years. Alternatively, the washed brains are homogenised in a suitable diluent approved by the competent authority to give 10 per cent suspension. It is then centrifuged lightly, distributed into sterile ampoules or sterile plastic vials and sealed. The sealed ampoules or plastic vials can be stored at –60° or below. When stored under prescribed conditions, the virus titre may be expected to be maintained for not less than one year. Storage time needs to be validated by the manufacturer.

Virus titre of the challenge virus. Prepare ten fold serial dilutions of the standard challenge virus suspension. Using the four groups of 10 mice each, inject 0.03 ml of the virus suspension intracerebrally into each mouse, using a different group for each suspension. Observe the mice for 14 days. Calculate the virus titre of the standard challenge virus suspension in LD_{50} per dose of 0.03 ml by standard statistical methods.

Determination of potency of the vaccine. Reconstitute the standard preparation with a suitable diluent. Prepare at least three 5-fold serial dilutions of the solution of the standard preparation and three 5-fold serial dilutions of the vaccine under examination. For both, the standard preparation and the preparation under examination, the serial dilutions should be prepared in such a way that the lowest dilution protects more than 50 per cent of the injected mice. Allocate one dilution to each of the six groups of 16 mice each. Inject intraperitoneally each mouse in each group with dilutions of the vaccine and reference preparation and repeat the injections. After 7 days, prepare identical dilutions of the vaccine and reference preparation and repeat the injections.

After a further 7 days, inject each vaccinated mouse intracerebrally with 0.03 ml of the standard challenge virus suspension such that on the basis of preliminary titration, 0.03 ml contains between 5 to 50 LD_{50}. Observe the mice for 14 days and record the number of mice surviving the challenge in each group. Calculate the potency of the preparation under examination by standard statistical methods.

The vaccine complies with the test if the estimated potency is not less than 2.5 IU per single human dose.

The test is not valid unless (a) for both the preparation under examination and the standard preparation, the 50 per cent protective dose lies between the largest and smallest doses given to the mice; (b) there is not deviation from linearity or parallelism of the dose response lines, the confidence limit (P = 0.95) are not less than 25.0 per cent and not more than 400 per cent of the estimated potency; (c) the titre of the challenge virus suspension lies between 5 to 50 LD_{50}.

Labelling. The label states the biological origin of the cells used for the preparation of the vaccine.

Rubella Vaccine (Live)

Rubella Vaccine (Live) is a freeze-dried preparation of a suitable attenuated strain of rubella virus. The vaccine is reconstituted immediately before use to give a clear liquid or may be coloured owing to the presence of a pH indicator.

Production

General provisions

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live rubella vaccines of adequate immunogenicity and safety in man. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies

to be satisfactory with respect to safety and efficacy.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Substrate for virus propagation

The virus is propagated in human diploid cells (2.7.2).

SEED LOT

The strain of rubella virus used in the production of rubella vaccine shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation.

To avoid unnecessary use of monkeys in the test for neurovirulence virus seed lots are prepared in large quantities and stored at temperatures below -20° if freeze-dried, or below -60° if not freeze-dried.

Only a seed lot that complies with the following tests may be used for virus propagation.

Identification

The master and working seed lots are identified as rubella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined to ensure consistency of production.

Extraneous agents (2.7.3). The working seed lot complies with the tests for seed lots.

Neurovirulence (2.7.5). The master/working seed lot complies with the test for neurovirulence of live virus vaccines. *Macaca* and *Cercopithecus* monkeys are suitable for the test.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Suitable animal (but not human) serum may be used in the growth medium, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. Not less than 500 ml of the production cell culture is set aside as uninfected cell culture (control cells). The temperature of incubation is controlled during the growth of the virus. The virus suspension is harvested, on one or more occasions, within 28 days of inoculation. Multiple harvests from the same production cell culture may be pooled and considered as a single harvest.

Only a single harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as rubella virus by serum neutralization in cell culture, using specific antibodies.

Virus concentration. The virus concentration in the single harvest is determined as prescribed under Assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (2.7.3). The single harvest complies with the tests for extraneous agents.

Control cells. The control cells comply with a test for identification and with the tests for extraneous agents (2.7.3).

FINAL BULK VACCINE

Single harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabilizer may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

A minimum virus concentration for release of the product is established such as to ensure, in the light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

Only a final lot that complies with the tests for minimum virus concentration for release, with the following requirement for thermal stability and with each of the requirements given below under Identification and Tests may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 7 days. Determine the virus concentration as described under Assay in parallel for the vaccine held at 37° for 7 days and for vaccine stored at 2° to 8°. The virus concentration of the heated vaccine is not more than 1.0 $\log_{10}$ lower than that of the unheated vaccine.

Identification

When the vaccine reconstituted as stated on the label is mixed with specific rubella antibodies, it is no longer able to infect susceptible cell cultures.

Tests

Sterility (2.2.11). The reconstituted vaccine complies with the test for sterility.

Water (2.3.43). Not more than 3.0 per cent, determined by Karl Fischer, semi-micro determination of water or by any suitable validated method.

Bovine serum albumin. Not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Assay

Titrate the vaccine for infective virus at least in triplicate, using at least five cell cultures for each 0.5 $\log_{10}$ dilution step or by a method of equal precision. Use an appropriate virus reference preparation to validate each assay. The estimated virus concentration is not less than that stated on the label; the minimum virus concentration stated on the label is not less than 1×10^3 $CCID_{50}$ per human dose. The assay is not valid if the confidence limits (P=0.95) of the logarithm of the virus concentration is greater than ± 0.3.

Rubella vaccine (Live) RS is suitable for use as a reference preparation.

Labelling. The label states (1) the strain of virus used for the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) the minimum virus concentration; (4) the time within which the vaccine must be used after reconstitution; (5) that the vaccine must not be given to a pregnant woman and that a woman must not become pregnant within two months.

Snake Venom Antiserum

Snake Antivenin; Snake Antivenom Serum; Snake Venom Antitoxin

Snake Venom Antiserum is a sterile preparation containing antitoxic globulins or their derivatives that have the power of specifically neutralising the venom of one or more species of snakes. It may be obtained from the serum or plasma of healthy equines or other suitable animals immunised against venoms of specific species usually elapine and viperine. It may be a purified liquid preparation or a freeze-dried product. It may contain a suitable preservative.

Description. A clear to slightly opalescent, colourless or pale yellow liquid, free from suspended particles or cream-coloured powder or pellet which when reconstituted with the diluent supplied by the manufacturer with the freeze-dried product yields a clear, colourless or pale yellow liquid.

Identification

Specifically renders the corresponding venom or venoms harmless to susceptible animals. It may also be identified by any other alternate suitable *in vitro* method.

Tests

Potency. In view of the numerous toxic fractions in venoms and varying requirements of different localities, no standard for potency is prescribed.

The potency of the snake venom antiserum is determined by estimating the ability of the antiserum to protect mice or other suitable animals against the lethal effect of a fixed dose of a reference preparation of snake venom of the relevant species or by comparing its ability to do so with that of a reference preparation of antiserum of established potency at two or more dose levels of the venom.

Other tests. Complies with the tests stated under Antisera.

Storage. Store the freeze-dried preparation in a cool, dark place and avoid exposure to excessive heat. Store the liquid preparation at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the volume of the contents and in case of freeze-dried preparation, the directions for reconstitution; (2) the species of snake against whose venom the antiserum is effective; (3) the animal species from which the antiserum has been obtained; (4) the name and proportion of any preservative added; (5) that in case of a liquid preparation, it should not be allowed to freeze.

Tetanus Antitoxin

Tetanus Antitoxin is a preparation containing the specific antitoxic globulins or their derivatives obtained by purification of hyperimmune serum of horses or other suitable animals and have the specific activity of neutralising the toxin formed by *Clostridium tetani*. The liquid preparation may contain a suitable antimicrobial preservative.

Tetanus Antitoxin has a potency of not less than 1000 Units per ml when intended for prophylactic use and not less than 3000 Units per ml when intended for therapeutic use.

Description. A clear, colourless or pale yellow liquid or a freeze-dried, cream-coloured powder or pellet.

Identification

Specifically neutralises and renders the toxin formed by *C. tetani* harmless to susceptible animals or by any other suitable *in vitro* test.

Tests

Other tests. Complies with the tests stated under Antisera.

Potency. Carry out the biological assay of tetanus antitoxin described below.

Biological Assay of Tetanus Antitoxin

The potency of tetanus antitoxin is determined by comparing the dose necessary to protect mice against the paralytic effects of a fixed dose of tetanus toxin with the dose of the Standard Preparation of tetanus antitoxin necessary to give the same protection. For this purpose the Standard Preparation of tetanus antitoxin and a suitable preparation of toxin, for use as a test toxin, are required. The test dose of the toxin is determined in relation to the Standard Preparation and the potency of the preparation under examination is then determined in relation to the Standard Preparation using the test toxin.

Standard Preparation

The Standard Preparation is the 2nd International Standard for Tetanus antitoxin, equine, established in 1969, consisting of freeze-dried hyperimmune horse serum (supplied in ampoules containing 1400 Units), or another suitable preparation the potency of which has been determined in relation to the International Standard.

Suggested Method

NOTE — The severity of the tetanic paralysis to be regarded as the end-point is such that the paralysis is readily recognised but not sufficiently extensive to cause significant suffering. For humane reasons the animals should be examined at least twice a day and should be killed as soon as the end-point is reached.

Test animals. Use healthy mice, weighing between 17 to 22 g, from the same stock.

Preparation of test toxin. Prepare tetanus toxin from a sterile filtrate of an 8 to 10 day culture of *C. tetani*. Test toxin may be prepared by adding this filtrate to *glycerin* in the proportion of 1 volume of the filtrate to 1 or 2 volumes of *glycerin*. This solution of test toxin is stored below 0°. Test toxin may also be prepared in stable form by saturating the filtrate with *ammonium sulphate*, collecting the resulting precipitate, drying it over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa and reducing it to a fine powder. The powder so obtained is preserved in the dry condition at a low temperature either in sealed ampoules or *over phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa.

Determination of test dose of toxin *(Lp/10 dose)*. First determine the Limes paralyticum/10 (Lp/10) dose of the test toxin. This is the smallest quantity of the toxin which when mixed with 0.1 Unit of the Standard Preparation and injected subcutaneously into mice causes tetanic paralysis within 4 days. Prepare a solution of the Standard Preparation in a suitable liquid such that 1 ml contains 0.5 Unit. Accurately measure or weigh a quantity of the test toxin and dilute it with, or dissolve it in, a suitable liquid. Prepare mixtures such that each contains 2.0 ml of the solution of the Standard Preparation (1 Unit), one of a series of graded volumes of the solution of the toxin, and sufficient of a suitable liquid to give a final volume of 5.0 ml. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject 0.5 ml of each mixture subcutaneously into mice, six mice being used for each mixture, and observe the mice for 4 days.

The test (Lp/10) dose of the toxin is the amount present in 0.5 ml of that mixture that contains the smallest amount of toxin sufficient to cause tetanic paralysis in all six mice injected with it within 4 days.

Determination of potency of the antitoxin. Prepare a solution of the Standard Preparation in a suitable liquid such that it contains 0.5 Unit per ml. Accurately measure or weigh a quantity of the test toxin and dilute it with, or dissolve it in, a suitable liquid so that 1.0 ml contains five times the Lp/10 dose as previously determined. Prepare mixtures such that each contains 2.0 ml of the solution of the test toxin and one of a series of graded volumes of the preparation under examination, and sufficient of a suitable liquid to give a final volume of 5.0 ml. Prepare similar mixtures containing 2.0 ml of the test toxin and one of a series of graded volumes of the solution of the Standard Preparation centred on that volume (2.0 ml) that contains 1 Unit. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inoculate 0.5 ml of each mixture subcutaneously into each mouse, six mice being used for each mixture, and observe the mice for 4 days. The mixture that contains the largest volume of the preparation under examination that fails to protect the mice from paralysis contains 1 Unit. The test is not valid unless all the mice injected with mixtures containing 2.0 ml or less of the solution of the Standard Preparation show paralysis and all those injected with more do not. Calculate the potency of the preparation under examination in Units per ml.

Storage. As stated under Antisera.

Labelling. The label states (1) the number of Units per ml; (2) species of animal from which the preparation has been made; (3) whether the preparation is for prophylactic or for therapeutic use; (4) the recommended dose; (5) the name and proportion of any added preservative; (6) that the preparation, if liquid, should not be allowed to freeze; (7) that the preparation, if dried, should be used immediately after reconstitution in the stated quantity of the diluent supplied by the manufacturer.

Tetanus Vaccine (Adsorbed)

Tetanus Vaccine (Adsorbed) is a preparation of tetanus formol toxoid adsorbed on mineral carrier. The formol toxoid is prepared from the toxin produced by the growth of *Clostridium tetani*.

Production

General provisions

The maximum number of Lf per single human dose of tetanus vaccine is 25. The production method is validated to demonstrate that the product, if tested, would comply with the following test.

BULK PURIFIED TOXOID

For the production of tetanus toxin, from which toxoid is prepared, seed cultures are managed in a defined seed-lot system in which toxinogenicity is conserved and, where necessary, restored by deliberate reselection. A highly toxinogenic strain of *C. tetani* with known origin and history is grown in a suitable liquid medium. At the end of cultivation, the purity of each culture is tested and contaminated cultures are discarded. Toxin-containing culture medium is collected aseptically. The toxin content (Lf per ml) is checked to monitor consistency of production. Single harvests may be pooled to prepare the bulk purified toxoid. The toxin is purified to remove components likely to cause adverse reactions in humans. The purified toxin is detoxified with formaldehyde by a method that avoids destruction of the immunogenic potency of the toxoid and reversion of toxoid to toxin, particularly on exposure to heat. Alternatively, purification may be carried out after detoxification.

Only bulk purified toxoid that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Absence of tetanus toxin. Inject subcutaneously at least 500 Lf of purified toxoid in a volume of 1 ml into each of five healthy guinea pigs, each weighing 250 to 350 g, that have not previously been treated with any material that will interfere with the test. None of the animals shows any symptoms of tetanus toxaemia or dies from tetanus within 21 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight repeat the test. If an animal dies or loses weight in the second test the toxoid fails the test.

Irreversibility of toxoid. Using the buffer for the final vaccine, prepare a dilution of the bulk purified toxoid containing the same toxoid concentration as the final vaccine. Divide the dilution into two equal parts. Keep one of them at 2° to 8° and the other at 37° for 6 weeks. Test both dilutions by a suitable sensitive assay for active tetanus toxin, such as inoculation into mice or guinea-pigs. The toxoid complies with the test if neither sample produces any sign of a toxic reaction attributable to tetanus toxin.

Antigenic purity. Not less than 1,000 Lf per mg of protein nitrogen.

FINAL BULK VACCINE

The final bulk vaccine is prepared by adsorption of a suitable quantity of bulk purified toxoid onto a mineral carrier such as hydrated aluminium phosphate or aluminium hydroxide; the resulting mixture is approximately isotonic with blood. Suitable antimicrobial preservatives may be added. Certain antimicrobial preservatives, particularly those of the phenolic type, adversely affect the antigenic activity and must not be used.

Only final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Specific toxicity. Use five normal, healthy guinea pigs weighing between 250 g and 350 g which have been maintained for at least one week on a uniform, unrestricted diet, have not lost weight during this period and have not been previously treated with any material that will interfere with the test. Weigh the animals separately and record their weights. Inject subcutaneously into each animal five times the dose stated on the label. Weigh all the animals at weekly intervals for 3 weeks. None of the animals shows any symptoms of tetanus toxaemia or dies from tetanus within 21 days or loses weight at the end of the test. If more than one animal dies from non-specific causes or loses weight repeat the test. If an animal dies or loses weight in the second test, the vaccine fails the test.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

pH (2.4.24). 6.0 to 7.0.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity for antisera and vaccine.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided the tests for

antimicrobial preservative, free formaldehyde and the assay have been carried out with satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

Tetanus toxoid is identified by a suitable immunochemical method (2.2.14). The following method, applicable to certain vaccines, is given as an example. Dissolve in the vaccine under examination sufficient *sodium citrate* to give a 10 per cent solution. Maintain at 37° for about 16 hours and centrifuge until a clear supernatant liquid is obtained. The clear supernatant liquid reacts with a suitable tetanus antitoxin, giving a precipitate.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if *aluminium hydroxide* or *hydrated aluminium phosphate* is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

Sterility (2.2.11). Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity for antisera and vaccine.

Potency of tetanus component

Assay. Determine by either of the following methods.

(1) Inject subcutaneously on each of two occasions separated by an interval of not more than 4 weeks, one-tenth of the stated human dose diluted to 1 ml with *saline solution* into each of 9 normal, healthy guinea pigs weighing between 250 and 350 g. Not more than 2 weeks after the second injection, collect the serum from each animal and carry out the biological test for tetanus antitoxin, described under Tetanus antitoxin or any other method approved by National Regulatory Authority.

Sera of at least 6 guinea pigs out of 9 should contain not less than 0.5 Unit of tetanus antitoxin per ml.

(2) Carry out the biological assay of adsorbed Tetanus Vaccine (Adsorbed) described below.

If the lower limit of the 95.0 per cent confidence interval of estimated potency is less than 40 IU per single human dose then the limits of the 95.0 per cent confidence interval of the estimater of potency shall be within 50 to 200 per cent of the estimated potency unless the lower limit of the 95.0 per cent confidence interval of the estimated potency is greater than 40 IU per single human dose.

Biological assay of adsorbed tetanus vaccine

The potency of adsorbed tetanus vaccine is determined by comparing the dose of the vaccine required to protect guinea-pigs or mice from the paralytic effects of a subcutaneous injection of tetanus toxin with the dose of the Standard preparation needed to give the same protection. For this comparison, the Standard preparation of adsorbed tetanus toxoid and a suitable preparation of tetanus toxin, for use as a challenge toxin, are necessary.

Standard preparation

The Standard preparation is International standard for Tetanus toxoid, adsorbed or another suitable preparation, the potency of which has been determined in relation to the International standard.

Suggested method

The paralysis method is not obligatory and the lethal method may be used. For this method the number of animals and the procedure are identical with those described for the paralysis method but the end-point is the death of the animal rather than the onset of paralysis and the LD_{10} dose is used instead of the LD_{50} dose.

(a) ***Test on guinea pigs***

Test animals. Use healthy guinea-pigs from the same stock and weighing between 250 and 350 g. Distribute them into six groups of sixteen each. The guinea-pigs should all be of the same sex or the males and females should be distributed equally between the groups. If the challenge toxin to be used has not been shown to be stable or has not been adequately standardised, include four further groups of five guinea pigs to serve as unvaccinated controls.

Challenge toxin. Select a preparation of tetanus toxin containing not less than 50 times the 50 per cent paralytic dose per ml. If the challenge toxin preparation has been shown to be stable, it is not necessary to verify the paralytic dose for every assay.

Preparation of the challenge toxin solution. Immediately prior to use, prepare from the challenge toxin by dilution with *phosphate buffered saline pH 7.4* a challenge toxin solution containing fifty times the 50 per cent paralytic dose per ml. If necessary, dilute portions of this challenge toxin solution 16, 50 and 160 fold with the same buffer solution.

Determination of potency. Prepare in *saline solution* three dilutions of the vaccine under examination and three dilutions of a solution of the Standard preparation such that, for each, the dilutions form a series differing by not more than 2.5 fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 1 ml volumes into guinea-pigs, protect approximately 50 per cent of the animals from the

paralytic effects of the subcutaneous injection of the quantity of tetanus toxin prescribed for this test. Allocate the six dilutions one to each of the six groups of sixteen guinea pigs and inject subcutaneously 1.0 ml of each dilution into each guinea pig in the group to which that dilution is allocated. After 28 days inject each animal subcutaneously with 1.0 ml of the *challenge toxin solution* containing fifty times the 50 per cent paralytic dose. If necessary, allocate the challenge toxin solution and the three dilutions made from it one to each of the four groups of five guinea pigs and inject subcutaneously 1.0 ml of each toxin solution into each guinea-pig in the group to which that toxin solution is allocated. Examine the guinea pigs twice daily, remove and kill all animals showing definite signs of tetanus paralysis. Count the number of guinea pigs without paralysis 5 days after injection of the challenge toxin and calculate the potency of the vaccine under examination relative to the potency of the Standard preparation on the basis of the number of animals without paralysis in each of the six groups of sixteen, using standard statistical methods.

The test is not valid unless (a) for both the vaccine under examination and the Standard preparation, the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the guinea pigs; (b) if applicable, the number of paralysed animals among the four groups of five injected with the challenge toxin solution and its dilutions indicate that the challenge was approximately 50 times the 50 per cent paralytic dose; (c) the fiducial limits of assay lie between 50.0 per cent and 200.0 per cent of the estimated potency; (d) the statistical analysis shows no deviations from linearity or parallelism. The test may be repeated any number of times but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

(b) ***Test on mice***

Test animals. Use healthy mice from the same stock, weighing between 14 and 20 g. Distribute them into six groups of sixteen each. If the challenge toxin to be used has not been shown to be stable or has not been adequately standardised, include four further groups of six mice to serve as unvaccinated controls. The mice should all be of the same sex or the males and females should be distributed equally among the groups.

Challenge toxin. Select a preparation of tetanus toxin containing not less than 100 times the 50 per cent paralytic dose per ml.

Preparation of the challenge toxin solutions. Immediately prior to use prepare from the challenge toxin by dilution with *phosphate buffered saline pH 7.4* a *challenge toxin solution* containing fifty times the 50 per cent paralytic dose in each 0.5 ml. If necessary, dilute portions of this challenge toxin solution 16, 50 and 160 fold with the same buffer solution.

Determination of potency. Prepare in *saline solution* three dilutions of the vaccine under examination and three dilutions of a solution of the Standard preparation such that, for each, the dilutions form a series differing by not more than 2.5 fold steps and in which the dilutions of intermediate concentration, when injected subcutaneously in 0.5 ml volumes into mice, protect approximately 50 per cent of the animals from the paralytic effects of the subcutaneous injection of the quantity of tetanus toxin prescribed for this test. Allocate the six dilutions one to each of the six groups of sixteen mice and inject subcutaneously 0.5 ml of each dilution into each mouse in the group to which the dilution is allocated. After 28 days inject each animal subcutaneously with 0.5 ml of the *challenge toxin solution* containing fifty times the 50 per cent paralytic dose. If necessary, allocate the challenge toxin solution and the three dilutions made from it one to each of the four groups of six mice and inject subcutaneously 0.5 ml of each *toxin solution* into each mouse in the group to which that toxin solution is allocated. Count the number of mice without paralysis 4 days after injection of the challenge toxin and calculate the potency of the vaccine under examination relative to the potency of the Standard preparation on the basis of the numbers of animals without paralysis in each of the six groups of sixteen, using standard statistical methods.

The test is not valid unless (a) for both the vaccine under examination and the Standard preparation, the 50 per cent protective doses lie between the largest and smallest doses of the preparations given to the mice; (b) if applicable, the number of paralysed animals among the four groups of six injected with the challenge toxin solution and its dilutions indicate that the challenge was approximately 50 times the 50 per cent paralytic dose; (c) the fiducial limits of the assay lie between 50.0 per cent and 200.0 per cent of the estimated potency; (d) the statistical analysis shows no deviation from linearity or parallelism. The test may be repeated any number of times but when more than one test is performed the results of all valid tests must be combined in the estimate of potency.

(c) ***Determination of antibodies in guinea pigs***

Preparation of serum samples. For preparation of serum samples, the following technique has been found suitable. Invert the tubes containing blood samples 6 times and allow to stand at 37° for 2 h, then at 4° for 2 hours, centrifuge at room temperature at 800 g for 20 min. transfer the serum to sterile tubes and store at a temperature below -20°. At least 40.0 per cent yield of serum is obtained by this procedure.

Determination of antibody titre. The ELISA and ToBI tests shown below are given as examples of immunochemical methods that have been found suitable for the determination of antibody tire.

Determination of antibody titre in guinea pig serum by enzyme-linked immunosorbent assay (ELISA). Dilutions of

test and reference sera are made on ELISA plates coated with tetanus toxoid. A positive guinea-pig serum control and a negative guinea-pig serum control are included on each plate to monitor the assay performance. Peroxidase-conjugated rabbit or goat antibody directed against guinea-pig-IgG is added followed by a peroxidase substrate. Optical density is measured and the relative antibody titre is calculated using the usual statistical methods.

Reagents and equipment

ELISA plates: 96 wells, columns 1-12, rows A-H.

Clostridium tetani guinea-pig antiserum (for vaccines-human use) reference preparation (positive control serum).

Peroxidase conjugate. Peroxidase-conjugated rabbit or goat antibody directed against guinea-pig IgG.

Tetanus toxoid.

Carbonate coating buffer pH 9.6. Dissolve 1.59 g of *anhydrous sodium carbonate* and 2.93 g of *sodium hydrogen carbonate* in 1000 ml of *water.* Distribute into 150 ml bottles and sterilise by autoclaving at 121° for 15 min.

Phosphate buffered saline pH 7.4 (PBS). Dissolve with stirring 80.0 g of *sodium chloride,* 2.0 g of *potassium dihydrogen phosphate,* 14.3 g of *disodium hydrogen phosphate dihydrate* and 2.0 g of *potassium chloride* in 1000 ml of *water.* Store at room temperature to prevent crystallisation. Dilute to 10 times its volume with *water* before use.

Citric acid solution. Dissolve 10.51 g of *citric acid* in 1000 ml of *water* and adjust the solution to pH 4.0 with a 400 g/l solution of *sodium hydroxide.*

Washing buffer. PBS *containing 0.5 g/l of polysorbate 20.*

Diluent block buffer. PBS containing 0.5 g/l of *polysorbate 20* and 25 g/l of dried skimmed milk.

Peroxidase substrate. Shortly before use, dissolve 10 mg of *diammonium 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate)* (ABTS) in 20 ml of *citric acid solution.* Immediately before use add 5 µl of *strong hydrogen peroxide solution.*

Method

The description below is given as an example of a suitable plate lay-out but others may be used. Wells 1A-H are for negative control serum and wells 2A-H and 3A-H are for positive control serum for assay monitoring. Wells 4-12A-H are for test samples.

Coat each well of the ELISA plates with 100 µl of *tetanus toxoid solution* (0.5 Lf/ml in carbonate coating buffer).

Allow to stand overnight at 4° in a humid atmosphere. To avoid interference from temperature gradient, do not stack more than 4 plates high. On the following day, wash the plates thoroughly with *washing buffer.* Block the plates by addition of 100 µl of *diluent block buffer* to each well. Incubate in a humid atmosphere at 37° for 1 h. Wash the plates thoroughly with *washing buffer.* Place 100 µl of *diluent block buffer* in each well of the plates, except those of row A. Prepare suitable dilutions of *negative control serum, positive control serum* (from about 0.01 IU/ml) and test sera. Allocate the *negative control serum* to column 1, *positive control serum* to columns 2 and 3 and test sera to columns 4-12 and add 100 µl of each serum to the first 2 wells of the column to which it is allocated. Using a multi channel micropipette, make twofold serial dilutions from row B down the plate to row H by transferring 100 µl to the following well. Discard 100 µl from the last row so that all wells contain 100 µl. Incubate at 37° for 2 h. Wash thoroughly with *washing buffer.* Prepare a suitable dilution (a 1 in 2000 dilution has been found suitable) of *peroxidase conjugate* in *diluent block buffer* and add 100 µl to each well. Incubate at 37° in a humid atmosphere for 1 h. Wash the plates thoroughly with *washing buffer.* Add 100 µl of peroxidase substrate to each well. Allow to stand at room temperature, protected from light, for 30 min. Read the plates at 405 nm in the same order as addition of substrate was made.

Determination of antibody titre in guinea-pig serum by toxin- or toxoid-binding inhibition (ToBI). Tetanus toxin or toxoid is added to serial dilutions of test and reference sera; the serum/antigen mixtures are incubated overnight. To determine unbound toxin or toxoid, the mixtures are transferred to an ELISA plate coated with tetanus antitoxin. *Peroxidase-conjugated equine anti-tetanus IgG* is added followed by a *peroxidase substrate.* Optical density is measured and the antibody titre is calculated using the usual statistical methods. A *positive control serum* and a *negative control serum* are included on each plate to monitor assay performance.

Reagents and equipment

Round-bottomed, rigid polystyrene microtitre plates.

Flat-bottomed ELISA plates.

Tetanus toxin or tetanus toxoid.

Clostridium tetani guinea-pig antiserum (for vaccines-human use) reference preparation.

Equine anti-tetanus IgG.

Peroxidase-conjugated equine anti-tetanus IgG.

Carbonate buffer pH 9.6. Dissolve 1.5 g of *anhydrous sodium carbonate,* 2.39 g of *sodium hydrogen carbonate* and 0.2 g of *sodium azide* in 1000 ml of *water,* adjust to pH 9.6 and autoclave at 121° for 20 min.

Sodium acetate buffer pH 5.5. Dissolve 90.2 g of *anhydrous sodium acetate* in 900 ml of *water,* adjust to pH 5.5 using a saturated solution of *citric acid monohydrate* and dilute to

1000 ml with *water.*

Phosphate buffered saline pH 7.2 (PBS). Dissolve 135.0 g of *sodium chloride,* 20.55 g of *disodium hydrogen phosphate dihydrate* and 4.80 g of *sodium dihydrogen phosphate monohydrate* in *water* and dilute to 15 litres with the same solvent. Autoclave at 100° for 60 min.

Diluent buffer. PBS containing 5 g/l of *bovine albumin* and 0.5 g/l of *polysorbate 80.*

Block buffer. PBS containing 5 g/l of *bovine albumin.*

Tetramethylbenzidine solution. 6 g/l solution of *tetramethylbenzidine* in *alcohol.* The substance dissolves within 30-40 min at room temperature.

Peroxidase substrate. Mix 90 ml of *water,* 10 ml of *sodium acetate buffer* pH 5.5, 1.67 ml of *tetramethylbenzidine solution* and 20 µl of *strong hydrogen peroxide solution.*

Washing solution. Tap water containing 0.5 g/l of *polysorbate 80.*

Method

Block the round-bottomed polystyrene microtitre plates by placing in each well 150 µl of *block buffer.* Cover the plates with a lid or sealer. Incubate in a humid atmosphere at 37° for 1 h. Wash the plates thoroughly with *washing solution.* Place 100 µl of *PBS* in each well. Place 100 µl of *reference guinea pig tetanus antitoxin* in the first well of a row. Place 100 µl of *undiluted test sera* in the first well of the required number of rows. Using a multichannel micropipette, make it two fold serial dilutions across the plate (up to column 10), by transfer of 100 µl to the following well. Discard 100 µl from the last column so that all wells contain 100 µl. Prepare 0.1 Lf/ml solution of *tetanus toxin* or toxoid using *PBS* a diluent. Add 40 µl of this solution to all wells except those column 12. The wells of row 11 are a positive control. Add 40 µl of *PBS* to the wells of column 12 (negative control). Shake the plates gently and cover them with lids. Coat the ELISA plates: immediately before use make a suitable dilution of *equine anti-tetanus IgG* in *carbonate buffer pH 9.6* and add 100 µl to all wells. Incubate the 2 series of plates overnight in a humid atmosphere at 37°. To avoid temperature gradient effects, do not stack more than 4 plate high. Cover the plates with lids. On the following day, wash the ELISA plates thoroughly with *washing solution.* Block the plates by placing in each well 125 µl of *block buffer.* Incubate at 37° in a humid atmosphere *for* 1 h. Wash the plates thoroughly with *washing solution.* Transfer 100 µl of the pre-incubation mixture from the polystyrene plates to the corresponding wells of the ELISA plates, starting with column 12 and then from 1 to 11. Cover the plates with a lid. Incubate at 37° in a humid atmosphere for 2 h. Wash the ELISA plates thoroughly with *washing solution.* Make a suitable dilution (a 1 in 4000 dilution has been found suitable) of the *peroxidase-conjugated equine anti-tetanus IgG* in *diluent buffer.* Add 100 µl of the dilution to each well and cover the plates with a lid. Incubate at 37° in a humid atmosphere *for* 1.5 h. Wash the ELISA plates thoroughly with *washing solution.* Add 100 µl of *peroxidase substrate* to each well. A blue colour develops. Incubate the plates at room temperature. Stop the reaction at a given time (within 10 min) by the addition of 100 µl of 2 M *sulphuric acid* to each well in the same order as the addition of substrate. The colour changes from blue to yellow. Measure the absorbance (2.4.7) at 450 nm immediately after addition of the *sulphuric acid* or maintain the plates in the dark until reading.

(d) Any other validated serological assay in guinea-pigs/mice approved by National Regulatory Authority.

Labelling. The label states (1) the human dose (ml); (2) the minimum units per single human dose or the minimum International Units per single human dose if potency test done by challenge method; (3) the name and the amount of the adsorbent and preservative; (4) that the vaccine must be shaken before use; (5) that the vaccine is not to be frozen.

Tick-borne Encephalitis Vaccine (Inactivated)

Tick-borne Encephalitis Vaccine (Inactivated) is a liquid preparation of a suitable strain of tick-borne encephalitis virus grown in cultures of chick-embryo cells or other suitable cell cultures and inactivated by a suitable, validated method.

Production

General provisions

The vaccine complies with the General Requirements of Vaccines for Human Use.

Production of the vaccine is based on a virus seed lot system. The production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man. The virus in the final vaccine shall not have undergone more passages from the master seed lot than the virus in the vaccine used in clinical trials.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity and immunogenicity.

Substrate for virus propagation

The virus is propagated in chick embryo cells prepared from eggs derived from a chicken flock free from specified pathogens (2.7.7) or in other suitable cell cultures.

SEED LOT

The strain of virus used is identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are stored at or below -60°.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification

Each seed lot is identified as containing the vaccine strain of tick-borne encephalitis virus by a suitable immunochemical method, preferably using monoclonal antibodies.

Virus concentration. The virus concentration of each seed lot is determined by titration in suitable cell cultures to monitor consistency of production.

Extraneous agents (2.7.3). Each seed lot complies with the requirements for extraneous agents in viral vaccines for human use; the tests in cell cultures are carried out in human and simian cells only.

PROPAGATION AND HARVEST

All processing of the cell cultures if performed under aseptic conditions in an area where no other cells are being handled. Serum and trypsin used in the preparation of cell suspensions and media used must be shown to be free from extraneous agents. The cell culture media may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. At least 500 ml of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells).

Only a single harvest that complies with the following requirements may be used in the preparation of the inactivated harvest.

Identification

The single harvest is shown to contain tick-borne encephalitis virus by a suitable immunochemical method, preferably using monoclonal antibodies, or by virus neutralization in cell cultures.

Sterility (2.2.11). Complies with the test for sterility carried out using 10 ml for each medium.

Mycoplasma (2.7.4). Complies with the test for mycoplasmas carried out using 1 ml for each medium.

Control cells. The control cells comply with the tests for extraneous agents (2.7.3). If the vaccine is produced using a cell-bank system, the control cells comply with a test for identification.

Virus concentration. Determine the virus concentration by titration in suitable cell culture to monitor consistency of production.

Inactivation

To avoid interference, viral aggregates are removed by filtration immediately before the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating tick-borne encephalitis virus without destroying the antigenic and immunogenic activity; as part of the validation studies, an inactivation curve is plotted representing residual live virus concentration measured on not fewer than three occasions. If formaldehyde is used for inactivation, the presence of an excess of free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Residual infective virus. Inoculate a quantity of the inactivated harvest equivalent to not less than ten human doses of vaccine in the final lot into primary chicken fibroblast cell cultures, or other cells shown to be at least as sensitive to tick-borne encephalitis virus with not less than 3 cm^2 of cell sheet per ml of inoculum. Incubate at 37 ± 1° for 14 days. No cytopathic effect is detected at the end of the incubation period. Collect the culture fluid and inoculate 0.03 ml intracerebrally into each of not fewer than ten mice about 4 weeks old. Observe the mice for 14 days. They show no evidence of tick-borne encephalitis virus infection.

Purification

Several inactivated single harvests may be pooled before concentration and purification by suitable methods, preferably by continuous-flow, sucrose density-gradient centrifugation.

Only a purified, inactivated harvest that complies with the following requirements may be used in the preparation of final bulk vaccine.

Sterility (2.2.11). Complies with the test for sterility, carried out using 10 ml for each medium.

Specific activity. Determine the antigen content of the purified, inactivated harvest by a suitable immunochemical method (2.2.14). Determine the total protein content by a suitable method. The specific activity, calculated as the antigen content per unit mass of protein, is within the limits approved for the specific product.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more purified, inactivated harvests.

Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the tests for free formaldehyde, bovine serum albumin (where applicable) and pyrogens and the assay have been carried out satisfactory results on the final bulk vaccine, they may be omitted on the final lot.

Identification

The vaccine is shown to contain tick-borne encephalitis virus antigen by a suitable immunochemical method using specific antibodies or by the mouse immunogenicity test described under Assay.

Tests

Aluminium (2.3.9). Maximum 1.25 mg per single human dose, if aluminium hydroxide or hydrated aluminium phosphate is used as the adsorbent.

Free formaldehyde (2.3.20). Maximum 0.1 g/l.

Bovine serum albumin. If bovine serum albumin has been used during production, the vaccine contains not more than 50 ng per single human dose, determined by a suitable immunochemical method (2.2.14).

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject into each rabbit, per kg of body mass, one dose of vaccine.

Assay

The potency is determined by comparing the dose necessary to protect a given proportion of mice against the effects of a lethal dose of tick-borne encephalitis virus, administered intraperitoneally, with the quantity of a reference preparation of tick-borne encephalitis vaccine necessary to provide the same protection. For this comparison an approved reference preparation and a suitable preparation of tick-borne encephalitis virus from an approved strain for use as the challenge preparation are necessary.

The following is cited as an example of a method that has been found suitable for a given vaccine.

Selection and distribution of test animals. Use healthy mice weighing between 11 and 17 g derived from the same stock. Distribute the mice into not less than six groups of a suitable size to meet the requirements for validity of the test; for titration of the challenge suspension, use not fewer than four groups of ten mice. Use mice of the same sex or distribute males and females equally between groups.

Determination of potency of the vaccine. Prepare not less than three suitable dilutions of the vaccine under examination and of the reference preparation; in order to comply with validity criteria four to five dilutions will usually be necessary. Prepare dilutions such that the most concentrated suspension is expected to protect more than 50 per cent of the animals and the least concentrated suspension less than 50.0 per cent. Allocate each dilution to a different group of mice and inject subcutaneously into each mouse 0.2 ml of the dilution allocated to its group. Seven days later make a second injection using the same dilution scale. 14 days after the second injection prepare a suspension of the challenge virus containing not less than 100 LD_{50} in 0.2 ml. Inject 0.2 ml of this virus suspension intraperitoneally into each vaccinated mouse. To verify the challenge dose, prepare a series of not fewer than three dilutions of the challenge virus suspension at not greater than one-hundred fold intervals. Allocate the challenge suspension and the four dilutions, one to each of the five groups of ten mice, and inject intraperitoneally into each mouse 0.2 ml of the challenge suspension or the dilution allocated to its group. Observe the animals for 21 days after the challenge and record the number of mice that die in the period between 7 and 21 days after the challenge.

Calculations. Calculate the results by the usual statistical methods for an assay with quantal responses (5.7).

Validity criteria. The test is not valid unless (1) the concentration of the challenge virus is not less than 100 LD_{50}; (2) for both the vaccine under examination and the reference preparation the 50.0 per cent protective dose (PD_{50}) lies between the largest and smallest doses given to the mice; (3) the statistical analysis shows a significant slope and no significant deviation from linearity and parallelism of the dose-response lines; (4) the fiducial limits (P = 0.95) are not less than 33.0 per cent and not more than 300.0 per cent of the estimated potency.

Potency requirement. Include all valid tests to estimate the mean potency and the fiducial limits (P = 0.95) for the mean potency; compute weighed means with the inverse of the squared standard error as weights. The vaccine complies with the test if the estimated potency is not less than that approved by the competent authority, based on data from clinical efficacy trials.

Labelling. The label states (1) the strain of virus used in preparation; (2) the type of cells used for production of the vaccine.

Tuberculin Purified Protein Derivative

Tuberculin PPD; Tuberculin Purified Protein Derivative for Human Use

Tuberculin Purified Protein Derivative is a preparation made from the heat-treated products of growth and lysis of one or more strains of *Mycobacterium tuberculosis* that reveal

delayed hypersensitivity in animals sensitised by a micro-organism of the same species.

Production

It is prepared from the water-soluble fraction obtained by heating in free-flowing steam or in an autoclave and subsequently filtering cultures of the mycobacteria grown in a suitable liquid medium. The active fraction in the filtrate, which is predominantly protein, is separated by precipitation, washed and redissolved. The preparation is free from mycobacteria. An antimicrobial preservative that does not give rise to false positive reactions, such as 0.5 per cent w/v of *phenol*, and a suitable stabiliser may be added. Phenol is not added to preparations that are to be freeze-dried. The final sterile product is distributed into sterile glass containers which are then sealed so as to prevent microbial contamination or alternatively it is freeze-dried and the containers subsequently sealed.*

* *To ensure availability of a preparation of uniform potency, Tuberculin Purified Protein Derivative is produced and issued by the Statens Serum Institute, Denmark as a powder to be reconstituted as stated on the label.*

The preparation may be issued either as a sterile liquid or as a freeze-dried product. If issued as a liquid, it is in a ready-to-use form and 0.1 ml constitutes one intradermal dose containing appropriate number of Units. If issued as a freeze-dried product, it should yield a ready-to-use preparation when reconstituted as per manufacturer's instructions.

Description. A colourless or pale, straw-coloured liquid, or dry cream-coloured powder, or pellet.

The preparation, reconstituted if necessary as stated on the label, complies with the following requirements.

Identification

A. When progressively increasing doses are injected intradermally into specifically sensitised guinea-pigs, reactions occur at the points of injection, varying from erythema to necrosis. When similar injections are administered to non-sensitised guinea pigs no such reactions occur.

B. The potency test described below serves as a test for identity if it is performed on material from the final containers.

Tests

pH (2.4.24). 6.5 to 7.5.

Phenol (*if present*) (2.3.36). Not more than 0.5 per cent w/v.

Sterility (2.2.11). Complies with the tests for sterility.

Potency. Carry out the *biological assay of tuberculin purified protein derivative* described below.

Tuberculin Purified Protein Derivative in freeze-dried form complies with the following additional requirements.

Live mycobacteria

A. Inject 5.0 ml intraperitoneally or subcutaneously into each of two guinea-pigs weighing between 300 and 400 g. Observe the animals for not less than 42 days. Kill the animals and carry out an autopsy. No guinea-pig shows signs of infection with mycobacteria.

B. Carry out tests for live mycobacteria in the preparation under examination using suitable culture media. No growth of mycobacteria should occur.

Sensitising effect. Inject intradermally into each of three guinea-pigs three times, at intervals of 5 days, a dose of the preparation under examination containing about 500 Units in a volume of 0.1 ml. Two to three weeks after the third injection inoculate the same dose intradermally into these animals and into a control group of three guinea-pigs of the same weight but that have not had any previous injections of tuberculin. The reactions of the two groups are not significantly different after 48 to 72 hours.

Toxicity. Inject subcutaneously into 2 healthy guinea-pigs, weighing not less than 250 g and which have not previously been treated with any material which will interfere with the test, 0.5 ml of a solution containing 1,00,000 Units per ml. No harmful effects are produced within 7 days.

Biological assay

The potency of tuberculin purified protein derivative is determined by comparing the dose necessary to reveal delayed hypersensitivity in guinea-pigs or other animals hypersensitised with mycobacteria of the same type as that used in the preparation of the tuberculin purified protein derivative with the dose of the appropriate Standard Preparation necessary to give the same effect.

The estimated potency is not less 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error are not less than 64 per cent and not more than 156 per cent of the stated potency.

Standard Preparation

The Standard Preparation is the 1st International Standard for Tuberculin, purified protein derivative (PPD), mammalian, established in 1951, consisting of PPD derived from cultures of *M. tuberculosis* (supplied in ampoules containing 5,00,000 Units) or another suitable preparation the potency of which has been determined in relation to the International Standard.

Method

Sensitise 12 guinea-pigs each weighing not less than 400 g by the intramuscular injection of a total of about 0.5 ml of a suspension in a suitable mineral oil with or without emulsifier and containing 0.1 mg per ml of heat-inactivated, dried mycobacteria of the same type as that used in the preparation

of tuberculin. Use *phosphate-buffered saline pH 7.4* containing 0.005 per cent w/v of *polysorbate 80* to prepare the dilutions of the Standard preparation and of the preparation under examination and to reconstitute freeze-dried preparations containing no stabiliser. Not less than 1 month and not more than 6 months later carry out the following test :

Shave the flanks to provide space for at least three reactions on each side, but not more than a total of 12 injection sites per animal. Use at least three doses of the Standard preparation and at least three doses of the preparation under examination, the highest dose being about 10 times as strong as the lowest. Dilute the preparations so that the lesions produced are 8 to 25 mm in diameter. Allocate the doses to the available sites in a random manner, in a Latin square design. Inject each dose intradermally in the same volume (0.1 or 0.2 ml) at the sites to which it has been allocated. Measure the diameters of the lesions after 24 to 48 hours and calculate the result of the test using standard statistical methods on the basis that the lesion diameters are directly proportional to the logarithm of the concentration of the tuberculin.

Storage. Store in light-resistant containers at a temperature between 2° and 8°. It should not be allowed to freeze.

Labelling. The label states (1) the number of Units per dose of 0.1 ml or per ml or per mg; (2) the total volume in the container (for liquid preparation); (3) the nature and quantity of the reconstituting liquid (for the freeze-dried preparation); (4) the name and proportion of any added substances; (5) the species or strain used; (6) the storage conditions; (7) the date after which the contents are not intended to be used; (8) that care should be taken to avoid inhaling the powder (for the freeze-dried preparation).

Typhoid (Strain Ty 21a) Vaccine, Live (Oral)

Typhoid Vaccine (Live, Oral, strain Ty 21a) is a freeze-dried preparation of the live *Salmonella typhi* strain Ty 21a grown in a suitable medium. When presented in capsules, the vaccine complies with the tests stated under Capsules.

Production

Choice of vaccine strain

The main characteristic of the strains is the defect of the enzyme uridine diphosphate-galactose-4 epimerase. The activities of galactopermease, galactokinase and galactose-1-phosphate uridyl-transferase are reduced by 50 to 90 per cent. Whatever the growth conditions, the strain does not contain Vi antigen. The strain agglutinates to anti-O : 9 antiserum only if grown in medium containing galactose. It contains the flagellar H:d antigen and does not produce hydrogen sulphide on Kligler iron agar. The strain is nonvirulent for mice. Cells of strain Ty 21a lyse if grown in the presence of 1.0 per cent of galactose.

SEED LOT

The vaccine is prepared using a seed-lot system. The working seed lots represent not more than one subculture from the master seed lot. The final vaccine represents not more than four subcultures from the original vaccine on which were made; the laboratory and clinical tests showing the strain to be suitable.

Only a master seed lot that complies with the following requirements may be used in the preparation of working seed lots.

Galactose metabolism

In a spectrophotometric assay, no activity of the enzyme uridine diphosphate-galactose-4-epimerase is found in the cytoplasm of strain Ty 21a compared to strain Ty 2.

Biosynthesis of lipopolysaccharide

Lipopolysaccharides are extracted by the hot-phenol method and examined by size-exclusion chromatography (2.4.16). Strain Ty 21a grown in medium free of galactose shows only the rough (R) type of lipopolysaccharide.

Serological characteristics

Strain Ty 21a grown in a synthetic medium without galactose does not agglutinate to specific anti-O:9 antiserum. Whatever the growth conditions, strain Ty 21a does not agglutinate to Vi antiserum. Strain Ty 21a agglutinates to H:d flagellar antiserum.

Biochemical markers

StrainTy 21a does not produce hydrogen sulphide on Kligler iron agar. This property serves to distinguish Ty 21a from other galactose-epimerase-negative *S. typhi* strains.

Cell growth

Strain Ty 21a cells lyse when grown in the presence of 1.0 per cent of galactose.

PROPAGATION AND HARVEST

The bacteria from the working seed lot are multiplied in a preculture, subcultured once and are then grown in a suitable medium containing 0.001 per cent of galactose at 30° for 13 to 15 hours. The bacteria are harvested. The harvest must be free from contaminating micro-organisms.

Only a single harvest that complies with the following requirements may be used for the preparation of the freeze-dried harvest.

pH (2.4.24). 6.8 to 7.5.

Optical density

The optical density of the culture, measured at 546 nm, is 6.5 to 11.0. Before carrying out the measurement, dilute the culture so that a reading in the range 0.1 to 0.5 is obtained and correct the reading to take account of the dilution.

Identification

Culture bacteria on an agar medium containing 1.0 per cent of *galactose* and *bromothymol blue*. Light blue, concave colonies, transparent due to lysis of cells, should be found. No yellow colonies (galactose-fermenting) should be found.

FREEZE-DRIED HARVEST

The harvest is mixed with a suitable stabilizer and freeze-dried by a process that ensures the survival of at least 10.0 per cent of the bacteria and to a water content shown to be favourable to the stability of the vaccine. No antimicrobial preservative is added to the vaccine.

Only a freeze-dried harvest that complies with the following tests may be used for the preparation of the final bulk.

Identification

Culture bacteria are examined on an agar medium containing 1.0 per cent of *galactose* and *bromoethymol blue*. Light blue, concave colonies, transparent due to lysis of cells, should be found. No yellow colonies (galactose-fermenting) should be found.

Number of live bacteria

Not less than 1×10^{11} live *S. typhi* strain Ty 21a per gram.

Water (2.4.43). 1.5 to 4.0 per cent, determined by the semi-micro determination of water or any other validated method.

FINAL BULK VACCINE

The final bulk vaccine is prepared by aseptically mixing one or more freeze-dried harvests with a suitable sterile excipient.

Only a final bulk that complies with the following requirement may be used in the preparation of the final lot.

Number of live bacteria. Not less than 40×10^9 live *S. typhi* strain Ty 21a per gram.

FINAL LOT

The final bulk vaccine is distributed under aseptic conditions into capsules with a gastro-resistant shell or into suitable containers.

Only a final lot that is satisfactory with respect to Identification, Tests and Number of live bacteria will be released for use, except that in the determination of number of live bacteria each dosage unit must contain not less than 4×10^9 live bacteria.

Identification

Culture bacteria from the vaccine under examination on an agar medium containing 1.0 per cent of *galactose* and *bromothymol blue*. Light blue, concave colonies transparent due to lysis of cells, should be found. No yellow colonies (galactose-fermenting) should be found.

Tests

Microbial contamination (2.2.9). Carry out the test using suitable selective media. Determine the total viable count using the plate-count method. The number of contaminating micro-organisms per dosage unit is not greater than 10^2 bacteria and 20 fungi. No pathogenic bacterium, particularly *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* other than strain Ty 21a are found.

Water (2.4.43). 1.5 to 4.0 per cent.

Number of live bacteria

Carry out the test using not less than five dosage units. Homogenise the contents of the dosage units in a 0.9 per cent w/v solution of *sodium chloride* at 4° using a mixer in a cold room with sufficient glass beads to emerge from the liquid. Immediately after homogenization prepare a suitable dilution of the suspension using cooled diluent and inoculate *brain heart infusion agar*, incubate at 36 ± 1° for 20 to 36 hours. The vaccine contains not less than 2×10^9 live *S. typhi* Ty 21a bacteria per dosage unit.

Labelling. The label states (1) the minimum number of live bacteria per dosage unit; (2) that the vaccine is for oral use only.

Typhoid Polysaccharide Vaccine

Typhoid Polysaccharide Vaccine is a preparation of purified Vi capsular polysaccharide obtained from *Salmonella typhi* Ty2 strain or some other suitable strain of known origin and history that has the capacity to produce Vi polysaccharide, and which have been characterized by suitable biochemical, physicochemical, serological or molecular methods.

Capsular polysaccharide is a partly 3-O-acetylated repeated units of 2-acetylamino-2-deoxy-D-galactopyranuronic acid with á-(1→4)-linkages.

Production

General provisions

The production of Vi polysaccharide is based on a defined seed-lot system. The method of production shall have been

shown to yield consistently Vi-polysaccharide typhoid vaccines of adequate immunogenicity and safety in man. The production method is validated to demonstrate that the product, if tested, would comply with the tests for abnormal toxicity.

SEED LOT

The strain of *S. typhi* used for the master seed lot shall be identified by historical records that include information on its origin and by its biochemical and serological characteristics. Cultures from the working seed lot shall have the same characteristics as the strain that was used to prepare the master seed lot, shall be demonstrated along with adequate documentation.

Only a strain that has the following characteristics may be used in the preparation of the vaccine: (a) stained smears from a culture are typical of *S. typhi*; (b) the culture utilises glucose without production of gas; (c) colonies on agar are oxidase-negative; (d) a suspension of the culture agglutinates specifically with an appropriate Vi antiserum or colonies form haloes on an agar plate containing a suitable Vi antiserum.

PROPAGATION AND HARVEST

The working seed lot is cultured on a solid medium, which may contain blood-group substances, or a liquid medium; the inoculum obtained is transferred to a liquid medium, which is used to inoculate the final medium. The liquid medium used and the final medium are semi-synthetic, free from substances that are precipitated by *cetrimonium bromide* and do not contain blood-group substances or high-molecular-mass polysaccharides, unless it has been demonstrated that they are removed by the purification process. The bacterial purity of the culture is verified by microscopic examination of Gram-stained smears and by inoculation into appropriate media. The culture is then inactivated at the beginning of the stationary phase by the addition of *formaldehyde*. Bacterial cells are eliminated by centrifugation; the polysaccharide is precipitated from the culture medium by addition of *hexadecyltrimethylammonium bromide* (*cetrimonium bromide*). The precipitate is harvested and may be stored at or below -20° before purification.

Purified Vi Polysaccharide

The polysaccharide is purified, after dissociation of the polysaccharide/cetrimonium bromide complex, using suitable procedures to eliminate successively nucleic acids, proteins and lipopolysaccharides. The polysaccharide is precipitated in its salt form and dried at 5±3°; the powder obtained constitutes the purified Vi polysaccharide. The loss on drying is determined by thermogravimetry, Karl Fischer or any other suitable method and is used to calculate the results of the chemical tests shown below with reference to the dried substance.

Only those pools of purified Vi-polysaccharide that comply with the following requirements may be used in the preparation of the final bulk:

Protein (2.7.1). Not more than 10 mg per gram of polysaccharide, calculated with reference to the dried substance.

Nucleic acids (2.7.1). Not more than 20 mg per gram of polysaccharide, calculated with reference to the dried substance.

O-Acetyl groups (2.7.1). Not less than 2 mmol per gram of polysaccharide, calculated with reference to the dried substance.

Molecular size. Examine by gel filtration or size-exclusion chromatography (2.4.16) using cross-linked agarose for chromatography. Use a column 0.9 m long and 16 mm in internal diameter equilibrated with a solvent having an ionic strength of 0.2 mol per kg and a pH of 7.0 to 7.5. Apply about 5 mg of polysaccharide in a volume of 1 ml to the column and elute at about 20 ml/h. Collect fractions of about 2.5 ml. Determine the point corresponding to K_0 = 0.25 and make two pools consisting of fractions eluted before and after this point. Determine O-acetyl groups on the two pools. Not less than 50 per cent of the polysaccharide is found in the pool containing fractions eluted before K_0 = 0.25.

Identification

Carry out an identification test using a suitable immunochemical method (2.2.14).

Bacterial endotoxins (2.2.3). Determine by a suitable method, the content should not be more than 150 IU per mg of polysaccharide.

FINAL BULK VACCINE

One or more batches of purified Vi polysaccharide are dissolved in a suitable solvent, which may contain an antimicrobial preservative, so that the volume corresponding to one dose contains 25 µg of polysaccharide and the solution is isotonic with blood (250 mosm/kg to 350 mosm/kg).

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot;

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount. If phenol has been used in the preparation, the content is not more than 2.5 g/l.

FINAL LOT

The final bulk vaccine is distributed and filled aseptically into sterile containers. The containers are closed so as to prevent contamination.

Only a final lot that is satisfactory with respect to each of the requirements prescribed below under Identification, Tests and Assay and with the requirements for Bacterial endotoxins may be released for use. Provided the tests for free formaldehyde and antimicrobial preservative have been carried out on the final bulk vaccine, they may be omitted on the final lot.

The vaccine contains minimum of 25 µg purified Vi-Polysaccharide per dose of 0.5 ml.

Identification

Carry out an identification test using a suitable immunochemical method (2.2.14).

Tests

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

pH (2.4.24). 6.5 to 7.5.

O-Acetyl groups (2.7.1). 0.085 (± 25 per cent) µmol per dose (25 µg of polysaccharide).

Test solution. Place 3 ml of the vaccine in each of three tubes (two reaction solutions and one correction solution).

Reference solutions. Dissolve 0.150 g of *acetylcholine chloride* in 10 ml of *water* (*stock solution* containing 15 g/l of *acetylcholine chloride*). Immediately before use, dilute 0.5 ml of the *stock solution* to 50 ml with *water* (working dilution containing 150 µg/ml of *acetylcholine chloride*). In ten tubes, place in duplicate (reaction and correction solutions) 0.1 ml, 0.2 ml, 0.5 ml, 1.0 ml and 1.5 ml of the working dilution.

Prepare a blank using 3 ml of *purified water*.

Make up the volume in each tube to 3 ml with *water*. Add 0.5 ml of a mixture of 1 volume of *water* and 2 volumes of *dilute hydrochloric acid* to each of the correction tubes and to the blank. Add 1.0 ml of *alkaline hydroxylamine solution* to each tube. Allow the reaction to proceed for exactly 2 min and add 0.5 ml of a mixture of 1 volume of *water* and 2 volumes of *dilute hydrochloric acid* to each of the reaction tubes. Add 0.5 ml of a 20 per cent w/v solution of *ferric chloride* in *0.2M hydrochloric* acid to each tube, stopper the tubes and shake vigorously to remove bubbles.

Measure the absorbance of each solution at 540 nm using the blank as the compensation liquid. For each reaction solution, subtract the absorbance of the corresponding correction solution. Draw a calibration curve from the corrected absorbance for the five reference solutions and the corresponding content of acetylcholine chloride and read from the curve the content of acetylcholine chloride in the test solution for each volume tested. Calculate the mean of the two values.

1 mol of acetylcholine chloride (181.7 g) is equivalent to 1 mol of O-acetyl (43.05 g).

Free formaldehyde (2.3.20). Maximum 0.2 g/l.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than 85.0 per cent and not greater than 115.0 per cent of the intended amount.

If phenol has been used in the preparation, the content is not more than 2.5 g/l.

Assay

Determine Vi polysaccharide content by a suitable immunochemical method (2.2.14) using a reference purified polysaccharide. The estimated amount of polysaccharide per dose is 80.0 per cent to 120.0 per cent of the content stated on the label. The fiducial limits of error (P = 0.95) of the estimated amount of polysaccharide are not less than 80.0 per cent and not more than 120.0 per cent.

Labelling. The label states (1) the number of micrograms of polysaccharide per human dose (25 µg); (2) the total quantity of polysaccharide in the container.

Typhoid Paratyphoid A Vaccine

Typhoid Paratyphoid A Vaccine is a sterile suspension or a freeze-dried solid prepared from one or more strains of *Salmonella typhi* and *S. paratyphi A* that are smooth and have the full complement of O, H and Vi antigens in S. *typhi strain*, and O and H antigens in S. *paratyphi A* strain. The bacteria are killed by heat or by a bactericide such as phenol or formaldehyde or by a chemical such as acetone. The liquid vaccine and any diluent supplied by the manufacturer with the freeze-dried vaccine contains a suitable preservative. The dried vaccine contains no preservative.

Typhoid Paratyphoid A Vaccine for adults contains not less than 1000 million *S. typhi* and 500 million *S. paratyphi A* organisms per ml. The vaccine meant for children contains 333 million *S. typhi* and 167 million of *S. paratyphi A* organisms per ml.

Description. The liquid or the reconstituted vaccine is a white or pale yellow turbid liquid free from clumps.

Identification

Identify by specific agglutination.

Tests

pH (2.2.24). 6.0 to 7.4.

Other tests. Complies with the tests stated under Vaccines with the following modifications.

Phenol (2.3.36) (*if present*). Not more than 0.5 per cent w/v.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity injecting subcutaneously, intramuscularly or intraperitoneally.

Storage. Store at a temperature 2° to 8°. The liquid vaccine must not be allowed to freeze.

Labelling. The label states (1) the number of organisms per ml; (2) whether the vaccine is meant for use in adults or for use in children; (3) the recommended dose; (4) the name and proportion of any added preservative; (5) that in case of liquid preparation, the vaccine should not be allowed to freeze.

Typhoid Vaccine

Typhoid Vaccine is a sterile suspension of inactivated *Salmonella typhi* containing not less than 5 x 10^8 and not more than 1 x 10^9 bacteria per human dose. The human dose does not exceed 1.0 ml.

Production

The vaccine is prepared using a seed-lot system from a suitable strain of *S. typhi* such as, Ty 2. The final vaccine represents not more than 3 subcultures from the strain on which were made the laboratory and clinical tests that showed it to be suitable. The bacteria are inactivated by *acetone*, by *formaldehyde*, by *phenol* or by heating or by a combination of the last two methods.

The production method is validated to demonstrate that the product, if tested, would comply with the test for abnormal toxicity (2.2.1) modified to the extent that 0.5 ml of the vaccine is injected subcutaneously or intramuscularly or intraperitoneally into each mouse and 1.0 ml into each guinea-pig.

Identification

It is identified by specific agglutination.

Phenol (2.3.36). If phenol has been used in the preparation, the concentration is not more than 0.5 per cent w/v.

Antigenic power. When injected into susceptible laboratory animals, it elicits anti-O, anti-H and, to a lesser extent, anti-Vi agglutinins.

Sterility (2.2.11). Complies with the test for sterility.

Labelling. The label states (1) the method used to inactivate the bacteria; (2) the number of bacteria per human dose.

Typhoid Vaccine (Freeze Dried)

Freeze Dried Typhoid Vaccine is a freeze-dried preparation of inactivated *Salmonella typhi*. The vaccine is reconstituted as stated on the label to give a uniform suspension containing not less than 5 x 10^8 and not more than 1 x 10^9 bacteria per human dose. The human dose does not exceed 1.0 ml of the reconstituted vaccine.

Production

The vaccine is prepared from a seed-lot system from a suitable strain of *S. typhi,* such as Ty 2. The final vaccine represents not more than 3 subcultures from the strain on which were made the laboratory and clinical tests that showed it to be suitable. The bacteria are inactivated either by *acetone* or by *formaldehyde* or by heat. Phenol is not used in the preparation. The vaccine is distributed into sterile containers and freeze-dried to a moisture content favourable to the stability of the vaccine.

The production method is validated to demonstrate that the product, if tested, would comply with test for abnormal toxicity (2.2.1), modified to the extent that 0.5 ml of the vaccine is injected subcutaneously or intramuscularly or intraperitoneally into each mouse and 1.0 ml into each guinea-pig.

Identification

The vaccine reconstituted as stated on the label is identified by specific agglutination.

Antigenic power. When injected into susceptible laboratory animals, the reconstituted vaccine elicits anti-O, anti-H and, to a lesser extent, anti-Vi agglutinins.

Sterility (2.2.11). The reconstituted vaccine complies with the tests for sterility.

Water. Not more than 5.0 percent.

Labelling. The label states (1) the method used to inactivate the bacteria; (2) the number of bacteria per human dose; (3) that the vaccine should be used within 8 hours of reconstitution.

Typhus Vaccine

Typhus Vaccine is a sterile suspension of killed rickettsiae of a strain or strains of epidemic typhus rickettsiae *(Rickettsia prowazeki)* selected for antigenic efficiency.

The vaccine is prepared by injecting virulent rickettsiae into the yolk sacs of fertile eggs which have been incubated for 7 days. Within 9 to 13 days after the injection, the yolk sacs are collected under aseptic conditions and subjected to suitable treatment to liberate the maximum number of rickettsiae. The

material is suspended in *saline solution* or other suitable solution isotonic with blood to which *formaldehyde solution* has been added so that the concentration of formaldehyde is 0.2 per cent to 0.5 per cent. The suspension so formed contains 10 per cent to 15 per cent w/w of yolk sac tissue. It is purified by treatment with ether. The aqueous middle layer of the resultant mixture is collected and distributed under aseptic conditions into sterile containers which are then sealed so as to exclude micro-organisms.

The vaccine may also be prepared from the lungs of small rodents in which rickettsial pneumonias have been caused by inhalation of massive doses of virulent rickettsiae, or from the peritoneal cavities of gerbils which have received intraperitoneal injections of rickettsiae.

Description. A slightly turbid liquid; a white deposit of the rickettsiae may separate on standing which can be readily redistributed on shaking.

Identification

The vaccine specifically protects laboratory animals against epidemic typhus.

Tests

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity injecting subcutaneously, intramuscularly or intraperitoneally.

Potency. Carry out the biological assay of typhus vaccine described below. The vaccine passes the test if the serum of immunised guinea-pigs, when diluted 32-fold, protects mice against the toxin of epidemic typhus rickettsiae.

Biological Assay of Typhus Vaccine

Inject subcutaneously 0.5 ml of the vaccine under examination into each of 10 or more healthy guinea-pigs, each weighing between 400 and 500 g. After 7 days inject once again 0.5 ml of the vaccine into the animals. Fourteen days after the second injection bleed the animals and pool aliquots of the sera. Test the pools against a toxic substance prepared from injected yolk sacs of living fertile eggs as described below.

Incubate for 4 to 10 days after inoculation with epidemic typhus rickettsiae. Harvest the yolk sac and stain a smear by the Macchiavello technique. Use only yolk sac of living embryos showing 4+ or more rickettsiae. Determine the wet weight of the yolk sac, grind the yolk sac with a suitable grade of *aluminium oxide* and suspend in sterile milk (pH adjusted to 7.6) or in a mixture of one part of fresh egg yolk and 99 parts of *sodium chloride injection* using, for each g of the yolk sac, 10 ml of the vehicle. Centrifuge this suspension for 5 minutes at low speed, decant the supernatant liquid and distribute in ampoules for freezing. Dilute a small amount of this suspension 8-, 16-, 32- and 64-fold with *saline solution*. Inject intravenously doses of 0.5 ml of each dilution into 8 white mice, each weighing between 11 and 15 g. 18 hours later, estimate the toxicity of the preparation as the LD_{50} per g of yolk sac, calculated from the numbers of mice killed by each dilution. Those toxic suspensions showing 160 or more LD_{50} per g of yolk sac are satisfactory for use in the neutralisation test. The remainder of the suspension may be sealed in ampoules and preserved for 18 months in liquid nitrogen.

Prepare mixtures of the toxic substance and the sera under test for the neutralisation test in mice and allow to stand for 2 hours before injection. Inject intravenously into mice, each weighing between 11 and 15 g, 0.5 ml containing 2 LD_{50} of the toxic substance together with serial 1 to 2 dilutions of serum. Keep the mice in an incubator at a temperature between 28° and 30° until the results of the test are recorded. Record the deaths at the end of 24 hours and express the results in terms of the highest dilution giving complete protection.

Storage. Store at a temperature 2° to 8°. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for at least 1 year.

Labelling. The label states (1) the nature of the preparation, i.e., whether it has been prepared in eggs, rodent's lungs or otherwise and whether it has been purified; (2) the name and proportion of any added preservative.

Varicella Vaccine, Live

Varicella Vaccine (Live) is a freeze-dried preparation of a suitable attenuated strain of *Herpesvirus varicellae*.

Production

General provisions

The production of vaccine is based on a virus seed-lot system and a cell-bank system. The production method shall have been shown to yield consistently live varicella vaccines of adequate immunogenicity and safety in man. The virus in the final vaccine shall not have been passaged in cell cultures beyond the 38th passage from the original isolated virus.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Substrate for virus propagation

The virus is propagated in human diploid cells (2.7.2).

SEED LOT

The strain of varicella virus shall be identified as being suitable by historical records which shall include information on the

origin of the strain and its subsequent manipulation. The virus shall at no time have been passaged in continuous cell lines. Seed lots are prepared in the same kind of cells as those used for the production of the final vaccine. To avoid the unnecessary use of monkeys in the test for neurovirulence, virus seed lots are prepared in large quantities and stored at temperatures below -20°, if freeze-dried, or below -60°, if not freeze-dried.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The virus concentration of the master and working seed lots is determined as prescribed under Assay to monitor consistency of production.

Extraneous agents (2.7.3). Complies with the requirements for seed lots for live virus vaccines; a sample of 50 ml is taken for the test in cell cultures.

Neurovirulence (2.7.5). Complies with the test for neurovirulence of live virus vaccines.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are handled. Approved animal (but not human) serum may be used in the media. Serum and trypsin used in the preparation of cell suspensions and media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and approved antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production. 5.0 per cent, but not less than 50.0 ml, of the cell cultures employed for vaccine production is set aside as uninfected cell cultures (control cells). The infected cells constituting a single harvest are washed, released from the support surface and pooled. The cell suspension is disrupted by sonication.

Only a virus harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Identification

The virus harvest contains virus that is identified as varicella virus by serum neutralisation in cell culture, using specific antibodies.

Virus concentration. The concentration of infective virus in virus harvests is determined as prescribed under assay to monitor consistency of production and to determine the dilution to be used for the final bulk vaccine.

Extraneous agents (2.7.3). Use 50 ml for the test in cell cultures.

Control cells. The control cells of the production cell culture from which the single harvest is derived comply with a test for identity and with the requirements for extraneous agents (2.7.3).

FINAL BULK VACCINE

Virus harvests that comply with the above tests are pooled and clarified to remove cells. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to prevent contamination and the introduction of moisture.

Only a final lot that is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use. Provided that the test for bovine serum albumin has been carried out with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Identification

When the vaccine reconstituted as stated on the label is mixed with specific *Herpesvirus varicellae* antibodies, it is no longer able to infect susceptible cell cultures.

Tests

Sterility (2.2.11) Complies with the test for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bovine serum albumin. Not more than 0.5 µg per human dose, determined by a suitable immunochemical method (2.2.14).

Water (2.3.43). Not more than 3.0 per cent, determined by the semi-micro determination of water.

Assay

Titrate for infective virus, using at least ten cell cultures for each fourfold dilution or by a technique of equal precision. Use a suitable virus reference preparation to validate each assay. The virus concentration is not less than the minimum stated on the label.

Labelling. The label states (1) the strain of virus used for the preparation of the vaccine; (2) the type and origin of the cells used for the preparation of the vaccine; (3) that contact with disinfectants is to be avoided; (4) the minimum virus concentration; (5) that the vaccine is not to be administered to pregnant women; (6) the time within which the vaccine must be used after reconstitution.

Viper Venom

Daboia Venom

Viper Venom is the dried secretion obtained from the poison glands of *Viperae russelli* and other species of *Viperae* (Fam. Viperidae).

Viper Venom contains not less than 50 Mouse Units per mg.

Production

Immediately after extraction, the poisonous secretion is dried from the frozen state. The dried venom is pooled, mixed, dissolved in ice-cold *water for injection* and then filtered through a bacteria-proof filter to give a stock solution. Further dilutions of the stock solution are made with *water for injection* under aseptic conditions to give solutions with the required number of Mouse Units per ml. These solutions are then distributed in single dose sterile glass containers, dried from the frozen state and sealed at a pressure not exceeding 2.75 kPa.

Description. An almost white or very light yellow, dry powder which when mixed with *water* yields a clear solution with some insoluble residue.

Identification

A. Produces almost immediate coagulation of blood and citrated human plasma.

B. Mix the soluble fraction from at least 0.6 mg with 1 ml of *polyvalent antisnake venom serum* and incubate the mixture at 37° for 30 minutes. Inject 0.5 ml of the mixture intravenously into a group of mice weighing between 18 and 20 g. Observe the animals for 24 hours; no animal dies.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Assay. Carry out the biological assay of snake venom described below:

Biological assay of snake venom

Dissolve a quantity of the freeze-dried venom equivalent to 50 Mouse Units in 25 ml of *saline solution*. Inject 0.5 ml intravenously into each of 10 mice weighing between 18 and 20 g and observe the animals for 24 hours. Not less than 3 and not more than 8 of the mice die in 2 to 24 hours. If the number of deaths is not within this range, change the dilution of the venom suitably.

Express the result in terms of number of Mouse Units per mg.

NOTE — The quantity in mg of the venom which will kill in 2 to 24 hours not less than 3 and not more than 8 mice represents one Mouse Unit.

Storage. Store in single dose, light-resistant containers.

Labelling. The label states (1) the number of Mouse Units per container; (2) the volume of *water for injection* to be used for reconstitution.

Yellow Fever Vaccine (Live)

Yellow Fever Vaccine (Live) is a freeze-dried preparation of the 17D strain of yellow fever virus grown in fertilised hen eggs.

Production

General provisions

The production of vaccine is based on a virus seed-lot system. The production method shall have been shown to yield consistently the yellow fever vaccine (live) of acceptable immunogenicity and safety for man.

The production method is validated to demonstrate that the product, if tested, would comply with the test for safety and efficacy.

Reference preparation. In the test for neurotropism, a suitable batch of vaccine known to have satisfactory properties in man is used as the reference preparation.

Substrate for virus propagation

Virus for the preparation of master and working seed lots and for all vaccine batches is grown in the tissues of chick embryos from a flock free from specified pathogens (2.7.7).

SEED LOT

The 17D strain shall be identified by historical records that include information on the origin of the strain and its subsequent manipulation. Virus seed lots are prepared in large quantities and stored at a temperature below -60°. Master and working seed lots shall not contain any human protein or added serum.

Unless otherwise justified and authorised, the virus in the final vaccine shall be between passage levels 204 and 239

from the original isolate of strain 17D. A working seed lot shall be only one passage from a master seed lot. A working seed lot shall be used without intervening passage as the inoculum for infecting the tissues used in the production of a vaccine lot, so that no vaccine virus is more than one passage from a seed lot that has passed all the safety tests.

Only a virus seed lot that complies with the following requirements may be used for virus propagation.

Identification

The master and working seed lots are identified as containing yellow fever virus by serum neutralisation in cell culture, using specific antibodies.

Extraneous agents (2.7.3). Each working seed lot complies with the test for extraneous agents.

PROPAGATION AND HARVEST

All processing of the fertilised eggs is done under aseptic conditions in an area where no other infectious agents or cells are handled at the same time. Two per cent but not less than twenty and not more than fifty eggs are set aside as uninfected control eggs. After inoculation and incubation at a controlled temperature, only living and typical chick embryos are harvested. The age of the embryo at the time of virus harvest is reckoned from the initial introduction of the egg into the incubator and shall not be more than 12 days. After homogenisation and clarification by centrifugation, the extract of embryonic pulp is tested as described below and kept at -70° or colder until further processing. Virus harvests that comply with the prescribed tests may be pooled. No human protein is added to the virus suspension at any stage during production. If stabilisers are added, they shall have been shown to have no antigenic or sensitising properties for man.

Only a single harvest that complies with the following tests may be used in the preparation of the final bulk vaccine.

Identification

The single harvest contains virus that is identified as yellow fever virus by serum neutralisation in cell culture, using specific antibodies.

Extraneous agents (2.7.3). Complies with the tests for extraneous agents.

Control eggs. Complies with the tests for extraneous agents (2.7.3).

Virus concentration. In order to calculate the dilution for formulation of the final bulk, each single harvest is titrated as described under Assay.

FINAL BULK VACCINE

Single harvests that comply with the tests prescribed above are pooled and clarified again. A test for protein nitrogen content is carried out. A suitable stabiliser may be added and the pooled harvests diluted as appropriate.

Only a final bulk vaccine that complies with the following tests may be used in the preparation of the final lot.

Sterility (2.2.11). Carry out test for sterility using 10 ml of bulk for each sterility medium.

Protein nitrogen content (2.3.30). The protein nitrogen content, before the addition of any stabiliser, is not more than 0.25 mg per human dose.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile, tamper-proof containers and freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to prevent contamination and the introduction of moisture.

Only a final lot that is satisfactory with respect to thermal stability and each of the tests given under Identification, Tests and Assay may be released for use. Provided that the test for ovalbumin has been performed with satisfactory results on the final bulk vaccine, it may be omitted on the final lot.

Thermal stability. Maintain samples of the final lot of freeze-dried vaccine in the dry state at 37° for 14 days. Determine the virus concentration as described under Assay in parallel for the heated vaccine and for unheated vaccine. The difference in the virus concentration between unheated and heated vaccine does not exceed 1.0 $\log_{10}$, and the virus concentration of the heated vaccine is not less than the number of $TCID_{50}$ or plaque-forming units (PFU) equivalent to 1×10^3 mouse LD_{50} per human dose.

Identification

When the vaccine reconstituted as stated on the label is mixed with specific yellow fever virus antibodies, there is a significant reduction in its ability to infect susceptible cell cultures.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Ovalbumin. Not more than 5 µg of ovalbumin per human dose, determined by a suitable immunochemical method (2.2.14).

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity.

Bacterial endotoxins (2.2.3). Not more than 5 IU of bacterial endotoxin per human dose.

Water (2.3.43). Not more than 3.0 per cent, determined by the semi-micro determination of water.

Assay

Titrate for infective virus in cell cultures. Use an appropriate virus reference preparation to validate each assay.

The virus concentration is not less than the equivalent in $TCID_{50}$ or PFU of 1×10^3 mouse LD_{50} per human dose. The relationship between mouse LD_{50} and $TCID_{50}$ or PFU is established by each laboratory and approved by the competent authority.

The method shown below, or another suitable technique, may be used to determine the mouse LD_{50}.

Mouse LD_{50}. The statistically calculated quantity of virus suspension that is expected to produce fatal specific encephalitis in 50 per cent of mice of a highly susceptible strain, 4 to 6 weeks of age, after intracerebral inoculation.

Appropriate serial dilutions of the reconstituted vaccine are made in diluent for yellow fever virus (0.75 per cent solution of *bovine albumin* in *phosphate-buffered saline pH 7.4,* or any other diluent that has been shown to be equivalent for maintaining the infectivity of the virus).

Mice of a highly susceptible strain, 4 to 6 weeks of age, are injected intracerebrally under anaesthesia with 0.03 ml of the vaccine dilution. Groups of not less than eight mice are used for each dilution; the series of dilutions is chosen so as to cover the range 0 to 100.0 per cent mortality of the mice. Injection of the mice is performed immediately after the dilutions have been made. The mice are observed for 21 days and all deaths are recorded. Only survivors and deaths caused by typical yellow fever infections are counted in the computations. Mice paralysed on the twenty-first day of observation are counted as survivors.

Tests in monkeys for Yellow Fever Vaccine

Each master and working seed lot complies with the following tests in monkeys for viraemia (viscerotropism), immunogenicity and neurotropism.

The monkeys shall be *Macaca* spp. susceptible to yellow fever virus and shall have been shown to be non-immune to yellow fever at the time of injecting the seed virus. They shall be healthy and shall not have received previously intracerebral or intraspinal inoculation. Furthermore, they shall not have been inoculated by other routes with neurotropic viruses or with antigens related to yellow fever virus. Not fewer than ten monkeys are used for each test.

Use a test dose of 0.25 ml containing the equivalent of not less than 5000 mouse LD_{50} and not more than 50,000 mouse LD_{50}, determined by a titration for infectious virus and using the established equivalence between virus concentration and mouse LD_{50} (see under Assay). Inject the test dose into one frontal lobe of each monkey under anaesthesia and observe the monkeys for not less than 30 days.

Viraemia (Viscerotropism). Viscerotropism is indicated by the amount of virus present in serum. Take blood from each of the test monkeys on the second, fourth and sixth days after inoculation and prepare serum from each sample. Prepare 1:10, 1:100 and 1:1000 dilutions from each serum and inoculate each dilution into a group of at least six cell culture vessels used for the determination of the virus concentration. The seed lot complies with the test if none of the sera contains more than the equivalent of 500 mouse LD_{50} in 0.03 ml and at most one serum contains more than the equivalent of 100 mouse LD_{50} in 0.03 ml.

Immunogenicity. Take blood from each monkey 30 days after the injection of the test dose and prepare serum from each sample. The seed lot complies with the test if at least 90.0 per cent of the test monkeys are shown to be immune, as determined by examining their sera in the test for neutralisation of yellow fever virus described below.

It has been shown that a low dilution of serum (for example, 1:10) may contain non-specific inhibitors that influence this test; such serum shall be treated to remove inhibitors. Mix dilutions of at least 1: 10, 1:40 and 1: 160 of serum from each monkey with an equal volume of 17D vaccine virus at a dilution that will yield an optimum number of plaques with the titration method used. Incubate the serum-virus mixtures in a water-bath at 37° for 1 h and then cool in iced water; add 0.2 ml of each serum-virus mixture to each of four cell-culture plates and proceed as for the determination of virus concentration. Inoculate similarly ten plates with the same amount of virus plus an equal volume of a 1:10 dilution of monkey serum known to contain no neutralising antibodies to yellow fever virus. At the end of the observation period, compare the mean number of plaques in the plates receiving virus plus non-immune serum with the mean number of plaques in the plates receiving virus plus dilutions of each monkey serum. Not more than 10 per cent of the test monkeys have serum that fails to reduce the number of plaques by 50.0 per cent at the 1:10 dilution.

Neurotropism. Neurotropism is assessed from clinical evidence of encephalitis, from incidence of clinical manifestations and by evaluation of histological lesions, in comparison with ten monkeys injected with the reference preparation. The seed lot is not acceptable if either the onset and duration of the febrile reaction or the clinical signs of encephalitis and pathological findings are such as to indicate a change in the properties of the virus.

Clinical evaluation. The monkeys are examined daily for 30 days by personnel familiar with clinical signs of encephalitis in primates (if necessary, the monkeys are removed from their cage and examined for signs of motor weakness or spasticity). The seed lot is not acceptable if in the monkeys injected with it the incidence of severe signs of encephalitis, such as paralysis or inability to stand when stimulated, or mortality is greater than for the reference vaccine. These and other signs of encephalitis, such as paresis, in-coordination, lethargy, tremors or spasticity are assigned numerical values for the

severity of symptoms by a grading method. Each day each monkey in the test is given a score based on the scale:

Grade 1 — rough coat, not eating,

Grade 2 — high-pitched voice, inactive, slow moving,

Grade 3 — shaky, tremors, unco-ordinated, limb weakness,

Grade 4 — inability to stand, limb paralysis or death (a dead monkey receives a daily score of 4 from the day of death until day 30).

A clinical score for a particular monkey is the average of its daily scores; the clinical score for the seed lot is the mean of the individual monkey scores. The seed lot is not acceptable if the mean of the clinical severity scores for the group of monkeys inoculated with it is significantly greater (P = 0.95) than the mean for the group of monkeys injected with the reference preparation. In addition, special consideration is given to any animal showing unusually severe signs when deciding on the acceptability of the seed lot.

Histological evaluation. Five levels of the brain are examined including :

Block I — the corpus striatum at the level of the optic chiasma,

Block II — the thalamus at the level of the mamillary bodies,

Block III — the mesencephalon at the level of the superior colliculi,

Block IV — the pons and cerebellum at the level of the superior olives,

Block V — the medulla oblongata and cerebellum at the level of the mid-inferior olivary nuclei.

Cervical and lumbar enlargements of the spinal cord are each divided equally into six blocks; 15 µm sections are cut from the tissue blocks embedded in paraffin wax and stained with gallocyanin. Numerical scores are given to each hemisection of the cord and to structures in each hemisection of the brain as listed below. Lesions are scored as follows:

Grade 1. Minimal: 1 to 3 small focal inflammatory infiltrates. Degeneration or loss of a few neurons.

Grade 2. Moderate: 4 or more focal inflammatory infiltrates. Degeneration or loss of neurons affecting not more than one third of cells.

Grade 3. Severe: moderate focal or diffuse inflammatory infiltration. Degeneration or loss of up to two third of the neurons.

Grade 4. Overwhelming: variable but often severe inflammatory reaction. Degeneration or loss of more than 90.0 per cent of neurons.

It has been found that inoculation of yellow fever vaccine into the monkey brain causes histological lesions in different anatomical formations of the central nervous system with varying frequency and severity (I. S. Levenbook *et al., Journal of Biological Standardization,* 1987, 15, 305-313). Based on these two indicators, the anatomical structures can be divided into target, spared and discriminator areas. Target areas are those which show more severe specific lesions in a majority of monkeys irrespective of the degree of neurovirulence of the seed lot. Spared areas are those which show only minimal specific lesions and in a minority of monkeys. Discriminator areas are those where there is a significant increase in the frequency of more severe specific lesions with seed lots having a higher degree of neurovirulence. Discriminator and target areas for *Macaca cynomolgus* and *Macaca rhesus* monkeys are shown in the table below:

Table 1 - The discriminator and target areas for monkey.

Type of monkey	Discriminator areas	Target areas
Macaca cynomolgus	globus pallidus putamen median thalamic nucleus lateral thalamic nucleus	substantia nigra anterior/
Macaca rhesus	caudate nucleus globus pallidus putamen anterior/ median thalamic nucleus lateral thalamic nucleus cervical enlargement lumbar enlargement enlargement	substantia nigra cervical lumbar enlargement

Scores for discriminator and target areas are used for the final evaluation of the seed lot. The individual monkey score is calculated from the sum of individual target area scores in each hemisection divided by the number of areas examined. A separate score is calculated similarly for the discriminator areas.

Mean scores for the test group are calculated in two ways: (1) by dividing the sum of the individual monkey discriminator scores by the number of monkeys and (2) by dividing the sum of the individual monkey target and discriminator scores by the number of monkeys. These two mean scores are taken into account when deciding on the acceptability of the seed lot. The seed lot is not acceptable if either of the mean lesion scores is significantly greater (P = 0.95) than for the reference preparation.

Labelling. The label states (1) the strain of virus used in preparation; (2) that the vaccine has been prepared in chick embryos; (3) the minimum virus concentration; (4) that contact with disinfectants is to be avoided; (5) the period of time within which the vaccine is to be used after reconstitution.

HERBS AND HERBAL PRODUCTS

Herbs and Herbal Products

Herbs and products containing herb(s) have been in trade and commerce and are currently used for a variety of purposes. As a country, India has a rich history of use of herbs, processed herbs and formulations containing herbs both from traditional wisdom as well as cultural usage. Herbs and herbal products are also regulated by various laws. For the purposes of pharmacopoeial standards various considerations have been given. This monograph provides a general outline and policies towards the same.

Crude Herbs

This term means, unless specified otherwise, mainly whole, fragmented or cut, plants, parts of plants, algae, fungi, and lichen in a form which is not processed. Herbs are usually in dried form, but sometimes, when specified, may also be in a fresh form. In specific cases exudates which have not been processed further also are covered under the term herbs. Processing, does not include, normally expected value addition steps like grading, sizing, removal of weeds or parts of plants other than those specified herb and removal of adulterants. The term herbs, though botanically generally refer to plants of specified height and nature, for the purposes of pharmacopoeial reference, shall mean and include plants and parts of plants not necessarily from herbs and shrubs, but cover the entire range namely creepers, climbers, trees etc. Each monograph of a herb in the pharmacopoeia shall specify the botanical scientific name according the binomial system specifying the genus, species, variety and author. In cases where there are controversial botanical identity, as is seen with mainly herbs known in the Indian traditional system, the monograph shall specify the official name of the herb along with its botanical scientific name and guidance is taken from Ayurvedic Pharmacopoiea of India* to decide the same. In cases where, the same herb is available in different grades or sizes, if found appropriate and necessary, separate monographs may be introduced in the pharmacopoeia to cover each of them with appropriate standards. For example- Pippali (large) and Pippali (small).

While deciding to introduce a monograph for a herb in the pharmacopoeia, the criteria that would be kept in mind, but not limited to are – herbs with specific name and a definitive botanical identity up to species, availability and usage in trade and commerce, regulatory interest, knowledge of and availability of a specific chemical compound of well characterised structure [either responsible for the biological activity of the herb (bio-marker) or a chemical compound known to be present in the herb even if not responsible for biological activity(chemical/analytical marker)], availability of a quantitative method for estimation of such a compound, knowledge of safety of the herb, and its sustainability. Herbs which may figure in a regulated list under appropriate forest and other laws, may still be taken up for a monograph for inclusion in pharmacopoeia, if there is knowledge of efforts to cultivate or take care of sustainability issues and /or specific permission is available under law for use of the herb. As already specified under "General Notices" in the pharmacopoeia, appearance of a monograph does not mean its approval as a drug under the law. Monograph of a herb in the pharmacopoeia is to provide qualitative and quantitative standards of quality for the herb for its use either as a food item or food ingredient or food supplement/ nutraceuticals, as a drug, and/or as an ingredient in cosmetics. Each such use would need to comply with applicable regulations. Each herb is regarded as one active substance, irrespective of the knowledge about the active constituents of the herb is available of not.

* Ayurvedic Pharmacopoeia of India, Vol. 1-6, Ministry of Health and Family Welfare, Govt. of India.

Herbs may be exposed to low dose gamma radiation for purposes of reducing their microbial contamination. Herbs treated with low dose gamma radiation shall meet national laws related to such treatment and shall be labeled as per law.

Processed Herbs

Processed herbs means preparations obtained by subjecting herbs to treatment such as extraction, distillation, expression, fractionation, purification, concentration and partial or full fermentation. Processing may also be done by way of powdering herbs, preparing tincture, preparing extract, distilling to get essential oils, fatty oils (either expressed or solvent extracted or a blend of both) expressed juices, extracted exudates, gums and oleo resins, liquid extract where the solvent is evaporated to yield concentrated semi solid mass or dried mass. Extraction may be performed by means of appropriate technology such as infusion, maceration, soxhleting, boiling under ambient or higher pressure, with or without specified enzymes, with or without agitation and combination thereof. Drying of liquid extracts for removal of the solvent may be done by using various appropriate technologies like air drying, sun drying, drying under vacuum or with forced air circulation, drying at low temperature with air circulation, by way of lyophilization or freeze drying. Extracts of herbs may also be prepared by using carbon di-oxide as a solvent-super critical fluid extraction.

Extracts may be liquid extracts and tinctures, semi solid (soft extracts) or solid dry extracts of known consistency obtained from herbs. Standardized extract, a term commonly employed, would for pharmacopoeial purposes, mean an extract adjusted with in an acceptable tolerance to a given content of bio-marker or chemical/ analytical marker. Standardization may be achieved by adjusting the extracts with approved inert material or by blending one or more batches of extracts. Wherever

possible, extracts shall specify the defined range of the constituents (bio-marker or chemical/ analytical marker). Extracts not covered in the above description would be defined by the process of production of the herb to the extract, solvent used and technology applied. The difference between extracts and tinctures would be, in the type of solvent used for extracting a herb, and tincture would normally mean an extract where aqueous-ethanol is used as a solvent for extraction. Dry extracts usually have a loss on drying or water content not greater than 5 per cent w/w, unless specified otherwise in any monograph. It is normal to extrapolate safety aspects and history of use information for extracts as long as the process, solvents, extraction ratios are comparable to the processes used in documented traditional knowledge. Additionally in cases of standardized extracts the inert excipients(s), if any used for standardization or adjustment of the content of constituents should also be declared on the label of such extracts. Extracts shall be free from solvent used for extraction and shall comply with the respective limts as given in Appendix 5.4. Harmful and carcinogenic solvents shall not be used for extraction purposes. Solvents and solvent systems may include use of propylene glycol, glycerin, sorbitol and such other polyhydroxy alcohols, as long as the content of such polyhydroxy alcohol are within safe limit in the final product.

In cases where extraction and fractionation process leads to preparation of an extract, which consists of a single chemical compound of more than at least 70 per cent purity, such extracts shall be treated as an active pharmaceutical ingredient or a food additive or a cosmetic ingredient and would be required to meet relevant laws.

Extracts may also be offered as purified or enriched extracts. Such extract of a herb is processed in such a way to provide higher than normal proportion of the active constituent (s) of the herb as long as the active constituent (s) is/are known. Such purified or enriched extracts may contain additional valuable components which may provide specific properties like enhanced efficacy or stability or solubility and availability of the active constituent (s). Purified and enriched extracts may also be prepared to reduce or remove other specific compound or group of compounds that is scientifically considered undesirable in the herb extracts. Pharmacological, toxicological, pharmaceutical considerations need to be applied while preparing such purified or enriched extracts. Mixed extracts may also be offered which would cover combination of more than one herb extract for purposes of providing simplification or economical way to manufacture herbal formulations.

Herbs may also be extracted using vegetable oils (approved by Food Law) for extraction purposes and such extracts shall specify the oil used for processing.

Approved preservatives or preservatives system may be used during preparation of extracts. The names of such preservatives used which would remain in the final extract shall be listed on the label of such extract, and the proportion of preservatives used shall not exceed normally accepted safe limits of their usage as per relevant laws or pharmacopoeial standards. No artificial colours may be used in extracts of herbs unless and otherwise specified in the specific monograph.Only approved colours shall be used.

Extracts may be exposed to ethylene oxide fumigation or low dose gamma radiation for purposes of reducing their microbial contamination. In cases where they are fumigated, the final extracts exposed shall meet residual levels of ethylene oxide limits as applicable. Herbs treated with low dose gamma radiation shall meet national laws related to such treatment and shall be labelled as per law.

Appearance of a monograph of an extract in the pharmacopoeia does not mean its approval as a drug under the law. Monograph of an extract in the pharmacopoeia is to provide qualitative and quantitative standards of quality for the extract for its use either as a food item or food ingredient or food supplement/ nutraceuticals, as a drug and / or as an ingredient in cosmetics. Each such use would need to comply with applicable regulations. Each extract is regarded as one active substance irrespective of the knowledge about the active constituents of the herb is available or not.

Herbal Formulations

Herbal formulation shall mean a dosage form consisting of one or more herbs or processed herb(s) in specified quantities to provide specific nutritional, cosmetic benefits, and/or other benefits meant for use to diagnose treat, mitigate diseases of human beings or animals and/or to alter the structure or physiology of human beings or animals. Dosage forms commonly employed for food or cosmetic or pharmaceuticals may be employed to formulate one or more herb or processed herbs. Dosage forms known in traditional medicines may also be adopted for preparing herbal formulations, either for external use or for internal administration. Adequate consideration for uniform distribution of herb or processed herbs as well as stability of the same in the dosage form shall be provided during formulation development.

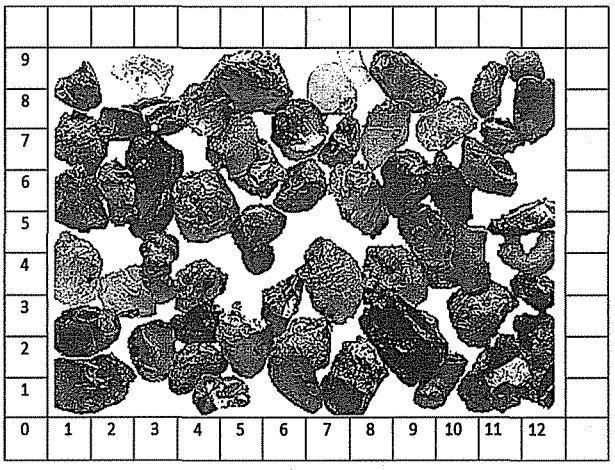
Herbal formulation shall be labelled to comply with relevant labelling requirements under food or drug or cosmetics laws as applicable. Additionally, adequate information shall be provided on label of such formulations to include the name of the herb, parts used, nature and type of extract or processed herb used, extraction ratios, quantity per unit dose or per serving, name (s) of inert excipients used and any preservatives added shall be provided on the label.

Appearance of a monograph of a herbal formulation in the pharmacopoeia does not mean its approval as a drug under

the law. Monograph of a herbal formulation in the pharmacopoeia is to provide qualitative and quantitative standards of quality for the formulation for its use either as a food item or food ingredient or food supplement/ nutraceuticals, as a drug and / or as a cosmetic. Each such use would need to comply with applicable regulations.

Acacia

Gum Acacia; Indian Gum



Acacia is the air-hardened, gummy exudation from the stem and branches of *Acacia nilotica* (Linn.) Del. subsp. *indica* (Benth.) Brenan (syn. *A. arabica* Willd. var. *indica* Benth.) (Fam. Leguminosae), or other species of *Acacia*. It is available as pieces (tears) or in the form of a powder.

Description

Tears — Irregular and broken pieces of varying size, yellowish-white, yellow or amber in colour, with numerous minute fissures; brittle fractured surface, glassy and occasionally iridescent; odourless.

Powder — A white or yellowish-white powder; odourless; on treatment with *water* it dissolves to give a mucilaginous liquid which is colourless or yellowish, dense, viscous, adhesive and translucent.

Identification

A. An aqueous solution is gelatinised by the addition of *lead subacetate solution*.

B. To 5 ml of a 10 per cent w/v solution add gradually, while shaking, 10 ml of *ethanol (95 per cent)*. The cloudy liquid, on addition of 0.5 ml of *acetic acid*, gives a white precipitate. Filter and add to the clear filtrate 50 ml of *ammonium oxalate solution*; the filtrate becomes cloudy.

C. A 10 per cent w/v solution is either dextro-rotatory or slightly laevo-rotatory.

Tests

Sterculia gum and agar. To 50 mg of the powdered substance under examination add 0.2 ml of freshly prepared *ruthenium red solution* and examine microscopically; the particles do not acquire a red colour after irrigation with *water*.

Agar and tragacanth. To 10 ml of a 10 per cent w/v solution add 0.2 ml of *lead acetate solution*; no precipitate is produced.

Starch and dextrin. Boil 10 ml of a 10 per cent w/v solution and cool, add 0.1 ml of *0.05 M iodine*; no blue or brown colour is produced.

Tannins. To 10 ml of a 10 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; a gelatinous precipitate is formed, but neither the precipitate nor the liquid shows a dark blue colour.

Sucrose and fructose. To 1 ml of a 10 per cent w/v solution add 4 ml of *water*, 0.1 g of *resorcinol* and 2 ml of *hydrochloric acid* and heat on a water-bath; no yellow or pink colour develops.

Water-insoluble matter. Dissolve 5 g, in fine powder, in 100 ml of *water* in a 250-ml flask, add 10 ml of *dilute hydrochloric acid* and boil gently for 15 minutes. Filter by suction while hot through a sintered-glass crucible, previously tared, wash thoroughly with hot *water*, dry at 105° and weigh; the residue does not exceed 50 mg.

Microbial contamination (2.2.9). 1.0 g is free from *Escherichia coli*.

Sulphated ash (2.3.18). Not more than 5.0 per cent.

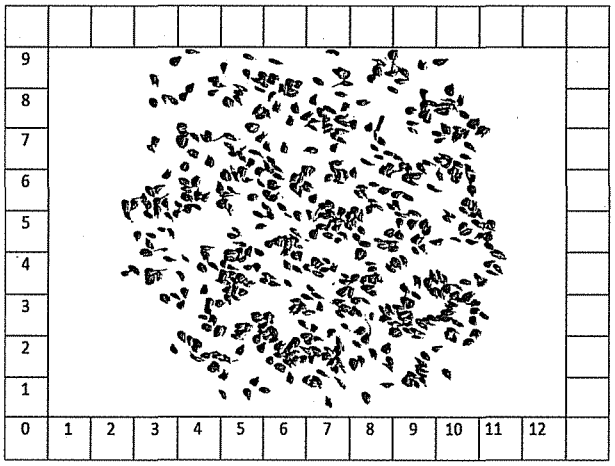
Acid-insoluble ash (2.3.19). Not more than 1.0 per cent, determined on 1.0 g by Method C.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Ajwain

Bishop's weed



Ajwain consists of the dried fruits of *Trachyspermum ammi* Mill. (Fam. Apiaceae).

Ajwain contains not less than 1.0 per cent w/w of thymol, calculated on the dried basis.

Description. The fruits are oval-oblong, greenish brown to yellowish brown in colour. They have an aromatic characteristic odour and the taste is sweet aromatic.

Identification

A. *Macroscopic* — The fruit consists of two portions each called mericarp and connected by central stalk (carpophore). A single seed is seen in each mericarp. Fruit surface is glabrous and the five primary ridges of each mericarp are prominent, straight and pale straw coloured.

B. *Microscopic* — Epicarp is composed of polygonal cells. In the mesocarpic region, reticulate and lignified parenchymas are seen at vascular strands. Tracheids show helical thickening. Endocarp consists of narrow elongated cells having a parquetry arrangement. Polyhedral, thick walled endosperm contains aleurone grains and oil globules. Vittae are six in number, four on the dorsal surface at the mesocarpic region below the secondary ridges and two on the commissural surface of the mericarp. Vittae long, slender composed of thin walled polygonal cells and is lined by an epithelium of small polygonal tubular cells; 10-15 separate, septum transverse or curved.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*..

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate.*

Test solution. To 1 g of the coarsely powdered substance under examination, add 25 ml of *dichloromethane,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *dichlōromethane*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. To 1 g of the *ajwain RS*, add 25 ml of *dichloromethane,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *dichloromethane*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat at 110° for 10 minutes and examine the plate in daylight. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 2.0 per cent.

Water-soluble extractive (2.6.3). Not less than 15.0 per cent by method I.

Total Ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 7.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 10 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh 2.0 g of the coarsely powdered substance under examination, add 50 ml of *methanol,* and reflux on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 100 ml. Dilute with *methanol* to 100.0 ml.

Reference solution. A 0.01 per cent w/v solution of *thymol RS* in *methanol.*

Chromatographic system

- a capillary column 30 m x 0.25 m x 0.25 mm, packed with methyl polysiloxane,
- temperature:
  oven 90° to 260° @10° per minute (initially and finally hold for 5 minutes respectively),
  injector 240°,

detector 280°,
- flow rate. 0.8 ml per minute,
- split flow 20 ml per minute.

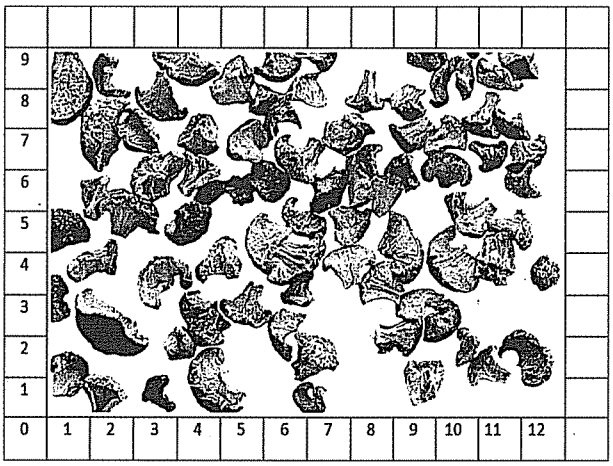
Inject the reference solution and the test solution.

Calculate the content of thymol.

Storage. Store protected from light in well-filled containers, at a temperature not exceeding 30°.

Amalaki

Emblic Myrobalan; Indian Gooseberry



Amalaki consists of the dried fruit pericarp of *Emblica officinalis* Gaertn. (*Phyllanthus emblica* Linn.) (Fam. Euphorbiaceae).

Amalaki contains not less than 1.0 per cent w/w gallic acid calculated on the dried basis.

Description. The dried fruit has a highly shriveled and wrinkled external surface. The taste is sour and astringent followed by delicately sweet tinge.

Identification

A. *Macroscopic* — The dried fruit shows a broad, highly shriveled and wrinkled external convex surface, lateral surface transversely wrinkled, external surface exhibits few whitish specks, occasionally some pieces show a portion of stony testa.

B. *Microscopic* — The epicarpic cells are rectangular in shape and their walls are highly cuticularized. Anomocytic type of stomata is found rarely. Collateral fibrovascular bundles are scattered throughout the inner mesocarp. Pitted and helical tracheids with tapering ends are seen. At places in the phloem, large cavities filled with crystal mass are present.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 20 volumes of *toluene*, 45 volumes of *ethyl acetate*, 20 volumes of *glacial acetic acid* and 5 volumes of *formic acid*.

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Reference solution. Reflux 0.4 g of the coarsely powdered *amalaki RS* with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the place at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 3 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 30 per cent.

Water-soluble extractive (2.6.3). Not less than 40 per cent by Method 1.

Total Ash (2.3.19). Not more than 5.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 0.5 g of coarsely powdered substance under examination, add 50 ml of *water*, sonicate for 3 minutes and heat on a boiling water-bath for 15 minutes, cool and dilute to 100.0 ml with *water* and filter.

Reference solution. A 0.01 per cent w/v solution of *gallic acid RS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: A. a solution prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*,
  B. *acetonitrile*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	100	0
18	55	45
25	20	80
30	100	0

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of gallic acid.

Storage. Store protected from light, heat, moisture and against attack by insects and rodents.

Amla Juice Powder

Amla Juice Powder is obtained by spray drying cold pressed juice of fresh fruits of *Phyllanthus emblica* Linn. (*Emblica officinalis* Gaertn, Fam. Euphorbiaceae)

Amla Juice Powder contains not less than 90.0 per cent w/w and not more than 110 per cent w/w of the stated amount of polyphenols, calculated on the anhydrous basis. It may contain suitable added substances.

Description. A beige to off-white powder.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 15 volumes of *ethyl acetate*, 1.5 volumes of *methanol* and 1 volume of *water*.

Test solution. Dissolve 0.5 g of the extract under examination with 10 ml of *water* and filter.

Reference solution. A 0.01 per cent w/v solution of *gallic acid RS* in *water*.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 10 minutes and examine the plate under 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Water - soluble extractive (2.6.3). Not less than 85.0 per cent by method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Water (2.3.43). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Gallic acid. Not less than 0.1 per cent and not more than 2.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 50 mg of the extract under examination or quantity equivalent to 10 mg of gallic acid in *water*, by frequent shaking and dilute to 50.0 ml with *water* and filter.

Reference solution. A 0.01 per cent w/v solution of *gallic acid RS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded porous silica (5 µm),
- mobile phase: A. 0.1 per cent *formic acid* in *water*,
  B. *methanol*,
- a linear gradient programme using the conditions given below,
- flow rate. 0.7 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
25	95	5
30	0	100
40	0	100
42	95	5

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of the gallic acid in extract.

Assay. Weigh accurately about 2 g of the extract, add 50 ml boiling *water* and heat it on a water-bath for 30 minutes with frequent stirring. Allow the solution to settle and carefully decant it through a piece of cotton wool to a 500-ml volumetric flask. Repeat the extraction for 5 times with 50 ml of boiling *water*. To confirm that all tannins have been extracted, add 3-4 drops of *ferric ammonium sulphate solution* to 5 ml of the extract. Blue colour will not be produced, if all tannins have been extracted. If blue colour develops extract again with 2 times with 50 ml of boiling *water* and check with *ferric ammonium sulphate solution* again. Cool the extracts and make up to the mark with *water*.Take 50 ml into a 250-ml conical flask and add 50 ml of *indigo sulphonic acid solution*, prepared by dissolving 1 g of *indigo carmin* in 50 ml of *sulphuric acid*, add 500 ml of *water* and dilute this solution to 1000.0 ml. Titrate, with *0.1 M potassium permanganate* using *indigo sulphonic acid* as indicator until a golden yellow colour is produced. Carry out a blank titration.

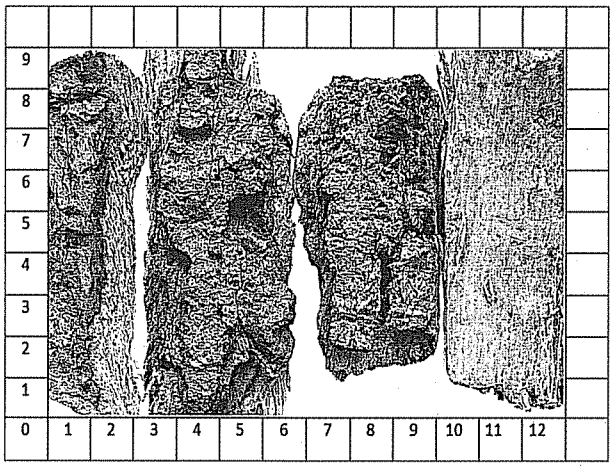
1 ml of *0.1 M potassium permanganate* is equivalent to 0.004157 g of polyphenols calculated as tannic acid.

Usual strength. 40 per cent w/w.

Storage. Store protected from heat and moisture.

Amra

Mango; *Mangifera indica*



Amra consists of dried stem bark of *Mangifera indica* L.(Fam. Anacardiaceae).

Amra contains not less than 1.5 per cent of mangiferin calculated on the dried basis.

Description. The dried stem bark occurs in pieces of variable size and thickness, surface rough. Odour pleasant and taste astringent.

Identification

A. *Macroscopic* — The surface is rough due to longitudinal cracks, fissures and scattered, raised lenticels, greyish to dark brown externally and yellowish-white to reddish internally.

B. *Microscopic* — The mature bark shows a wide cork which has tangentially elongated cells a few outer layers are brown and inner lighter in colour, resin canals and yellow coloured tannin sacs are found in the phloem region, stone cells are thick walled and lignified, prismatic calcium oxalate crystals are present.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 11 volumes of *formic acid*, 11 volumes of *acetic acid* and 25 volumes of *water*.

Test solution. To 5.0 g of the coarsely powdered substance under examination, add 50 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further for three times with 50 ml of *methanol*, cool and filter. Combine all the filtrates and evaporate under vacuum to 10 ml.

Reference solution. Weigh about 2.0 g of *amra RS*, add 50 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further three times with 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 4 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *vanillin glacial acetic acid reagent*. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 10.0 per cent.

Water-soluble extractive (2.6.3). Not less than 10.0 per cent by method I.

Total ash (2.3.19). Not more than 16.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of coarsely powdered substance under examination, add 10 ml of *dimethylformamide*, sonicate for 5 minutes and add 50 ml of *methanol* and boil on a water-bath for 10 minutes, cool and dilute to 100.0 ml with *methanol* and filter. Dilute 5.0 ml of this solution to 50.0 ml with *methanol.*

Reference solution. Dissolve 10 mg of *mangiferin RS* in 10 ml of *dimethylformamide* and dilute to 100.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: filtered and degassed mixture of 15 volumes of *acetonitrile* and 85 volumes of buffer pH 2.8 prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* in 950 ml of *water*, adjust the pH 2.8 with *orthophosphoric acid* and make upto 1000 ml,
- flow rate. 1 ml per minute,
- spectrophotometer set at 365 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

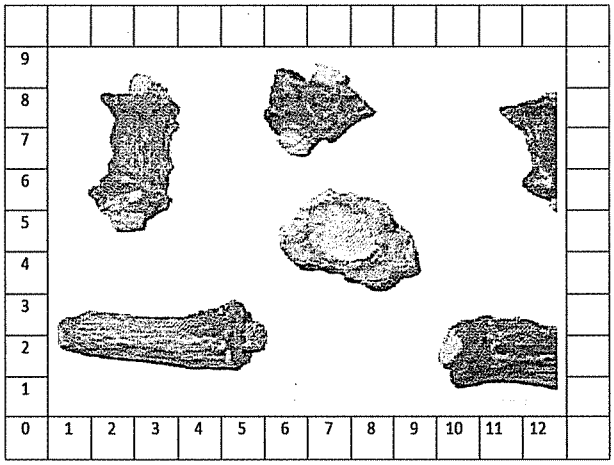
Inject the test solution and the reference solution.

Calculate the content of mangiferin.

Storage. Store protected from moisture and against attack by insects and rodents.

Anantmula

Sariva; Indian Sarsaparilla



Anantmula consists of the dried roots of *Hemidesmus indicus* (Linn) R.Br. (Fam. Asclepiadaceae).

Anantmula contains not less than 0.020 per cent of iso-vanillin, calculated on the dried basis.

Description. The roots are cylindrical, yellowish brown in colour. They have vanillin like odour and the taste is acrid.

Identification

A. *Macroscopic* — Root is 30 cm or more long and from 3 to 6 mm thick, rigid, tortuous, cylindrical, and little branched, consisting of a ligneous center, and a brownish, corky bark, marked with longitudinal furrows and transverse fissures. The odour is aromatic, recalling that of tonqua bean, the taste is aromatic and sweetish. On one side of the root the cork is frequently separated from and raised above the cortex, and is transversely fissured.

B. *Microscopic* — Transverse section of root shows 2-3 layered cork cambium having compressed cells filled with brown contents. Xylem traversed by narrow medullary rays. Pith absent and central region occupied by woody tissues. Vessel shows pitted thickening. The transverse section exhibits numerous laticiferous cells in the secondary cortex. Powder in chloral hydrate solution shows unicellular and branched latex ducts; lignified elements and vessels with pitted thickenings.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF* 254.

Mobile phase. A mixture of 75 volumes of *toluene,* 15 volumes of *ethyl acetate,* 5 volumes of *glacial acetic acid* and 5 volumes of *methanol.*

Test solution. To 2 g of the coarsely powdered substance under examination, add 40 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. To 2 g of the *anantmula RS*, add 40 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat at 105° for 5-10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 8.0 per cent.

Water-soluble extractive (2.6.3). Not less than 12.0 per cent by method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12 per cent, determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of the coarsely powdered substance under examination, add 30 ml of *water,* and reflux on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *water* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 50 ml. Dilute to 50.0 ml with *methanol.*

Reference solution. A 0.002 per cent w/v solution of *iso-vanillin RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent w/v solution of *formic acid* in *water*
  B. *acetonitrile,*
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	90	10
10	70	30
35	10	90
36	90	10
40	90	10

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of iso-vanillin.

Storage. Store protected from heat, moisture and against attack of insects and rodents.

Arachis Oil

Groundnut Oil; Peanut Oil

Arachis Oil is the refined fixed oil obtained from the seed kernels of one or more of the cultivated varieties of *Arachis hypogaea* Linn. (Fam. Leguminosae) and may contain suitable antioxidants as stabilisers.

Description. A clear, colourless or pale yellow oily liquid; odour, faint and nutlike.

Identification

Determine by the thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G.*

Mobile phase. Glacial acetic acid.

Test solution. Dissolve 20 mg (one drop) of the substance under examination in 4 ml of *chloroform.*

Reference solution (a). Dissolve 20 mg (one drop) of arachis oil in 4 ml of *chloroform.*

Reference solution (b). Dissolve 20 mg (one drop) of cottonseed oil in 4 ml of *chloroform.*

Reference solution (c). Dissolve 20 mg (one drop) of sesame oil in 4 ml of *chloroform.*

Impregnate the plate by placing it to a depth of about 5 mm in a tank containing a shallow layer of a mixture of 95 volumes of *light petroleum (60° to 80°)* and 5 volumes of *liquid paraffin,* allowing the solvent to rise at least 14 cm, removing the plate and allowing it to dry in air for 5 minutes; use with the flow of the mobile phase in the direction in which impregnation was carried out.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 110° for 10 minutes, allow to cool and expose it to iodine vapour in a saturated chamber. Remove the plate and allow it to stand for a few minutes until the brown background colour has disappeared. Spray the plate with *starch solution;* blue spots appear which become brown on drying and become blue again on spraying with *water.* The spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with reference solution (a).

Tests

Weight per ml (2.4.29). 0.908 g to 0.920 g.

Refractive index (2.4.27). 1.467 to 1.470.

Alkaline impurities. Mix 10 ml of recently distilled *acetone* and 0.3 ml of *water* in a test-tube, add 0.05 ml of a 0.04 per cent w/v solution of *bromophenol blue in ethanol (95 per cent)* and neutralise the solution, if necessary, with *0.01 M hydrochloric acid* or *0.01 M sodium hydroxide.* Add 10 ml of the substance under examination, shake and allow to stand. Not more than 0.1 ml of *0.01 M hydrochloric acid* is required to change the colour of the upper layer to yellow.

Semi-drying oils. Boil 1 g in a flask under a reflux condenser for 5 minutes with 5 ml of a mixture of 3 volumes of *2 M ethanolic potassium hydroxide* and 1 volume of *ethanol (95 per cent),* add 1.5 ml of *6 M acetic acid* and 50 ml of *ethanol (70 per cent),* warm until the solution is clear. Cool

slowly with a thermometer in the liquid; the temperature at which the solution becomes turbid is not lower than 36°.

Peroxide value (2.3.35). Not more than 5.0.

Acid value (2.3.23). Not more than 0.5.

Iodine value (2.3.28). 85 to 105.

Saponification value (2.3.37). 185 to 196.

Rancidity. Shake 1 ml of a 10 per cent v/v solution in *ether* with 1 ml of *hydrochloric acid,* add 1 ml of a 0.1 per cent w/v solution of *phloroglucinol* in *ether*; no red or pink colour is produced.

Unsaponifiable matter (2.3.39). Not more than 1.0 per cent.

Cottonseed oil. In the Identification test, the chromatogram obtained with the test solution does not correspond to that obtained with reference solution (b)

Sesame oil. In the Identification test, the chromatogram obtained with the test solution does not correspond to that obtained with reference solution (c).

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Arachis Oil intended for use in the manufacture of parenteral preparations complies with the following additional requirement.

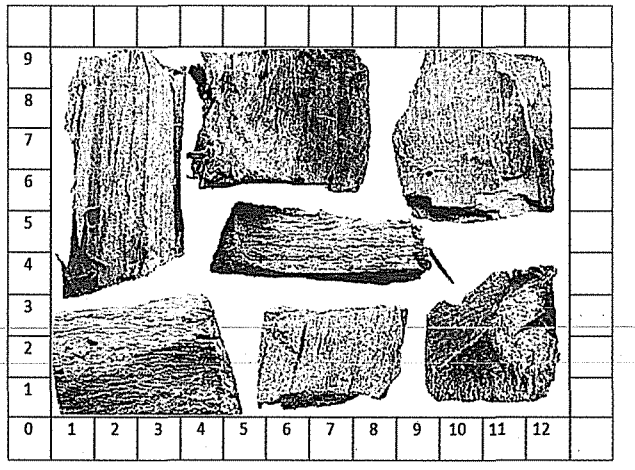
Water (2.3.43). Not more than 0.3 per cent, determined on 3.0 g.

Storage. Store protected from light in a well-filled container.

Labelling. The label states (1) whether the contents are suitable for use in the manufacture of parenteral preparations; (2) when the addition of antioxidants is authorised, the name and quantity of the added antioxidants.

Arjuna

Terminalia arjuna Bark



Arjuna consists of dried stem bark of *Terminalia arjuna* (Roxb) Wight & Arn (Fam. Combretaceae)

Arjuna contains not less than 0.02 per cent of arjungenin calculated on the dried basis.

Description. A flat or minutely curved thick pieces of bark with reddish gray colour and astringent taste.

Identification

A. *Macroscopic* — Stem bark pieces, flat or minutely curved, with reddish gray external surface and darker inner surface. Internal surface has longitudinal minute ridges. Fractures longitudinal.

B. *Microscopic* — Cork consisting of 6-10 layers of elongated cells, phloem broad, medullary rays uniseriate. Calcium oxalate clusters abundant. Few of the parenchyma cells contain colouring matter.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *toluene*, 5 volumes of *ethyl acetate* and 0.5 volume of *acetic acid*.

Test solution. Reflux 2 g of coarsely powdered substance under examination with 50 ml of *chloroform* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *chloroform*. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in 10 ml of *ethanol* at 50° for 10 minutes and filter.

Reference solution. Reflux 1 g of *arjuna RS* with 50 ml of *chloroform* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *chloroform*. Combine the filtrate and concentrate under vacuum to dryness. Dissolve the residue in 5 ml of *ethanol* at 50° for 10 minutes and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with 10 per cent w/v solution of *sulphuric acid* in *methanol*. Heat the plate at 110° for 5 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 20.0 per cent.

Water-soluble extractive (2.6.3). Not less than 20 per cent by method I.

Total ash (2.3.19). Not more than 30.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5 g of coarsely powdered substance under examination with 50 ml of *chloroform* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *chloroform*, cool and filter. Combine the filtrates and concentrate under vacuum to dryness, then extract dried residue with 10 ml of *ethanol* at 50° for 10 minutes and filter.

Reference solution. A 0.1 per cent w/v solution of *arjungenin RS* in *ethanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: A. *acetonitrile (70 per cent)* in *water*,
  B. *acetonitrile (30 per cent)* in *water,*
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume.20 µl

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	30	70
10	50	50
30	70	30
50	30	70

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections for the analyte peak corresponding to arjungenin is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the contents of arjungenin.

Storage. Store protected from light, heat, moisture and against attack by insects and rodents.

Arjuna Dry Extract

Arjuna Dry Extract is obtained by extracting Arjuna (*Terminalia arjuna* Wight and Arn,Fam. Combretaceae) bark with *methanol* or any other suitable solvent and evaporation of solvent.

Arjuna Dry Extract contains not less than 90.0 per cent w/w and not more than 110.0 per cent w/w of stated amount of the arjunolic acid on the dried basis. It may contain suitable added substances.

Description. A light brown to beige powder with or without red tinge.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 92 volumes of *chloroform* and 8 volumes of *methanol.*

Test solution. Dissolve 50 mg of the extract under examination with 50.0 ml of *methanol* and filter.

Reference solution. A 0.1 per cent w/w solution of *arjunolic acid RS* in the *methanol.*

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with *anisaldehyde sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine the plate in ultraviolet light at 365 nm and day light. The chromatographic profile of the test solution is similar to that of the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 35 volumes of *chlorofom,* 10 volumes of *methanol* and 2 volumes of *water.*

Test solution. Dissolve 50 mg of the extract under examination with 10.0 ml of *methanol* and filter.

Reference solution. A 0.05 per cent w/w solution of *arjugenin RS* in the *methanol.*

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with *vanilline sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine the plate in ultraviolet light at 365 nm and day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Ethanol-soluble extractive (2.6.2). Not less than 80.0 per cent.

Total ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the extract under examination containing about 50 mg of arjunolic acid in 50.0 ml of the *methanol*, filter.

Reference solution. A 0.1 per cent w/v solution of *arjunolic acid RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *5 mM α-cyclodextrin* and 65 volumes of *methanol,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

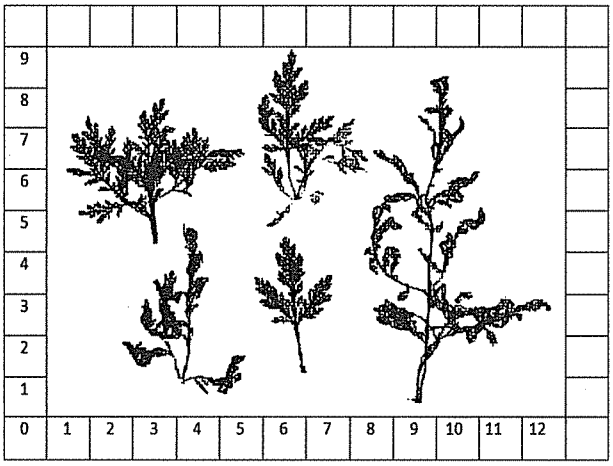
Calculate the content of the arjunolic acid in the extract.

Usual strength. 60 per cent w/w.

Storage. Store protected from heat and moisture.

Artemisia

Artemisia annua



Artemisia consists of dried leaves or the dried leaves and flowering tops of *Artemisia annua* L. (Fam. Asteraceae), known as *Qinghao.*

Artemisia contains not less than 0.8 per cent of artemisinin, calculated on the dried basis.

Description. Slightly camphoraceous odour and bitter in taste.

Identification

A. The leaves grayish green, slightly darker upper surface, glabrous to sparsely hairy, break easily into small fragments, 3-pinnatipartite, petiolate and much variable in size (2.5-10.0 cm long). Leaf lobes narrow, oblong to elliptical with acuminate tip, about 1.0 mm wide. Petioles up to 1.0 cm long, base amplexicaul. Inflorescence panicled raceme. Dry capitula yellowish brown, pedicellate, heterogamous globose to sub-globose or disc shaped, 2.0 mm in diameter, flower heads arranged in lax or drooping, involucral bracts 3- seriate, greenish yellow, glabrous and oblong in shape, measure 1.0-1.2 x 0.5 mm in size. Inner involucral bracts elliptic having a median greenish streak on its outer surface. Ray florets pistillate, 6-8 in number per capitulum and 1.0-1.2 mm long. Disc florets hermaphrodite, 20-36 florets per capitulum and 0.8-1.0 mm long. All florets possess capitate oil glands on the middle of the outer surface that are 54-83 µm in diameter. Stamens 0.7 mm long attached to the corolla base, anther appendages lanceolate to triangular with acuminate tip. Anthers oblong and introrse. Pollen grains tricolpate, rounded 21-33 µm in diameter. In dry condition, capitula become empty because florets/achenes come out of it. Receptacle globular to oblong. Achenes minute; possess striated surface, yellowish brown in colour, 0.7-1.0 mm long, and oval to elliptic in shape. Stomatal index: 8-10-12.

B. The powder of the herb is grayish green to greenish yellow. Examined under a microscope using chloral hydrate solution. The powder shows the following diagnostic characteristics: T-shaped and globular trichomes, epidermal cells with wavy walls, anomocytic to anisocytic stomata, minute druse crystals, tricolpate rounded pollen grains, stone cells, annular vessels, rod shaped palisade cells and stigma with small and club shaped papillae.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 1 volume of *hexane* and 1 volume of *diethyl ether.*

Test solution. Boil 0.1 g of the coarsely powdered substance under examination with 10 ml of *hexane* for 10 minutes and filter. Evaporate the filterate to 1 ml.

Reference solution. A 0.1 per cent w/v solution of *artemisia RS* in *hexane.*

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat the plate at 100° for 15 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Total ash (2.3.19). Not more than 11.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Assay. Determine by high performance thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 1 volume of *hexane* and 1 volume of *diethyl ether*.

Test solution. To 0.1 g of the coarsely powdered substance under examination, add 10 ml of *hexane* and keep for 12 hours, filter. Repeat the process of extraction 3 times. Combine the extracts, evaporate and dissolve in 1.0 ml of *hexane*.

Reference solution. A 0.1 per cent w/v solution of *artemisinin RS* in *hexane*.

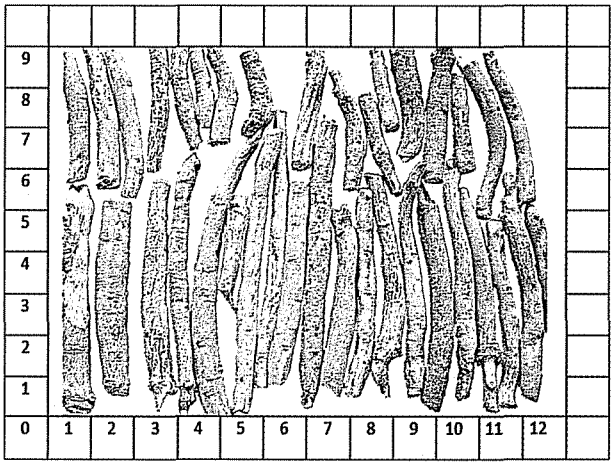
Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with a mixture of 50 volumes of *glacial acetic acid*, 1 volume of *sulphuric acid* and 0.5 volume of *anisaldehyde*. Heat the plate at 100° for 15 minutes, scan the plate in absorbance mode at 540 nm. Record the chromatograms and measure the responses for the analyte peak.

Calculate the content of artemisinin.

Storage. Store protected from light and moisture and against attack by insects and rodents.

Ashwagandha

Indian Ginseng; *Withania somnifera*



Ashwagandha consists of the dried mature roots of *Withania somnifera* (L.) Dunal (Fam. Solanaceae).

Ashwagandha contains not less than 0.02 per cent w/w of total withanolide (sum of withanolide glycosides and withanolide aglycones), calculated on the dried basis.

Description. Buff to greyish-yellow roots. Taste, slightly mucilaginous, bitter and acrid.

Identification

A. *Macroscopic* — Primary roots are straight, conical or finger like in shape, variable in thickness with the age. Secondary roots are thin and fibrous. Surface buff to greyish-yellow with longitudinal wrinkles.

B. *Microscopic* — Vessels with bordered pits and horizontal perforations. Fibres aseptate with pointed ends. Wood elements lignified. Starch grains abundant, simple, mostly spherical, reniform – oval with central hilum. Microcrystals in parenchyma cells.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 9 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. Reflux 3 g of coarsely powdered substance under examination with 50 ml *methanol* for 15 minutes, cool and filter.

Reference solution. Reflux 0.6 g of coarsely powdered *ashwagandha RS* with 10 ml *methanol* for 15 minutes, cool and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° for 5-10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 10.0 per cent.

Water-soluble extractive (2.6.3). Not less than 15 per cent by Method I.

Total ash (2.3.19). Not more than 7.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.2 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 5 g of the coarsely powdered substance under examination with 50 ml of *acetonitrile* on a water-bath for 15 minutes, cool and filter. Reflux the residue

further with *acetonitrile* till the last extract turns colourless, cool and filter. Combine all the filtrates and concentrate to 50.0 ml.

Reference solution. A solution containing 0.01 per cent w/v each of *withanolide A RS* and *withanoside IV RS* in *acetonitrile,* prepared by heating gently on a water-bath.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 900 ml *water*, adjust pH to 2.8 with *dilute phosphoric acid* and diluting it to 1000 ml with *water*,
  B. *acetonitrile,*
- a linear gradient programme using the conditons given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 227nm,
- injection volume.20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
18	55	45
25	20	80
27	95	5
37	95	5

Inject the reference solution.The relative retention time of *withanolide A* is 1.0 and *withanoside IV* is about 0.7.The test is not valid unless the relative standard deviation for the replicate injections for both the analyte peaks is not more than 2.0 per cent and resolution is not less than 2.0. The relative retention time of *withanolide glycosides* and *withanolide aglycones* are as follows.

Withanolide glycoside

Withanoside IV	0.7
Withanoside V and VI	0.89

Withanolide aglycones

12, deoxywithastramonolide	0.96
Withanolide A	1.0
Withanolide B	1.14
Withanone	1.01

Inject the reference solution and the test solution.

Calculate the contents of *withanolide glycosides* in the sample as withanoside IV by summing the peak areas of *withanoside IV, V* and *VI.* Calulate the content of withanolide aglycones in the sample as withanolide A by summing the peak areas of *12, deoxywithastramonolide, withanolide A, withanolide B* and *withanone.*Sum the content of *withanolide glycosides* and *withanolide aglycones* to get total withanolides.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Ashwagandha Dry Extract

Ashwagandha Dry Extract is obtained by extracting Ashwagandha (*Withania somnifera* (L.) Dunal, Fam. Solanaceae) roots with *methanol* or any other suitable solvent and evaporation of solvent.

Ashwagandha Dry Extract contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of withaferin A, not less than 85.0 per cent and not more than 110.0 per cent w/w of the total withanolides calculated as the sum of free withanolides and glycowithanolides calculated on the dried basis. The extract also contains not less than 1.0 per cent of alkaloids, calculated on the dried basis.

Description. A pale brown to light brown powder.

Identification

Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 5 volumes of *toluene*, 5 volumes of *ethyl acetate* and 1 volume of *formic acid.*

Test solution. Dissolve about 0.2 g of the extract under examination with 10 ml of *methanol* and filter.

Reference solution. A 0.05 per cent w/v solution of *withaferin A RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with *anisaldehyde-sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine the plate at 365 nm and under day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Water-soluble extractive (2.6.3). Not less than 70.0 per cent by method I.

Sulphated ash (2.3.18). Not more than 10.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay

A. Total alkaloids

Test solution. Shake about 20 g of the extract under examination with 400 ml of a solution containing 4 volumes of *ether* and 1 volume of *ethanol.* Add 20 ml of 5 per cent v/v *ammonia solution* into 1000-ml conical flask and shake for one hour. Decant and filter through cotton.

Transfer the filtrate into a separator. Wash the residue with a mixture containing 80 volumes of *ether* and 20 volumes of *ethanol.* To the filtrate add 100 ml of *0.5 M sulphuric acid.* Collect the lower layer into another separator. To the ether layer; add 100 ml of a mixture containing 80 volumes of *0.25 M sulphuric acid* and 20 volumes of *ethanol* and extract. Continue the extraction 3 times with 80 ml of the *0.25 M sulphuric acid* and a mixture containing 80 volumes of *ether* and 20 volumes of *ethanol* until aqueous layer is colourless.

Combine the acid solution and wash with 40 ml of *chloroform* followed by 2 times with 20 ml of *chloroform.* Wash, the combined chloroform layer, with acid alcohol mixture. Discard the chloroform layer. Combine the acid alcohol solutions and make it alkaline with 5 per cent v/v *ammonia solution* and add 10 ml excess. Extract 3 times with 100 ml of *chloroform.* Add 2 ml of *0.1 M hydrochloric acid* to 0.5 ml of the extract, remove the *chloroform* by evaporation, transfer the aqueous residue to a test tube and add 0.05 ml of *potassium mercury-iodide solution*; not more than a very faint opalescence is produced. If the opalescence is more, the extraction is not complete. Extract further 2 times with 100 ml of *chloroform.*

Combine the chloroform extract and wash with 20 ml of *distilled water.* Filter the chloroform layer through cotton plug into a tared beaker. Wash the residue with a little chloroform, transferring the chloroform layer to the same tared beaker. Evaporate on a water-bath. Add 5 ml of *alcohol* to the residue and dry to constant weight at 105°. Finally weigh the residue and calculate the content of total alkaloids.

B. Total withanolides. Shake about 5 g of dry extract with 25 ml of *methanol* and 25 ml of *water* in a separating funnel. It is defatted by extraction 4 times with 50 ml of *hexane.* This fraction is discarded. The remaining *aqueous methanolic solution* is extracted with *hexane* and then with 5 times with 25 ml of *ether.* The ether extracts are combined, washed twice with *water* and dried under vacuum to give the free withanolides content. Finally weigh the residue and calculate the content of free withanolides.

C. Glycowithanolides. The above remaining *aqueous methanolic solution* is extracted 3 times with 2 volumes of *chloroform* and 1 volumes of *methanol.* The chloroform-methanol extracts are combined and evaporated on water-bath and dried under vacuum at 80° to constant weight. Finally weigh the residue and calculate the content of glycowithanolides.

Calculate the content of total withanolides as the sum of free withanolides and glycowithanolides.

D.Withaferin A

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the extract under examination containing about 25 mg of withaferin A in 25.0 ml of *water*, filter.

Reference solution. A 0.1 per cent w/v solution of *withaferin A RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (10 µm),
- mobile phase: a mixture of 65 volumes of 0.1 per cent v/v solution of *orthophosphoric acid* and 35 volumes of *acetonitrile,*
- flow rate. 1ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

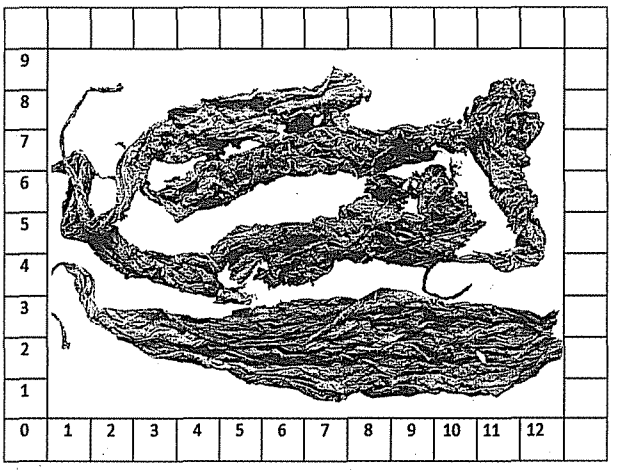
Inject the test solution and the reference solution.

Calculate the content of the withaferin A in the extract.

Usual strength. 2.5 per cent withaferin A and 7.5 to 10 per cent w/w of total withanolides and glycowithanolides.

Storage. Store protected from heat and moisture.

Belladonna Leaf



Belladonna Leaf consists of the dried leaf and flowering tops of *Atropa belladonna Linn.* or of *A. acuminata* Royle ex Lindley (Fam. Solanaceae) or a mixture of both species.

Belladonna Herb contains not less than 0.30 per cent of total alkaloids, calculated as hyoscyamine with reference to the material dried at 100° to 105°.

Description. Green to greenish-brown leaves, slightly darker on the upper surface, often crumpled and rolled and partly matted together in the drug. When whole, the lamina is 5 to 25 cm long and 3 to 12 cm wide, elliptical to ovate; acuminate at the apex, narrowing at the base; margin entire. Petiole 0.5 to 4 cm in length. The young leaves are highly pubescent, the older leaves are slightly pubescent along the veins. In the flowering tops, the stems are hollow and flattened, with leaves in pairs of unequal size, in the axils of which are single flowers with campanulate corolla, about 2 cm long and 1.5 cm wide, purple or yellow-brown in colour, with five short, reflexed lobes, five epipetalous stamens and one bilocular ovary with numerous ovules.

Identification

A. When examined under a microscope it shows epidermal cells with sinuate anticlinal walls and cuticle which is often striated and furrowed. Covering and glandular hairs infrequent, though more frequent in the young leaves and around the veins; covering hairs, multicellular, uniseriate, with thin smooth walls; glandular hairs; short clavate glands with multicellular heads and glands with a long uniseriate stalk and ovoid unicelluar head. Stomata, anisocytic, more frequent on the lower epidermis. The midrib is characterised by an open arc of vascular bundles with isolated groups of perimedullary phloem. Mesophyll dorsiventral with a single palisade layer. Throughout the parenchyma and particularly just below the palisade layer are cells containing microsphenoidal crystals of calcium oxalate or, very rarely, cluster crystals. The stems show pericyclic fibres and perimedullary bundles of phloem, few trichomes; the cortical parenchymatous cells and the pith cells contain microsphenoidal crystals of calcium oxalate.

B. Powder 1 g and shake for 2 minutes with 10 ml of *0.05 M sulphuric acid.* Filter and add to the filtrate 1 ml of *strong ammonia solution* and 5 ml of *water*. Extract with 15 ml of *ether,* taking care to prevent the formation of an emulsion. Dry the ether extract over *anhydrous sodium sulphate* and filter. Evaporate the filtrate to dryness, add 0.5 ml of *fuming nitric acid* and evaporate to dryness. Add 10 ml of *acetone* and, dropwise, a 3 per cent w/v solution of *potassium hydroxide* in *ethanol (95 per cent);* a deep violet colour develops.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 90 volumes of *acetone*, 7 volumes of *water* and 3 volumes of *strong ammonia solution.*

Test solution. Add 15 ml of *0.05 M sulphuric acid* to 0.6 g of the material under examination, in fine powder, shake for 15 minutes, filter and wash the filter with *0.05 M sulphuric acid* until 20 ml of filtrate is obtained; add 1 ml of *strong ammonia solution* to the filtrate, extract with two quantities, each of 10 ml, of *peroxide-free ether*, separate the ether layer, by centrifugation if necessary, dry the combined ether extracts over *anhydrous sodium sulphate*, filter, evaporate to dryness on a water-bath and dissolve the residue in 0.5 ml of *methanol.*

Reference solution. Add 15 ml of *0.05 M sulphuric acid* to 0.6 g of the *belladonna leaf RS*, shake for 15 minutes, filter and wash the filter with *0.05 M sulphuric acid* until 20 ml of filtrate is obtained, add 1 ml of *strong ammonia solution* to the filtrate, extract with two quantities, each of 10 ml, of *peroxide-free ether*, separate the ether layer, by centrifuging if necessary, dry the combined ether extracts over *anhydrous sodium sulphate*, filter, evaporate to dryness on a water-bath and dissolve the residue in 0.5 ml of *methanol.* or

Dissolve 50 mg of *hyoscyamine sulphate* in 9 ml of *methanol* (solution A) and dissolve 15 mg of *hyoscine hydrobromide* in 10 ml of *methanol* (solution B), mix 8 ml of solution A with 1.8 ml of solution B.

Apply to the plate 10 µl and 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate at 105° for 15 minutes, allow to cool and examine in ultraviolet light at 254 nm and 365 nm, spray with 10 ml of *modified potassium iodobismuthate solution* until the bands become visible as orange or brown on a yellow background. The bands in the chromatogram obtained with test solution have similar Rf values to those in the chromatogram obtained with reference solution (hyoscyamine in the lower third of the chromatogram; hyoscine in the upper third) and are similar in colour and atleast equal in size. Faint secondary bands may appear, particularly in the middle of the chromatogram obtained with 20 µl of the test solution or near the line of application in the chromatogram obtained with 10 µl of test solution. Spray the plate with a freshly prepared 10 per cent w/v solution of *sodium nitrite* until transparent and examine after 15 minutes. The colours due to hyoscyamine in the chromatogram change from brown to reddish-brown but not to greyish-blue (atropine); any secondary bands are no longer visible.

Tests

Foreign organic matter (2.6.1). Not more than 3 per cent.

Acid-insoluble ash (2.3.19). Not more than 3 per cent.

Assay. Powder 50 g and determine the loss on drying (2.4.19), by drying 2 g, accurately weighed, in an oven at 105°. From the remaining sample, weigh accurately about 10 g, moisten with a mixture of 5 ml of *dilute ammonia solution*, 10 ml of *ethanol (95 per cent)* and 30 ml of *ether* and mix thoroughly. Transfer the mixture to a percolator with the aid of an extracting solvent mixture consisting of 3 volumes of *ether* and 1 volume of *chloroform*. Allow to macerate for 4 hours and percolate

with the solvent mixture until complete extraction of the alkaloids is effected, (2.6.4).

Concentrate the percolate to about 50 ml by distilling off the solvent mixture on a water-bath, and transfer to a separator, previously rinsed with *ether*. Add a quantity of *ether* at least equal to 2.1 times the volume of the percolate and extract with three quantities, each of 20 ml, of *0.5 M sulphuric acid*. Transfer each acid extract to another separating funnel. Combine the acid extracts, make the solution alkaline with *dilute ammonia solution* and extract with *chloroform* until complete extraction of the alkaloids has been effected. Wash the combined chloroform extracts with 10 ml *water*, discard the *water*, evaporate the chloroform layer to dryness and heat the residue for 15 minutes on a water-bath. Redissolve the residue in successive small quantities of *chloroform*, evaporating to dryness on a water-bath each time before adding the solvent. Heat for 15 minutes on a water-bath and dissolve the residue in 5 ml of *chloroform*. Add 20.0 ml of *0.01 M sulphuric acid*, remove the chloroform by evaporation on a water-bath and titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.01 M sulphuric acid* is equivalent to 0.005788 g of total alkaloids calculated as hyoscyamine. Calculate the content of total alkaloids with reference to the dried material.

Storage. Store protected from light and moisture.

Belladonna Dry Extract

Belladonna Dry Extract is obtained from the dried leaf and flowering top of *Atropa belladonna Linn or of A. acuminata* Royle ex Lindley (Fam. Solanaceae) by extraction with ethanol or any other suitable solvent.

Belladonna Dry Extract contains not less than 0.95 per cent and not more than 1.05 per cent w/w of alkaloids, calculated as hyoscyamine.

Tests

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Assay. Weigh accurately about 3 g and wash into a separating funnel with 12 ml of a mixture of equal volumes of *ethanol (95 per cent)* and *water*, shake-well and frequently for 30 minutes, add 2 ml of *dilute ammonia solution* and 25 ml of *chloroform*. Shake well, allow to separate and filter the chloroform layer into a second separating funnel through a plug of absorbent cotton moistened with *chloroform*. Continue the extraction with further quantities, each of 25 ml, of *chloroform* until complete extraction of the alkaloids is effected (2.6.4), running each chloroform solution through the same plug of absorbent cotton. Extract the combined chloroform solutions with successive quantities of a mixture of 3 volumes of *0.1 M sulphuric acid* and 1 volume of *ethanol (95 per cent)* until complete extraction of the alkaloids is effected, filtering each extract through a plug of absorbent cotton previously moistened with *water*. Wash the mixed acid solutions with 10, 5 and 5 ml of *chloroform*, extracting each chloroform solution with the same 20 ml of *0.05 M sulphuric acid* and discard the chloroform. Combine the acid solutions, neutralise with *dilute ammonia solution*, add 5 ml in excess and shake with successive quantities, each of 25 ml, of *chloroform* until complete extraction of the alkaloids is effected, washing each chloroform solution with the same 10 ml of *water* and filtering into a flask through a plug of cotton wool previously moistened with *chloroform*. Distil most of the *chloroform* from the combined extracts and transfer the remainder of the chloroform to a shallow open dish. Evaporate the remainder of the *chloroform* without the aid of a current of air, heat the residue in an oven at 100° for 15 minutes, dissolve in a little *chloroform*, evaporate to dryness without the aid of a current of air and again heat in an oven at 100° for 15 minutes. Dissolve the residue in 2 ml of *chloroform*, add 5.0 ml of *0.025 M sulphuric acid*, warm to remove the *chloroform*, cool and titrate the excess of acid with *0.05 M sodium hydroxide* using *methyl red solution* as indicator.

1 ml of *0.025 M sulphuric acid* is equivalent to 0.01447 g of alkaloids, calculated as hyoscyamine.

Storage. Store in small, wide-mouthed, tightly-closed containers in a cool place.

Belladonna Tincture

Belladonna Tincture obtained from *Belladonna leaf* or roots of one or more of the cultivated varieties of *Atropa belladonna Linn.* or *A. acuminata* Royle ex Lindley (Fam. Solanaceae) or a mixture of both species.

Belladonna Tincture contains not less than 90 per cent w/w and not more than 110 per cent w/w of total alkaloids, calculated as hyoscyamine, $C_{17}H_{23}NO_3$.

Description. A clear green or brownish green liquid.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 10 volumes of *anhydrous formic acid*, 10 volumes of *water*, 30 volumes of *methyl ethyl ketone* and 50 volumes of *ethyl acetate*.

Test solution. Evaporate 10 ml of the tincture under examination in a water-bath at 40° under reduced pressure. Dissolve the residue in 1.0 ml of the *methanol*.

Reference solution. Dissolve 1 mg of *chlorogenic acid RS* and 2.5 mg of *rutin RS* in 10 ml of the *methanol.*

Apply to the plate 40 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 15 cm. Dry the plate in air, heat the plate at 110° for 10 minutes. Spray the warm plate with 1 per cent v/v solution of *diphenylboric acid aminoethyl ester* in *methanol.* Allow to cool and spray with 5 per cent v/v solution of *macrogol 400* in *methanol,* allow the plate to dry in air for 30 minutes and examine in ultraviolet light at 365 nm. The chromatographic profile of the test solution is similar to that of the reference solution.

B. **Atropine**. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase . A mixture of 3 volumes of *strong ammonia solution*, 7 volumes of *water* and 90 volumes of *acetone.*

Test solution. To 15.0 ml of the tincture under examination, add 15 ml of *0.05 M sulphuric acid* and filter. Add 1 ml of *strong ammonia solution* to the filtrate, with two quantities, each of 10 ml, of *peroxide-free ether*, separate the ether layer by centrifugation if necessary, dry the combined ether extracts over *anhydrous sodium sulphate*, filter and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 ml of *methanol.*

Reference solution. Dissolve 50 mg of *hyoscyamine sulphate* in 9 ml of *methanol* and 15 mg of *hyoscine hydrobromide* in 10 ml of *methanol,* separately. Mix 1.8 ml of the *hyoscine hydrobromide solution* and 8 ml of the h*yoscyamine sulphate solution.*

Apply to the plate 20 µl and 40 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 10 cm. Dry the plate at 105° for 15 minutes, spray with *potassium iodobismuthate solution*, dry the plate and spray with *sodium nitrite solution* until the plate is transparent. Examine the plate after 15 minutes in day light. The chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Ethanol (2.3.45). 64 to 69 per cent v/v by method III.

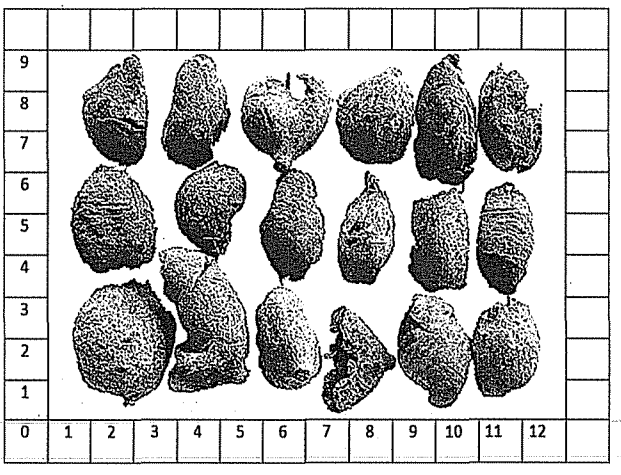
Assay. Evaporate 50.0 g of the tincture under examination to a volume of about 10 ml. Transfer quantitatively to a separating funnel, with the minimum volume of *ethanol (70 per cent v/v)*. Add 5 ml of strong *ammonia solution* and 15 ml of *water.* Extract with three quantities, each of 40 ml of a mixture of 1 volume of *dichloromethane* and 3 volumes of *peroxide-free ether*, carefully to avoid emulsion, until the alkaloids are completely extracted. Combine the *dichloromethane* and *ether* extracts, concentrate the solution to a volume of about 50 ml by heating on a water-bath. Transfer the resulting solution quantitatively to a separating funnel, rinsing with *peroxide-free ether.* Add a quantity of *peroxide-free ether* equal to at least 2.1 times the volume of the solution to produce a layer having a density well below that of water. Extract the resulting solution with minimum of three quantities, each of 20 ml of *0.25 M sulphuric acid* until the alkaloids are completely extracted. Separate the layers by centrifugation if necessary and transfer the layers to a separating funnel. Make the combined layers alkaline with *strong ammonia solution* and extract with minimum of three quantities, each of 30 ml of *dichloromethane* until the alkaloids are completely extracted. Combine the *dichloromrthane* and *ether* extracts, add 4 g of *anhydrous sodium sulphate* and allow to stand for 30 minutes with occasional shaking. Decant the *dichloromethane* and filter. Wash the *sodium sulphate* with three quantities, each of 10 ml of *dichloromethane.* Combine the *dichloromethane* and *ether* extracts, evaporate to dryness on a water-bath. Heat the residue in an oven at 105° for 15 minutes. Dissolve the residue in a few ml of *dichloromethane*, evaporate to dryness on a water-bath and heat the residue in an oven at 105° for 15 minutes again. Dissolve the residue in a few ml of *dichloromethane.* Add 20.0 ml of *0.01 M sulphuric acid* and remove the *dichloromethane* by evaporation on a water-bath. Titrate the excess of acid with *0.02 M sodium hydroxide* using *methyl red mixed solution* as indicator.

1 ml of *0.01 M sulphuric acid* is equivalent to 0.005788 g of total alkaloids calculated as hyoscyamine. Calculate the content of total alkaloids with reference to the dried material.

Usual strength. 0.03 per cent w/w.

Bhibhitaki

Belliric Myrobalan; *Terminalia bellirica*



Bhibhitaki consists of the dried fruit pericarp of *Terminalia bellirica* (Gaertn.) Roxb. (Fam. Combretaceae).

Bhibhitaki contains not less than 0.3 per cent w/w of ellagic acid and 0.75 per cent w/w of gallic acid, calculated on the dried basis.

Description. The dried pericarp appears as curved pieces of irregular shapes, the external surface is velvety, wrinkled grey to brown in colour and has astringent taste

Identification

A. *Macroscopic* – The dried pericarp of the ripe fruit occurs as curved pieces of irregular shapes with convex external surface. The external surface appears velvety, slightly wrinkled grey to brown in colour. Internal surface is pale yellow. The cut surface is with occasional projecting threads, representing the vascular bundles.

B. *Microscopic* – The cells of the epidermis has a characteristic and a slightly bulged based with a hair like prolongation. Several vascular strands traverse the mesocarp in various directions. Peripheral layers of mesocarp have tangentially elongated cells, devoid of starch grains, containing rosettes of calcium oxalate crystals and few small stone cells.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 20 volumes of *toulene*, 45 volumes of *ethyl acetate* and 20 volumes of *glacial acetic acid* and 5 volumes of *formic acid.*

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Reference solution. Reflux 0.4 g of the coarsely powdered *bhibhitaki RS* with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 25 per cent.

Water-soluble extractive (2.6.3). Not less than 35 per cent by Method 1.

Total ash (2.3.19). Not more than 8 per cent.

Acid-insoluble ash (2.3.19). Not more than 2 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 0.5 g of coarsely powdered substance under examination, add 50 ml of *water*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with *water* and filter.

Reference solution. A solution containing 0.01 per cent w/v each of *gallic acid RS* and *ellagic RS* in *water.*

NOTE — Use freshly prepared solution and protected from light.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a gradient mixtures of *acetonitrile* and a buffer solution prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and make upto 1000 ml with *water,*

  B. *acetonitrile*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	95	5
18	65	35
25	45	55
30	95	5

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of *gallic acid* and *ellagic acid.*

Storage. Store protected from light, heat, moisture and against attack by insects and rodents.

Bhibhitaki Aqueous Extract

Bhibhitaki Aqueous Extract is obtained by extracting Bhibhitaki (*Terminalia bellirica* (Gaertn.) Roxb., Fam. Combretaceae) fruit with *water.*

Bhibhitaki Aqueous Extract contains not less than 90 per cent w/w and not more than 120.0 per cent w/w of the stated amount of gallic acid and ellagic acid.

Description. A light brown to dark brown powder.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.F 254.*

Mobile phase. A mixture of 35 volumes of *benzene*, 10 volumes of *methanol*, 4 volumes of *acetone* and 1 volume of *water.*

Test solution. Dissolve 0.5 g of the extract under examination in 20 ml of *methanol* under reflux at 80° on a water bath and filter.

Reference solution. A 0.01 per cent w/v solution of *gallic acid RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Acid insoluble ash (2.3.19). Not more than 4.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss of drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.2 g of the extract under examination or a quantity containing 10 mg of gallic acid and 1 mg of ellagic acid in 80 ml of *methanol* and dilute to 100.0 ml with *water* and filter.

Reference solution (a). A 0.01 per cent w/v solution of *gallic acid RS* in 80 per cent v/v *methanol* in *water.*

Reference solution (b). A 0.001 per cent w/v solution of *ellagic acid RS* in 80 per cent v/v *methanol* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A mixture of *acetonitrile* and a 0.1 per cent v/v *orthophosphoric acid* in water,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 252 nm for ellagic acid and at 271 nm for gallic acid,
- injection volume 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-8	95	5
8-25	95→ 0	5 →100
25-30	0	100
30-32	0 → 95	5 →5

Inject reference solution (a) and (b). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution, reference solution (a) and (b).

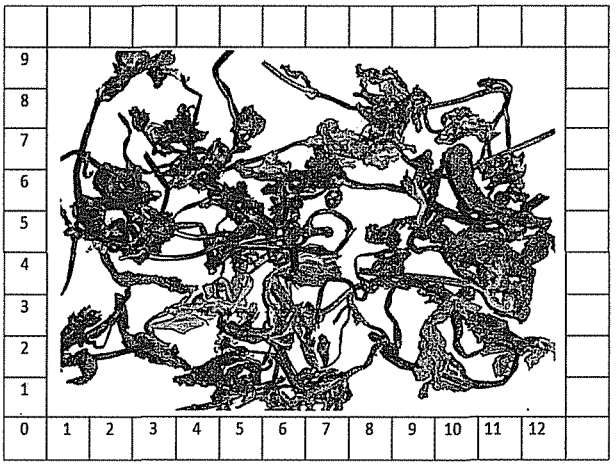
Calculate the content of gallic acid and ellagic acid in the extract

Usual strength. 7 per cent w/w.

Storage. Store protected from heat and moisture.

Bhringraj

Eclipta alba



Bhringraj consists of the dried whole plant of *Eclipta alba* (L.) Hassk. (Fam. Asteraceae).

Bhringraj contains not less than 0.1 per cent of wedelolactone, calculated on the dried basis.

Description. A green to greenish brown colour when completely dry.

Identification

A. *Macroscopic* — Root. Well developed, a number of secondary branches arise from main root up to about 7 mm in dia, cylindrical, greyish.

Stem. Herbaceous, branched occasionally rooting at nodes, cylindrical or flat, rough due to oppressed white hairs, node distinct, greenish, occasionally brownish.

Leaf. Opposite, sessile to sub sessile, usually oblong, lanceolate, sub-entire, sub-acute or acute, strigose with appressed hairs on both surfaces.

Flower. Solitary or 2, together on unequal axillary peduncles, involucral bracts about 8, ovate, obtuse or acute, herbaceous, strigose with oppressed hairs; ray flowers ligulate, ligule small, spreading, scarcely as long as bracts, not toothed; white disc flowers tubular, corolla often 4 toothed; pappus absent, except occasionally very minute teeth on the top of achene; stamen 5, filaments epipetalous, free, anthers united into a tube with base obtuse; pistill bicarpellary; ovary inferior; unilocular with one basal ovule.

Fruit. Achenial cypsella, one seeded, cuneate, with a narrow wing, covered with warty excrescences, brown.

Seed. Dark brown, hairy and non endospermic.

B. *Microscopic* — Powder. Dark green; shows vessels in large groups or single broken pieces with pitted walls, numerous fibres entire or in pieces, trichomes entire or in pieces, warty, a few attached with epidermal and subsidiary cells, anomocytic and anisocytic stomata.

Root. The cells of outer one or two rows of secondary cortex, elongated or rounded with air cavities, while cells of inner secondary cortex, elongated to irregular in shape. Stone cells scattered in secondary cortex. Phloem rays broader towards the periphery, cells rounded. Xylem rays distinct, run straight in tangential section, rarely uniseriate and biseriate, cells pitted.

Stem. A few epidermal cells elongate to form characteristic non-glandular trichomes. Secondary cortex composed of large, rounded parenchymatous cells having wide air space. Vascular bundle in a ring, collateral, endarch, of varying size. Vessels barrel-shaped, some elongated with simple perforations, pitted with spiral thickening. A few xylem fibres bifurcate. Xylem rays uniseriate or biseriate.

Leaf. Anomocytic and anisocytic stomata and non-glandular hairs are present on both surface, more abundant on lower side. Vascular bundle, fine in mid rib, central one largest while four other small flanking either side of central bundle.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 9 volumes of *toluene*, 6 volumes of *acetone* and 1 volume of *formic acid.*

Test solution. Reflux 1g of the coarsely powdered substance under examination with 25 ml of *methanol* for 30 minutes, cool and filter. Reflux the residue further with 3 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Reference solution. Reflux 0.5 g *bhringraj RS* with 25 ml of *methanol* for 30 minutes, cool and filter. Reflux the residue further with 3 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat the plate at 100° for 5-10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2 .0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 5.0 per cent.

Water- soluble extractive (2.6.3). Not less than 15.0 per cent by Method I.

Total ash (2.3.19). Not more than 22 per cent.

Acid-insoluble ash (2.3.19). Not more than 11 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5 g of the coarsely powdered substance under examination with 30 ml of *methanol* on a water- bath for 30 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colourless, cool and filter. Combine all the filtrates and concentrate to 100 ml.

Reference solution. 0.01 per cent w/v solution of *wedelolactone RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 35 volumes of *acetonitrile* and 60 volumes of 0.1 per cent v/v *phosphoric acid* prepared by diluting 1ml of *phosphoric acid* to 1000 ml with *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 249 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

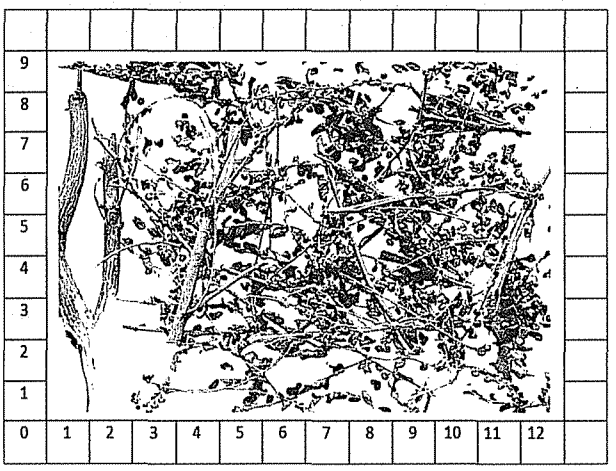
Inject the reference solution and the test solution.

Calculate the content of wedelolactone.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Bhuiamla

Phyllanthus amarus



Bhuiamla consists of the dried aerial parts of *Phyllanthus amarus* Schum. & Thom. (Fam. Euphorbiaceae).

Bhuiamla contains not less than 0.25 per cent w/w of total phyllanthin and hypophyllanthin, calculated on the dried basis.

Description. A green to greenish yellow in colour, taste, slightly bitter.

Identification

A. *Macroscopic* — Stem teret, 1-4 mm in diameter. Leaves oblong 5 × 3 mm, short stalked, greenish brown in colour.

B. *Microscopic* — Stem, inner cortex chlorenchymatous; xylem rays 1-2-seriate. Leaf stomata mostly paracytic; epidermal cell wall markedly sinuous; rosette and prismatic crystals of calcium oxalate along the veins and midrib.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 6 volumes of *toluene*, 2 volumes of *ethyl acetate*, 1 volume of *formic* acid and 0.2 volume of *methanol*.

Test solution. Reflux 2 g of coarsely powdered substance under examination with 50 ml *methanol* on a boiling water-bath for 30 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Reference solution. Reflux 1 g of *bhuiamla RS* with 50 ml *methanol* on a boiling water-bath for 30 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *methanolic sulphuric acid* (10 per cent, v/v) Heat the plate at 120° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 6.0 per cent.

Water-soluble extractive (2.6.3). Not less than 15.0 per cent by Method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 10.0 ml.

Reference solution (a). A 0.020 per cent w/v solution of *phyllanthin RS* in *methanol*.

Reference solution (b). A 0.020 per cent w/v solution of *hypophyllanthin RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 65 volumes of *methanol* and 35 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 230 nm,
- injection volume. 20 µl.

Inject the reference solution (a) and (b). The test is not valid unless the relative standard deviation for the replicate injections for both the analyte peaks corresponding to phyllanthin and hypophyllanthin is not more than 2.0 per cent.

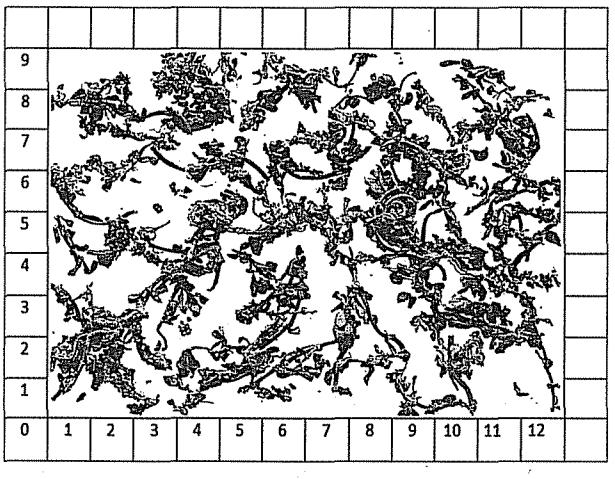
Inject the test solution, reference solutions (a) and (b).

Calculate the contents of phyllanthin and hypophyllanthin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Brahmi

Bacopa monnieri



Brahmi consists of the dried whole plant, preferably leaves and stem of *Bacopa monnieri* (Linn.) Pennell (Fam. Scrophulariaceae).

Brahmi contains not less than 2.5 per cent w/w of bacoside A, calculated on the dried basis.

Description. A brown to reddish brown colour when completely dried or green colour when partially dried with slightly bitter taste.

Identification

A. *Macroscopic* — Herbaceous comprising of stems, runner stems and leaves. Stems glabrous, leafless towards the base; internodes long. Leaves spatulate-obovate, sessile, and glabrous.

B. *Microscopic* — Cortex in stem composed of parenchyma cells enclosing large air spaces; xylem vessels radially arranged xylem rays uniseriate; pith parenchyma collapsed. In leaf, midrib indistinct, mesophyll isobilateral of spongy cells, a few prismatic crystals of calcium oxalate in mesophyll; stomata anomocytic on both the surfaces of leaf.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *chloroform* and 30 volumes of *methanol.*

Test solution. Reflux 2 g of coarsely powdered substance under examination with 25 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 x 25 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. Reflux 0.4 g of coarsely powdered *brahmi RS* with 5 ml *methanol* for 15 minutes, cool and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *methanolic sulphuric acid* (20 per cent v/v). Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

D. In the Assay, the chromatogram obtained with test solution corresponds to the chromatogram obtained with reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 6.0 per cent.

Water-soluble extractive (2.6.3). Not less than 22 per cent by Method I.

Total ash (2.3.19). Not more than 18 per cent.

Acid-insoluble ash (2.3.19). Not more than 6.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution. A 0.2 per cent w/v solution of *bacoside A RS* in *methanol,* prepared by heating gently on a water bath.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A.a gradient mixtures of *acetonitrile* and a buffer solution prepared by dissolving 0.5 g *phosphoric acid* in 800 ml of *water*, adjust pH to 2.8 with *dilute phosphoric acid* and dilute to 1000 ml with *water*,

  B. *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

Time (min)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	70	30
25	60	40
35	40	60
36	70	30
45	70	30

Inject the reference solution. The relative retention times are 1.00 for bacoside A3, about 1.04 for bacopaside II, 1.13 for jujubogenin isomer of bacopasaponine C and 1.19 for bacopasaponine C as bacoside A. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of bacoside A.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Brahmi Extract

Brahmi Extract is obtained by extracting Brahmi (*Bacopa monnieri,* Fam. Plantaginaceae) from the dried leaves and stems of with aqueous ethanol or any other suitable solvent.

Brahmi Extract contains not less than 90.0 per cent w/w and not more than 120.0 percent w/w of bacoside-A (sum of bacoside-A_3, bacopaside-II, bacopasaponin-C, jujubogenin isomer of bacopasaponin-C).

Description. Light green to dark green powder with characteristic odour and bitter taste.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 7 volumes of *Ethyl acetate,* 2 volumes of *methanol* and 1 volume of *water.*

Test solution. Shakewell 0.5 g of the extract under examination with 50 ml *methanol* and filter.

Reference solution. A 0.1 per cent w/v solution of *bacoside-A RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm.Spray the plate with *vanillin sulphuric acid* solution.Heat the plate at 60° to70° for 10 minutes and examine the plate at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Loss of drying (2.4.19). Not more than 5.0 per cent, determined on 2.0 g by drying in an oven at 105° for 3 hours.

Total ash (2.3.19). Not more than 5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, (Method B) 20 ppm.

Microbial contamination (2.2.9). Complies with the microbial contamination test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.5 g of the extract in *methanol* by gentle heating, dilute to 100 ml and filter.

Reference solution. A 0.1 per cent w/v solution of *bacoside-A RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.60 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.5 g of *phosphoric acid* in *water*,
  B. Acetonitrile
- a linear gradient programme using the conditions given below,
- flow rate 1.5 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume.20 µl.

Time (in min)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	70	30
25	60	40
35	40	60
36	70	30
45	70	30

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.The relative retention time with refernce to bacoside-A_3 for Bacopaside II is about 1.04, for jujubogenin isomer of bacopasaponin C is about 1.13 and for bacopasaponin C is about 1.19.

Inject the reference solution and the test solution.

Calculate the content of *bacoside-A.*

Usual strengths. 10 per cent w/w; 20 per cent w/w.

Storage. Store protected from heat and moisture.

Castor Oil

Castor Oil is the fixed oil obtained by cold expression from the seeds of *Ricinus communis* Linn. (Fam. Euphorbiaceae). It may contain suitable antioxidants.

Description. A pale yellowish or almost colourless, transparent, viscid liquid; odour, slight and characteristic.

Tests

Light absorption (2.4.7). Absorbance of a 1.0 per cent w/v solution in *ethanol (95 per cent)* at the maximum at about 269 nm, not more than 1.0.

Weight per ml (2.4.29). 0.945 g to 0.965 g.

Refractive index (2.4.27). 1.4758 to 1.4798.

Optical rotation (2.4.22). +3.5° to +6.0°.

Peroxide value (2.3.35). Not more than 5.0.

Acid value (2.3.23). Not more than 2.0.

Acetyl value (2.3.22). Not less than 143.

Hydroxyl value (2.3.27). Not less than 150.

Saponification value (2.3.37). 176 to 187.

Iodine value (2.3.28). 82 to 90.

Foreign fatty substances. A mixture of 2 ml of the substance under examination and 8 ml of *ethanol (95 per cent)* is clear.

B. Shake 10.0 ml with 20.0 ml of *light petroleum (60° to 80°)* and allow to separate; the volume of the lower layer is not less than 16.0 ml.

Storage. Store protected from light and moisture at a temperature not exceeding 15°.

Labelling. The label states (1) the name and quantity of any added antioxidant; (2) whether the contents are suitable for use in the manufacture of parenteral preparations.

Clove Oil

Clove oil is the oil distilled from the dried flower buds of *Syzygium aromaticum* (Linn.) Merrill and Perry *Eugenia caryophyllus* (Spreng.) Bull. and Harr.(Fam. Myrtaceae).

Description. A clear, colourless or pale yellow liquid when freshly distilled, becoming darker and thicker by ageing or exposure to air; odour as of clove.

Clove Oil contains not less than 85.0 per cent w/w and not more than 95.0 per cent w/w of phenolic substances, chiefly eugenol, $C_{10}H_{12}O_2$.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. Toluene

Test solution. Dissolve 20 µl of the substance under examination in 2 ml of *toluene*.

Reference solution. Dissolve 20 µl of *eugenol* RS in 2 ml of *toluene*.

Apply to the plate 20 µl of the test solution and 10 µl of the reference solution as bands 20 mm by 3 mm. Use an unlined tank, develop the chromatogram immediately after pouring the mobile phase into the tank and allow the mobile phase to rise 10 cm. Dry the plate, allow to stand for 5 minutes and again allow the mobile phase to rise 10 cm under the same conditions. Following the second development, dry the plate in air, examine in ultraviolet light at 254 nm and mark the quenching bands. In the chromatogram obtained with the test solution there is a quenching band in the middle of the plate corresponding to the quenching band due to eugenol in the chromatogram obtained with the reference solution. A weak quenching band may also be seen in the chromatogram obtained with the test solution with an Rf value slightly lower than that of the band corresponding to eugenol (acetyleugenol). Spray the plate with about 10 ml of *anisaldehyde solution*, heat at 100° to 105° for 10 minutes and examine in daylight. In the chromatogram obtained with the test and reference solutions the bands corresponding to eugenol are strongly coloured brownish-violet and any band corresponding to acetyleugenol in the chromatogram obtained with the test solution is faintly violet-blue. Other coloured bands may be visible in the chromatogram obtained with the test solution, in particular a faint red band in the lower part of the chromatogram and a reddish-violet band in the upper part (caryophyllene).

Tests

Optical rotation (2.4.22). 0° to –1.50°.

Weight per ml (2.4.29). 1.038 g to 1.060 g.

Refractive index (2.4.27). 1.527 to 1.535, determined at 20°.

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

Phenol. Shake 1 ml with 20 ml of hot *water*; the mixture shows not more than a scarcely perceptible acid reaction with *blue litmus paper*. Cool the mixture, pass the aqueous layer through a wetted filter and treat the clear filtrate with 1 drop of *ferric chloride test solution*. The mixture has only a transient greyish-green colour but not a blue or violet colour.

Alkali-soluble matter. Place 80 ml of a 5 per cent w/v solution of *potassium hydroxide* in a 150-ml flask with a long neck which is graduated in tenths of a ml and is of such a diameter that not less than 15 cm in length has a capacity of 10 ml. Clean the flask with *sulphuric acid* and rinse well with water before use. Add 10 ml of the oil and shake thoroughly at 5 minute intervals for 30 minutes at ambient temperature. Raise the undissolved portion of the oil into the graduated part of the neck of the flask by the gradual addition of more of the *potassium hydroxide solution*; allow to stand for not less than 24 hours and read off the volume of the undissolved portion of the oil which measures between 1.0 and 1.5 ml.

Assay. Determine by gas chromatography (2.4.14).

Test solution (a). A 0.2 per cent w/v solution of the oil under examination in *ethanol (95 per cent)*.

Test solution (b). A 0.2 per cent w/v solution of the oil under examination and 0.15 w/v of *1-decanol* (internal standard) in *ethanol (95 per cent)*.

Reference solution. A solution containing 0.2 per cent w/v solution of *eugenol RS* and 0.15 per cent w/v of the internal standard in *ethanol (95 per cent)*.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with 3 per cent w/w of dimethyl silicone fluid on acid-washed diatomaceous support (120 mesh),
- temperature:
  column.110° for 18 minutes, then increased to 170° at a rate of 12° per minute and maintained at this temperature for 2 minutes,
- inlet port at 220° and
  detector at 300°,
- flow rate 40 ml per minute of the carrier gas.

Calculate the eugenol content in the oil under examination using the ratios of the area of the peak corresponding to eugenol to the area of the peak due to the internal standard in the chromatogram obtained with test solutions (b) and the reference solution.

Storage. Store protected from light in well-filled containers at a temperature not exceeding 30°.

Coconut Oil

Coconut Oil is the refined fixed oil obtained from the dried, solid part of the endosperm of *Cocos nucifera* L. (Fam.Arecaceae).

Coconut Oil contains not less than 1.5 per cent w/w of caproic acid, not less than 5.0 per cent and not more than 11.0 per cent w/w of *caprylic acid*, not less than 4.0 per cent and not more than 9.0 per cent w/w of capric acid, not less than 40.0 per cent and not more than 50.0 per cent w/w of lauric acid, not less than 15.0 per cent and not more than 20.0 per cent w/w of myristic acid, not less than 7.0 per cent and not more than 12.0 per cent w/w of palmitic acid, not less than 1.5 per cent and not more than 5.0 per cent w/w of stearic acid, not less than 4.0 per cent and not more than 10 .0 w/w per cent of oleic acid, not less than 1.0 per cent and not more than 3.0 per cent w/w of linoleic acid, not less than 0.2 per cent w/w of linolenic acid, not less than 0.2 per cent w/w of arachidic acid and not less than 0.2 per cent w/w of eicosenoic acid.

Description. A white or almost white, unctuous mass.

Identification

A. It complies with the test for melting range (2.4.21).

B. It complies with the test for composition of fatty acids.

Tests

Melting range (2.4.21). 23° to 26°.

Refractive index (2.4.27). About 1.449, at 40°.

Peroxide value (2.3.35). Not more than 5.0.

Acid value (2.3.23). Not more than 0.5, determined on 20.0 g

Unsaponifiable matter (2.3.39). Not more than 1.0 per cent, determined on 5.0 g.

Iodine value (2.3.28). 82 to 90.

Composition of fatty acids

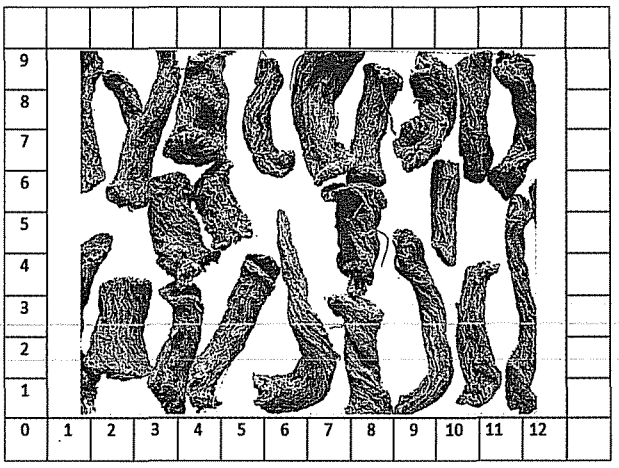
Test Solution. Refined coconut oil is melted under gentle heating to a homogeneous liquid.

Reference solution. Dissolve 15 mg of *tricaproin RS*, 80 mg of *tristearin RS*, 0.15 g of *tricaprin RS*, 0.2 g of *tricaprylin RS*, 0.45 g of *trimyristin RS* and 1.25 g of *trilaurin RS* in a mixture of 2 volumes of *dichloromethane* and 8 volumes of *heptane*, then dilute to 50 ml with the same mixture of solvents heat at 45°to 50°. Transfer 2 ml of this mixture to a 10 ml centrifuge tube with a screw cap and evaporate the solvent in a current of *nitrogen*. Dissolve with 1 ml of *heptane* and 1 ml of *dimethyl carbonate* and mix vigorously under gentle heating (50° to 60°). Add, while still warm, 1 ml of a 1.2 per cent w/v solution of *sodium* in *anhydrous methanol*, prepared with the necessary precautions, and mix vigorously for about 5 minutes. Add 3 ml of *distilled water* and mix vigorously for about 30 seconds. Centrifuge for 15 minutes at 1500 rpm. Inject 1 µl of the organic phase.

Storage. Store protected from light in well-filled containers.

Coleus

Coleus forskohlii



Coleus consists of the whole or cut dried roots of *Coleus forskohlii* Briq. (Fam. Lamiaceae).

Coleus contains not less than 0.4 per cent w/w of forskolin, calculated on the dried basis.

Description. The roots are light brown in color, generally long and radially spread. They have an aromatic characteristic odor and the taste is slightly pungent.

Identification

A. *Macroscopic* — Roots are brown, longitudinally wrinkled, fracture short, cut surface yellowish white.

B. *Microscopic* — The outermost layer consists of rectangular cork cells, cork cambium, rectangular parenchymatous region containing sclereids and calcium oxalate crystals. Vascular cambium is present in the form of a continuous ring. The tracheids and tracheidal fibres have bordered pits.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *benzene,* and 25 volumes of *ethyl acetate.*

Test solution. To 5 g of the coarsely powdered substance under examination, add 50 ml of *acetonitrile* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 50 ml of *acetonitrile*, cool and filter. Combine all the filtrates and concentrate under vacuum to 100 ml.

Reference solution. To 1 g of *coleus RS* add 50 ml of *acetonitrile* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 50 ml of *acetonitrile*, cool and filter. Combine all the filtrates and concentrate under vacuum to 20 ml.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *vanillin glacial acetic acid reagent.* Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 15.0 per cent.

Water-soluble extractive (2.6.3). Not less than 18.0 per cent by method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 3 g of coarsely powdered substance under examination, add 50 ml of *acetonitrile* and reflux on a water bath for 15 minutes, cool and filter. Reflux the residue two times with 75 ml of *acetonitrile*, cool and filter, Concentrate the filterate to 100.0 ml.

Reference solution. A 0.1 per cent w/v solution of *forskolin RS* in *acetonitrile*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1.8 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of forskolin.

Storage. Store protected from moisture and against attack by insects and rodents.

Coleus Dry Extract

Coleus Dry Extract is obtained by extracting Coleus (*Coleus Forskohlii* Willd. Briq.,Fam. Lamiaceae) roots with *methanol* or any other suitable solvent and evaporation of solvent.

Coleus Dry Extract contains not less than 90 per cent w/w to not more than 120 per cent w/w of the stated amount of forskolin, calculated on the dried basis. It may contain suitable added substances.

Description. A light brown to brown colour powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 40 volumes of *ethyl acetate* and 60 volumes of *hexane.*

Test solution. Dissolve about 0.5 g of the extract under examination with 10 ml *methanol*, filter.

Reference solution. A 0.1 per cent w/v solution of *forskolin RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray the plate with a *anisaldehyde-sulphuric acid reagent*. Heat the plate at 110° for 10 minutes and examine in ultraviolet light at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Total ash (2.3.19). Not more than 10.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the extract under examination containing about 50 mg of forskolin in 50.0 ml of *methanol and* filter.

Reference solution. A 0.1 per cent w/v solution of *forskolin RS* in the *methanol*.

Chromatographic system
- a stainless steel column, 25 cm x 0.46 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 50 volumes of *water* and 50 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of the forskolin in the extract.

Usual strengths. 10 per cent w/w; 20 per cent w/w.

Storage. Store protected from heat and moisture.

Coriander Oil

Coriander Oil is obtained by steam distillation from the fruits of *Coriandrum sativum* L.(Fam.Apiaceae).

Coriander Oil contains not less than 65.0 per cent and not more than 78.0 per cent of *linalol*, not less than 3.0 per cent and not more than 7.0 per cent of *α-pinene*, not less than 1.5 per cent and not more than 5.0 per cent of *limonene*, not less than 1.5 per cent and not more than 8.0 per cent of *γ-terpinene*, not less than 0.5 per cent and not more than 4.0 per cent of *p-cymene*, not less than 3.0 per cent and not more than 6.0 per cent of *camphor*, not less than 0.1 per cent and not more than 1.5 per cent of *α-terpineol*, not less than 0.5 per cent and not more than 4.0 per cent of *geranyl acetate* and not less than 0.5 per cent and not more than 3.0 per cent of *geraniol*.

Description. A clear, colourless or pale yellow liquid; characteristic and spicy odour .

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 95 volumes of *toluene* and 5 volumes of *ethyl acetate*.

Test solution. Dissolve 10 µl of the substance under examination in 1 ml of *toluene*.

Reference solution. Dissolve 10 µl of *linalol* and 2 µl of *geranyl acetate* in 1ml of *toluene*.

Apply to the plate 10 µl of each solution as bands of 10 mm by 2 mm. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° to 105° for 10 to 15 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Relative density (2.4.29.). 0.860 to 0.880.

Refractive index (2.4.27). 1.462 to 1.470.

Optical rotation (2.4.22). + 7° to + 13°.

Acid value (2.3.23). Not more than 3.0.

Enantiomeric purity. Not more than 14 per cent w/w of –(R) - linalol.

Determine by gas chromatography (2.4.13).

Test solution. Dissolve 0.02 g of the substance under examination in 10 ml of *pentane*.

Reference solution. Dissolve 10 µl of *linalol* and 5 mg of *borneol* in 10 ml of *pentane*.

Chromatographic system
- a fused silica column 25 m x 0.25 mm, packed with modified α-cyclodextrin (0.25 µm),
- temperature:
  column. 50° for 5 minutes, then increased to 180° at a rate of 12° per minute and maintained at this temperature for 65 minutes,
- inlet port and detector at 230°,
- flow rate 1.3 ml per minute of the carrier gas.

Inject 1 µl of the reference solution. The test is not valid unless the resolution between the peaks due to (R)-linalol

and (S) linalol is not less than 5.5 and the resolution between the peaks due to (S)-linalol and borneol is not less than 2.9.

Inject 1 µl of the reference solution and the test solution.

Assay. Determine by gas chromatography (2.4.13).

Test solution. The substance under examination dissolve in I ml of *hexane.*

Reference solution (a). Dissolve 10 µl of *α-pinene*, 10 µl of *limonene*, 10 µl of *α-terpinene*, 10 µl of *p-cymene*, 10 mg of *camphor*, 20 µl of *linalol*, 10 µl of *α-terpineol*, 10 µl of *geranyl acetate* and 10 µl of *geraniol* in 1 ml of *hexane.*

Reference solution (b). Dissolve 5 µl of *geraniol* in *hexane* and dilute to 10 ml with the same solvent.

Chromatographic system

- a fused silica column 60 m x 0.25 mm, packed with macrogol 20000 (0.25 µm),
- temperature:
  column. 60° for 10 minutes, then increased to 190° at a rate of 12° per minute and maintained at this temperature for two minutes,
- inlet port at 220° and detector at 240°,
- flow rate 1ml per minute of the carrier gas.

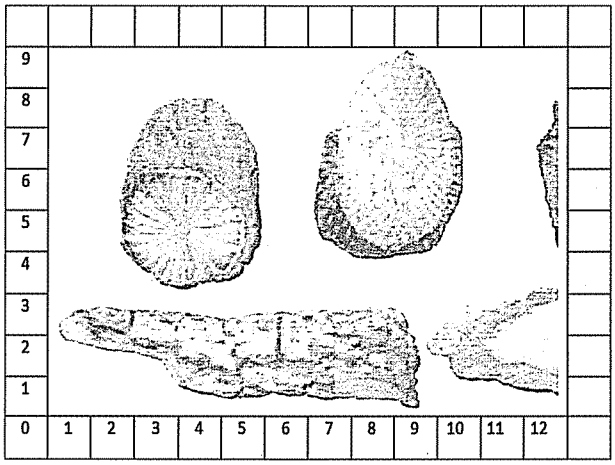
Inject 1 µl of the reference solution: The test is not valid unless the resolution between the peaks due to linalol and camphor is not less than 1.5.

Inject 1 µl of the refernce solution and the test solution.

Storage. Store protected from light, at a temperature not exceeding 30°.

Daruharidra Roots

Berberis, *Berberis arisata*



Daruharidra Roots consist of the dried roots of *Berberis aristata* DC (Fam. Berberidaceae).

Daruharidra Roots contain not less than 0.70 per cent w/w of berberine, calculated on the dried basis.

Description. The roots are cylindrical, yellowish brown in colour. They have a slightly characteristic odour and the taste is bitter.

Identification

A. Macroscopic — Root cylindrical, more or less knotty, strongly branched, usually cut into pieces of varying length and up to 45 mm in diameter; externally light yellowish-brown, longitudinally wrinkled and short scaly; fracture hard and tough; bark 1 mm. in thickness, easily separable into layers; wood yellow.

B. *Microscopic* — The powder is yellowish-brown; composed chiefly of fragments of wood fibers associated with a few tracheae and medullary rays; wood fibers yellowish, scarcely giving any reaction with *phloroglucinol* and *hydrochloric acid*, and with large, simple, transverse pores; trachea chiefly scalariform with bordered pits, occasionally reticulate; medullary rays one to twelve cells wide, and in very long rows; starch grains simple or two- to three-compound, the individual grains being irregularly spherical.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 80 volumes of *ethyl acetate,* 10 volumes of *formic acid,* 10 volumes of *glacial acetic acid* and 20 volumes of *water.*

Test solution. To 2 g of the coarsely powdered substance under examination, add 40 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. To 1 g of the *daruharidra roots RS*, add 40 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm and also under day light The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 2.0 per cent.

Water-soluble extractive (2.6.3). Not less than 6.0 per cent by method I.

Total ash (2.3.19). Not more than 5.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (60 ppm).

Loss on drying (2.4.19). Not more than 10.0 per cent determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Perform the Assay by following method I or by method II.

Method I

Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of the coarsely powdered substance under examination, add about 20 ml *2 M hydrochloric acid* and 30 ml of *methanol,* reflux on a water-bath for 15 minutes, cool and filter. Reflux the residue further with 2 *M hydrochloric acid* and *methanol*, till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 50 ml. Dilute to 50.0 ml with *methanol.*

Reference solution. A 0.01 per cent w/v solution of *berberine hydrochloride RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution pH 2.5 prepared by dissolving 0.136 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and make upto 1000 ml with *water*,

  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 346 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
25	50	50
26	80	20
30	80	20

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of berberine.

1 mg of *berberine hydrochloride* is equivalent to 0.9045 mg of berberine.

Method II

Determine by liquid chromatography (2.4.14.).

Test solution. Boil under reflux 1.0 g of the coarsely powdered substance under examination with 40 ml of a 0.1 per cent v/v solution of *strong ammonia solution* in *methanol* for 30 minutes, cool and filter. Boil under reflux the residue with further 2 quantities, each of 30 ml, of 0.1 per cent v/v *strong ammonia solution* in *methanol*, cool and filter. Combine all the filtrates and dilute to 100 ml with 0.1 per cent v/v solution of *strong ammonia solution* in *methanol.* Dilute 5.0 ml of this solution to 50 ml with a 0.1 per cent v/v *strong ammonia solution* in *methanol.*

Reference solution. A 0.002 per cent w/v solution of *berberine hydrochloride RS* in 0.1 per cent v/v *strong ammonia solution* in *methanol.*

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 73 volumes of 1.36 per cent w/v solution of *potassium dihydrogen orthophosphate* in *water* and 27 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

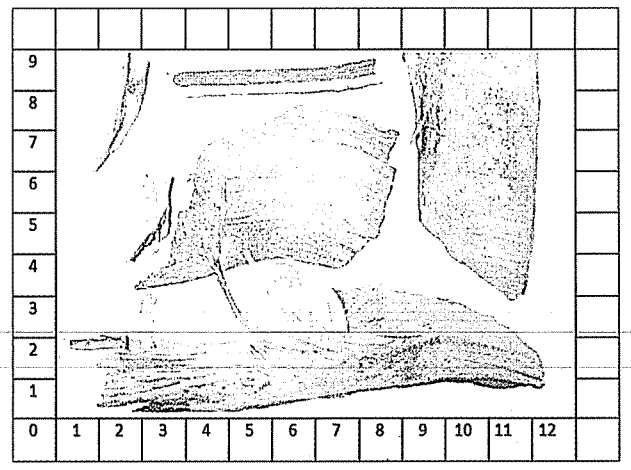
Calculate the content of berberine.

1 mg of berberine hydrochloride is equivalent to 0.9045 mg of berberine.

Storage. Store protected from heat, moisture and against attack of insects and rodents.

Daruharidra Stems

Berberis; *Berberis aristata*



Daruharidra Stems consist of cut dried stems of *Berberis aristata*, DC (Fam. Berberidaceae).

Daruharidra Stems contain not less than 0.5 per cent w/w of berberine, calculated on the dried basis.

Description. Yellowish-brown, cut chips of varying sizes, fracture fibrous, and taste bitter.

Identification

A. *Macroscopic* — Cut tortuous pieces of varying length and thickness. Bark thick, yellowish to brownish grey, wrinkled irregularly, deeply furrowed; surface hard, rough, fracture short and fibrous.

B. *Microscopic* — Cork consists of rectangular to squarish yellow coloured thin walled cells, radially arranged; sieve elements irregularly shaped, few cells containing yellowish brown to orange brown masses; phloem fibers thick walled arranged tangentially; phloem ray cells have calcium oxalate crystals, scattered stone cells; secondary phloem broad, sieve elements arranged as tangential bands, phloem fibers short, thick walled; secondary xylem broad, consisting of small to medium sized xylem vessels, tracheids, xylem fibers.

Powder is greenish yellow and shows the following diagnostic characters when observed under a microscope using chloral hydrate solution. Abundant thin walled parenchyma, fragments of yellowish-brown cork; ovoid or spherical orange brown granular masses. Examine under a microscope using 50 per cent v/v solution of *glycerol*. The powder shows starch granules, simple, small, spherical to ovoid.

C. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *n-butanol*, 10 volumes of *glacial acetic acid* and 20 volumes of *water*.

Test solution. Shake 1 g of powdered substance under examination with 10 ml *methanol* for 10 to 15 minutes and filter. Wash the residue with 5 ml of *methanol* and add washing to the filtrate.

Reference solution. Shake 1 g of powdered *daruharidra stem RS* with 10 ml of *methanol* for 10-15 minutes and filter. Wash the residue with 5 ml of *methanol* and add washing to the filtrate.

Apply to the plate, 10 µl of each solution as bands of 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and observe in ultraviolet light at 254 nm and 365 nm and also under day light. The chromatographic profile of the test solution is similar to that of reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 2.0 per cent.

Water-soluble extractive (2.6.3). Not less than 5 per cent by Method I.

Total ash (2.3.19). Not more than 5 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (60 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Perform the Assay by following method I or by method II.

Method I

Determine by liquid chromatography (2.4.14).

Test solution. Boil under reflux 1.0 g of the coarsely powdered substance under examination with 40 ml of a 0.1 per cent v/v solution of *strong ammonia solution* in *methanol* for 30 minutes, cool and filter. Boil under reflux the residue with further 2 quantities, each of 30 ml, of 0.1 per cent v/v *strong ammonia solution* in *methanol*, cool and filter. Combine all the filtrates and dilute to 100 ml with 0.1 per cent v/v solution of *strong ammonia solution* in *methanol*. Dilute 5.0 ml of this solution to 50 ml with a 0.1 per cent v/v *strong ammonia solution* in *methanol*.

Reference solution. A 0.002 per cent w/v solution of *berberine hydrochloride RS* in 0.1 per cent v/v *strong ammonia solution* in *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 73 volumes of 1.36 per cent w/v solution of *potassium dihydrogen orthophosphate* in *water* and 27 volumes of *acetonitrile*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 235 nm,
- injection volume. 10 µl.

Method II

Determine by liquid chromatography (2.4.14.).

:Test solution. Weigh 2 g of the coarsely powdered substance under examination, add about 20 ml *2 M hydrochloric acid* and 30 ml of *methanol*, reflux on a water-bath for 15 minutes, cool and filter. Reflux the residue further with 2 *M hydrochloric acid* and *methanol*, till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 50 ml. Dilute to 50.0 ml with *methanol*.

Reference solution. A 0.01 per cent w/v solution of *berberine hydrochloride RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution pH 2.5 prepared by dissolving 0.136 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and make upto 1000 ml with *water*,

  B. *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 346 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
25	50	50
26	80	20
30	80	20

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of berberine.

1 mg of *berberine hydrochloride* is equivalent to 0.9045 mg of berberine.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of berberine.

1 mg of berberine hydrochloride is equivalent to 0.9045 mg of berberine.

Storage. Store protected from heat, moisture and against attack of insects and rodents.

Eucalyptus Oil

Nilgiri Oil

Eucalyptus Oil is the essential oil obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branches of various species of eucalyptus like *Eucalyptus globulus* Labill., *E. fruticetorum* F. von Muell., and *E. smithii* (R. T. Baker) (Fam. Myrtaceae).

Eucalyptus Oil contains not less than 60 per cent w/w of cineole, $C_{10}H_{18}O$.

Description. A colourless or pale yellow liquid; odour, aromatic and camphoraceous; taste, pungent and camphoraceous, followed by a sensation of cold.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 90 volumes of *toluene* and 10 volumes of *ethyl acetate.*

Test solution. Dissolve 1 g of the substance under examination in 100 ml of *toluene.*

Reference solution. A 1 per cent w/v solution of *cineole RS* in *toluene.*

Apply to the plate 2 µl of each solution. After development, dry the plate in air, spray with *anisaldehyde solution*, using about 10 ml for a 200 mm x 200 mm plate, heat at 105° for 10 minutes and examine in daylight and in ultraviolet light at 365 nm. In the chromatogram obtained with the reference solution a dark brown spot due to cineole is visible in daylight in the middle part; when examined in ultraviolet light at 365 nm, the spot shows a brown fluorescence. The principal spot in the chromatogram obtained with the test solution corresponds to that of cineole; no carmine-brown spot appears in daylight in the upper third of the chromatogram and when examined in ultraviolet light at 365 nm no spot showing a greenish brown fluorescence appears in the upper third (citronellal). Other spots may be visible in the upper and lower thirds of the chromatogram.

Tests

Optical rotation (2.4.22). 0° to +10°.

Refractive index (2.4.27). 1.457 to 1.469.

Weight per ml (2.4.29). 0.897 g to 0.924 g.

Aldehydes. Place 10 ml in a glass-stoppered tube (150 mm x 25 mm) add 5 ml of *toluene* and 4 ml of *ethanolic hydroxylamine solution*, shake vigorously and titrate immediately with *0.5 M potassium hydroxide* in *ethanol (60 per cent)* until the red colour changes to yellow. Continue the shaking and neutralising until the pure yellow colour of the indicator is permanent in the lower layer after shaking vigorously for 2 minutes and allowing separation to take place; the reaction is complete in about 15 minutes. Repeat the operation using a further 10 ml of the substance under examination, and as the standard for the end-point, the titrated liquid of the first determination with the addition of 0.5 ml of *0.5 M potassium hydroxide in ethanol (60 per cent).* Not

more than 2.0 ml of *0.5 M potassium hydroxide in ethanol (60 per cent)* is required in the second determination.

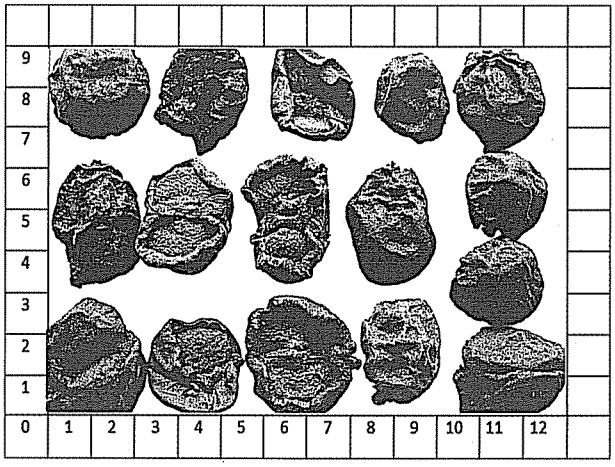
Phellandrene. Mix 1 ml with 2 ml of *glacial acetic acid* and 5 ml of *light petroleum (40° to 60°)*, add 2 ml of a saturated solution of *sodium nitrite* and shake gently; no crystalline precipitate is produced in the upper layer within 1 hour.

Assay (2.3.24) Determine the content of cineole in the oil.

Storage. Store in well-filled, tightly-closed containers at a temperature not exceeding 30°.

Garcinia

Vilayati Imli; *Garcinia cambogia*



Garcinia is the dried deseeded fruit of *Garcinia cambogia* Desr. {*Garcinia gummi-gutta* (L.) N: Robson} (Fam. Guttiferae).

Garcinia contains not less than 12.0 per cent of total hydroxycitric acid and hydroxycitric acid lactone, calculated on the dried basis.

Description. Dark brown to blackish brown fruits. Taste, acidic.

Identification

A. *Macroscopic* — Dark brown to blackish brown fruits, ovoid, longitudinally grooved.

B. *Microscopic* — Mesocarp very wide composed of parenchymatous cells of various sizes and shapes. Compound starch grains and prismatic crystals of calcium oxalate traverse throughout the parenchymatous cells of the mesocarp.

C. In the Assay, the principal peak in the chromatogram obtained with test solution has a retention time similar to that of the peak due to hydroxycitric acid in the chromatogram obtained with reference solution.

Tests

Citric Acid. Not more than 2.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution, reference solutions (a), (b) and chromatographic system as described under Assay.

Inject test solution and reference solution (b).

Calculate the content of citric acid.

Foreign organic matter (2.6.1). Not more than 5.0 per cent.

Water-soluble extractive (2.6.3). Not less than 40.0 per cent by Method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh about 5 g of the coarsely powdered substance under examination and transfer to a 250-ml beaker. Add 5 ml of *dilute phosphoric acid* and 100 ml of *water*, concentrate to half the volume by heating, cool and filter. Extract the residue further with water till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate under vacuum to 250.0 ml.

Reference solution (a). A 0.8 per cent w/v solution of *hydroxycitric acid calcium salt RS* in *dilute phosphoric acid.*

Reference solution (b). A 0.1 per cent w/v solution of *citric acid* in *dilute phosphoric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 900 ml of *water*, adjusting the pH to 2.5 with *dilute phosphoric acid* and diluting to 1000.0 ml with *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Inject the reference solution (a). The test is not valid unless the relative retention times are about 0.8 for *hydroxycitric acid lactone,* 1.0 for *hydroxycitric acid* and 2.0 for *citric acid,* the resolution factor between *hydroxycitric acid lactone* and *hydroxycitric acid* is not less than 1.8, the tailing factor is

not more than 1.5 and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the sum of the contents of *hydroxycitric acid* and *hydroxycitric acid lactone.*

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Garcinia Aqueous Extract

Garcinia Aqueous Extract is obtained by extracting Garcinia (*Garcinia cambogia* Desr., *Garcinia gummi-gutta* (L.) N: Robson, Fam. Guttiferae) fruit rinds with *water* as calcium salts.

Garcinia Aqueous Extract contains not less than 90.0 per cent w/w to not more than 120.0 per cent w/w of the stated amount of hydroxy citric acid, and calcium not less than 15.0 per cent w/w calculated on the dried basis. It may contain suitable added substances.

Description. A pale brown to brown powder.

Tests

Total ash (2.3.19). Not more than 10.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 6.0 per cent determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. *hydroxy citric acid* — Determine by liquid chromatography (2.4.14).

Test Solution. Shake well 0.2 g of the extract under examination add 2 ml of *3 M hydrochloric acid* and add 10 ml of *water*, sonicate it to dissolve, make up to volume 100.0 ml with *water* and mix.

Reference solution. A 0.1 per cent w/v solution of *hydroxy citric acid RS* with 2 ml of *3 M hydrochloric acid* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: 0.1 per cent *orthophosphoric acid* in *water*, filter and degas,
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the referene solution and the test solution.

Calculate the content of the hydroxy citric acid in the extract.

Calcium —

Test solution. Weigh accurately about 0.2 g of the extract and transfer into a 500 ml conical flask. Dissolve in 2 ml of *3 M hydrochloric acid* and add 200 ml of *water.* Add 15 ml of *1 M Sodium hydroxide* and titrate with *0.05 M EDTA* using *hydroxy naphthol blue* as indicator until deep blue colour persist.

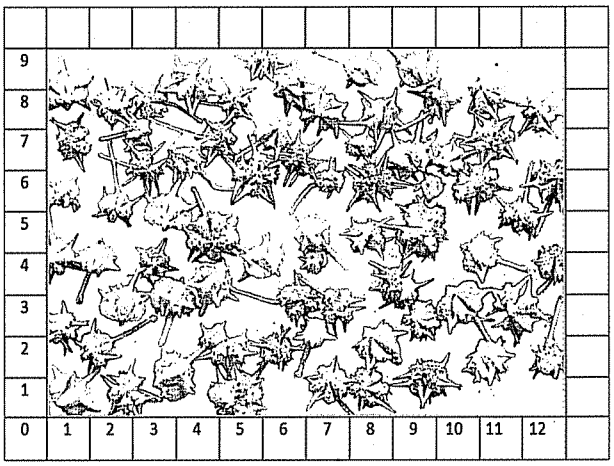
Each ml of *0.05 M EDTA* solution is equivalent to 0.002004 g of calcium.

Usual strengths. 50 per cent; 60 per cent.

Storage. Store in air-tight containers, protected from light.

Gokhru

Tribulus terrestris



Gokhru consists of dried fruits of *Tribulus terrestris* L.(Fam.Zygophyllaceae).

Gokhru contains not less than 0.5 per cent of diosgenin, calculated on the dried basis.

Description. Pedicellate and globose fruits, having wedge-shaped cocci, covered with short and stiff spines. Possesses faintly aromatic smell and acrid taste.

Identification

A. *Macroscopic* — Fruit is pedicellate, having wedge-shaped cocci, covered with spines. Surface of schizocarp is rough.

B. *Microscopic* — Pericarp is differentiated into epicarp, mesocarp and endocarp. Epicarp is surrounded by non-glandular trichomes.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 8 volumes of *toluene* and 2 volumes of *ethyl acetate.*

Test solution. Reflux 5 g of coarsely powdered substance under examination with 50 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol*, cool and filter. Combine both the filtrates and concentrate under vacuum to dryness. Extract the dried residue with 10 ml of *methanol* at 50° for 10 minutes, filter the solution and use filtrates for analysis.

Reference solution. Reflux 2.5 g of *gokhru RS* with 50 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol*, cool and filter. Combine both the filtrates and concentrate under vacuum to dryness. Extract the dried residue with 5 ml of *methanol* at 50° for 10 minutes, filter the solution and use the filtrates for analysis.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent.* Heat the plate at 105° for 5 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 3.0 per cent.

Water-soluble extractive (2.6.3). Not less than 15.0 per cent by method I.

Total ash (2.3.19). Not more than 11.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5.0 g of the substance under examination with 50 ml of *sulphuric acid (10 per cent)* for 4 hours. Cool and transfer to separating funnel. Extract with 50 ml of *ethyl acetate.* Repeat the extraction 3 times. Pass the ethyl acetate layer through *sodium sulphate* and evaporate. Dissolve the residue with 50 ml of *methanol.*

Reference solution. A 0.1 per cent w/v solution of *diosgenin RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 80 volumes of *acetonitrile* and 20 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution.

Calculate the content of diosgenin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Guar Gum

Guar Gum is a gum obtained from the ground endosperms of the seeds of *Cyamopsis tetragonolobus* (Linn.) Taub or other species of *Cyamopsis* (Fam. Leguminosae). It consists mainly of a high molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan units combined through glycosidic linkages.

Description. An almost white to pale yellowish white powder; odour, characteristic.

Identification

A. When mounted in *lactophenol* and examined under a microscope, irregular, angular particles of various sizes and shapes are seen.

B. To 0.1 g add 1 ml of *0.2 M iodine*; the mixture does not acquire an olive-green colour.

C. Dissolve 0.1 g in 20 ml of *water* by shaking and add 0.5 ml of *hydrogen peroxide solution (20 vol)* and 0.5 ml of a 1 per cent w/v solution of *benzidine* in *ethanol (90 per cent)*, shake and allow to stand; no blue colour is produced (distinction from acacia).

D. Mount a small quantity in *ruthenium red solution* and examine under a microscope; the particles do not acquire a pink colour (distinction from sterculia gum and agar).

E. To 2 ml of a 0.5 per cent w/v solution add 2 ml of a 20 per cent w/v solution of *lead acetate*; a flocculent precipitate is produced (distinction from acacia, ghatti gum and sterculia).

Tests

Acidity or alkalinity. A 0.5 per cent w/v solution is neutral to *litmus paper.*

Tannin. To 5 ml of a 0.5 per cent w/v solution add 0.1 ml of *ferric chloride test solution*; no bluish black colour is produced.

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate,* add 10 ml of *bromine solution* and mix thoroughly.

Evaporate to dryness on a water-bath, gently ignite and dissolve the cooled residue in a mixture of 16 ml of *brominated hydrochloric acid* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Protein. Not more than 5.0 per cent, determined by the following method. Carry out the determination of nitrogen (2.3.30), using about 3.5 g, accurately weighed, and multiplying the percentage of nitrogen determined by 6.25 to obtain the percentage of protein.

Acid-insoluble matter. Not more than 3.0 per cent, determined by the following method. Weigh accurately about 1.5 g and disperse in 150 ml of *water* and 1.5 ml of *sulphuric acid*. Warm on a water-bath for 6 hours, replacing the water lost by evaporation. Add about 0.5 g of a suitable filter-aid, accurately weighed, and filter through a suitable ashless filter paper. Wash the residue several times with hot *water*; dry the filter and its contents at 105° for 3 hours. Cool in a desiccator, weigh and subtract the weight of the filter aid.

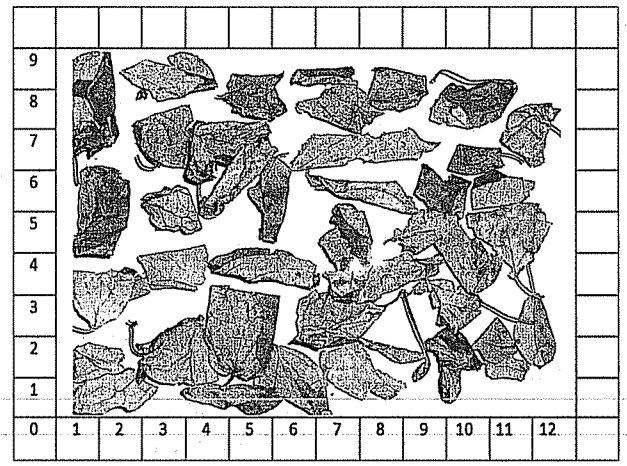
Microbial contamination (2.2.9). Total bacterial count: Not more than 5000 per g. 1 g is free from *Escherichia coli* and 10 g is free from *salmonellae*.

Total ash (2.3.19). Not more than 2.0 per cent, determined on 1.0 g.

Loss on drying (2.4.19). Not more than 13.0 per cent, determined on 0.5 g by drying in an oven at 105°.

Gudmar

Gymnema sylvestre



Gudmar consists of the dried mature leaves of *Gymnema sylvestre* R.Br. (Fam. Asclepiadaceae).

Gudmar contains not less than 1.0 per cent w/w of gymnemic acids (calculated as gymnemagenin), calculated on dried basis.

Description. Greenish-yellow in colour, surface pubescent on both sides and characteristic odour with extremely bitter and acrid taste.

Identification

A. *Macroscopic* — Leaves, simple, petiolate about 2 to 6 cm long and 1 to 4 cm broad, yellowish brown on adaxial and dark green on abaxial side.

B. *Microscopic* — Upper and lower epidermis covered with cuticle having uni to tri cellular covering trichomes which are slightly curved at the bulbous base. Below the epidermis is single layer of palisade cells followed by 2-3 layered spongy parenchyma. Starch gains are simple and present in spongy parenchyma. Midrib region shows 2-7 layers of collenchymatous cells. Stomata are of paracytic type, mostly on lower the surface. There is a fan shaped vascular bundle in the centre. Each vascular bundle is collateral, closed and surrounded by parenchymatous sheath. Rosette crystal of calcium oxalate present in the spongy parenchyma.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 5 volumes of *chloroforms*, 1 volume of *methanol* and 1 volume of *ethyl acetate*.

Test solution. Reflux 5 g of the coarsely powdered substance under examination with 50 ml of *ethanol (50 per cent v/v)* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *ethanol (50 per cent v/v)*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml. Take 5 ml of resulting solution, add 5 ml *ethanol* and 2 ml of *potassium hydroxide* and reflux for 1 hour. Cool and add 1.8 ml of *12 M hydrochloric acid* and heat on water bath. Cool and adjust the pH to 7.5-8.5 with 11 per cent *potassium hydroxide*. Dilute the solution with *ethanol (50 per cent v/v)* to 100 ml and filter.

Reference Solution. Reflux 1 g of *gudmar RS* with 50 ml of *ethanol (50 per cent v/v)* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *ethanol (50 per cent v/v)*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml. Take 5 ml of resulting solution, add 5 ml *ethanol* and 2 ml of *potassium hydroxide* and reflux for 1 hour. Cool and add 1.8 ml of *12 M hydrochloric acid* and heat on water bath. Cool and adjust the pH to 7.5-8.5 with 11 per cent *potassium hydroxide*. Dilute the solution with *ethanol (50 per cent v/v)* to 50 ml and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent* . Heat the plate at

100° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 5.0 per cent.

Water-soluble extractive (2.6.3). Not less than 20.0 per cent by method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 6.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 14.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5 g of the coarsely powdered substance under examination with 50 ml of *ethanol (50 per cent v/v)* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *ethanol (50 per cent v/v)*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml. Take 5 ml of resulting solution, add 5 ml *ethanol* and 2 ml of *potassium hydroxide* and reflux for 1 hour. Cool and add 1.8 ml of *12 M hydrochloric acid* and heat on water bath. Cool and adjust the pH to 7.5-8.5 with 11 per cent *potassium hydroxide*. Dilute the solution with *ethanol (50 per cent v/v)* to 100 ml and filter.

Reference solution. A 0.01 per cent w/v solution of *gymnemagenin RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase (A). 80 per cent v/v *acetonitrile,*
  (B). 0.1 per cent w/v *dihydogen potassium phosphate,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	25	75
20	50	50
30	25	75

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

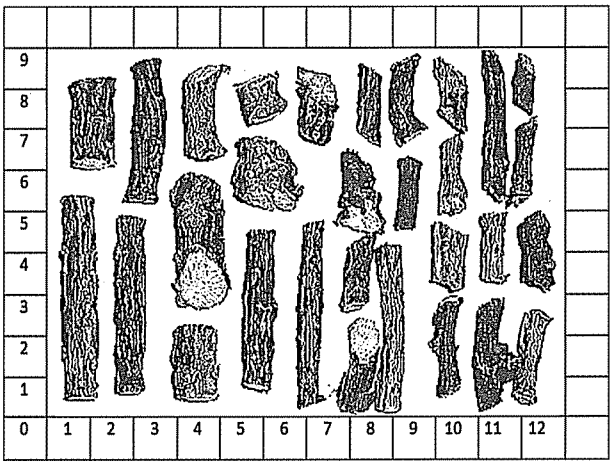
Inject the test solution and reference solution.

Calculate the content of gymnemagenin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Guduchi

Giloe; Amrita; *Tinospora cordifolia*



Guduchi consists of the dried, mature pieces of stem of *Tinospora cordifolia* (Willd.) Miers (Fam. Menispermaceae).

Guduchi contains not less than 0.02 per cent w/w of cordifolioside A, calculated on the dried basis.

Description. A greyish-black in colour, fibrous fracture and no distinct odour with bitter taste.

Identification

A. *Macroscopic* — Stem-pieces glabrous, cylindrical, solid, lenticillate, 5-15 mm in diameter having light brown surface marked with warty protuberances due to circular lenticels. Transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues.

B. *Microscopic* — Transverse section of stem shows outermost layer of cork which is differentiated in to outer zone of thick walled, compressed cells and inner zone of thin walled, tangential cells. Cork broken at some places due to lenticels. Cortex consists of 3-5 rows of irregularly arranged tangential, chlorenchymatous cells with numerous intercellular spaces. Inner cortex filled with plenty of starch gains. Vascular zone consists of 10-12 wedge-shaped strips of xylem externally surrounded by semi-circular strips of phloem. Cambium

composed of one to two layers of tangentially elongated cells. Primary phloem appears crushed and obliterated; secondary phloem groups are massive. Pith composed of large, thin walled cells mostly containing starch gains.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 85 volumes of *chloroform* and 15 volumes of *methanol*.

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Reference solution. Reflux 2 g of the *guduchi RS* with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 110° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 1.5 per cent.

Water-soluble extractive (2.6.3). Not less than 9.0 per cent by method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 25 ml. Dilute with *methanol* to 25.0 ml.

Reference solution. A 0.004 per cent w/v solution of *cordifolioside A RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a gradient mixtures of *water*
  B. *.acetonitrile*,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
25	20	80
30	80	20

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

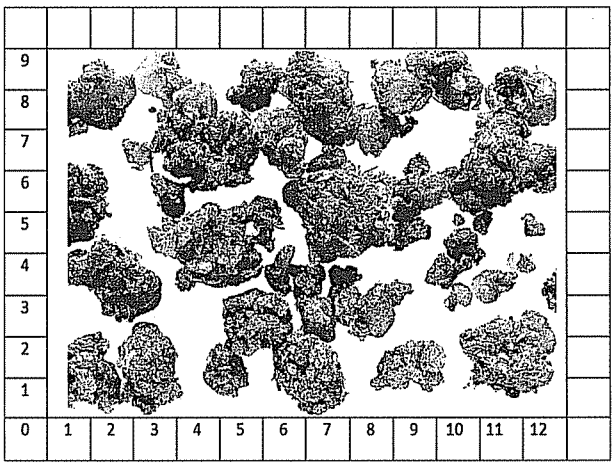
Inject the test solution and reference solution.

Calculate the content of cordifolioside A.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Guggul Resin

Guggul; *Commiphora wightii*



Guggul Resin is the oleoresin exudation from *Commiphora wightii* (Arnott) Bhandari (*Commiphora mukul* (Arn.) Bhandari, *Balsamodendron mukul* Hook. ex Stocks) (Fam. Burseraceae).

Guggul Resin contains not less than 1.0 per cent w/w and not more than 1.5 per cent w/w of gugulsterones (Z and E).

Description. Light to dark-brown conglomerates of tears, rounded or irregular, slightly sticky to touch; odour, faintly balsamic.

Identification

A. Prepare a 0.005 per cent w/v solution in *ethanol (95 per cent)* of the residue obtained in the test for Ethanol-soluble extractive.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 245 nm and 327 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *light petroleum (60° to 80°)* and 1 volume of *ethyl acetate*.

Test solution. Dissolve 0.5 g of the residue obtained in the test for Ethanol-soluble extractive in 100 ml of *ethanol (95 per cent)*.

Reference solution. A 0.5 per cent w/v solution of the residue obtained similarly from *guggul resin RS* in *ethanol (95 per cent)*.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air until the odour of the solvent is no longer detectable and examine in ultraviolet light at 254 nm and 365 nm, spray with a 10 per cent v/v solution of *sulphuric acid* in *methanol*. Heat the plate at 100° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Ethyl acetate-soluble extractive. Not less than 25.0 per cent, determined by the following method. Crush the substance under examination to a coarse powder. Shake 5.0 g of the powder with 25 ml of *light petroleum (60° to 80°)* for 1 hour and separate the liquid by filtration. Repeat the extraction twice and dry the defatted material over *phosphorus pentoxide* at room temperature at a pressure not exceeding 2.75 kPa for 8 hours. Crush the dried material and extract with four quantities, each of 25 ml, of *ethyl acetate* by shaking each time for 1 hour followed by filtration through a sintered-glass funnel (porosity No. 3) and combining the filtrates. Evaporate the combined filtrates, dry over *phosphorus pentoxide* at room temperature at a pressure not exceeding 2.75 kPa for 12 hours and weigh.

Ethanol-soluble extractive (2.3.46). Not less than 35.0 per cent, determined by the following method. Crush the substance under examination to a coarse powder. Macerate 5.0 g of the powder with 100 ml of *ethanol (95 per cent)* in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing to stand for 18 hours. Filter rapidly taking care to avoid loss of ethanol, evaporate 25 ml of the filtrate to dryness, dry at 105° and weigh.

Sulphated ash (2.3.18). Not more than 10.0 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.3.14).

Test solution. Weigh accurately about 3.0 g of the substance under examination, add 50 ml of *acetonitrile*, reflux on a water-bath for 30 minutes, cool and filter. Reflux the residue further with three portions, each of 30 ml, of *acetonitrile*, cool and filter. Combine the filtrates and concentrate to 100.0 ml.

Reference solution. A 0.02 per cent w/v solution of *gugulsterones (Z and E) RS* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times are about 0.69 for gugulsterone E and 1.0 for gugulsterone Z and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution. Calculate the contents of gugulsterones (Z and E).

Storage. Store protected from light at a temperature not exceeding 30°.

Gugulipid

Gugulipid is the ethyl acetate extractive of Guggul Resin.

Gugulipid contains not less than 4.0 per cent w/w and not more than 6.0 per cent w/w of gugulsterones (*Z* and *E*).

Description. A brown, viscous liquid.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *chloroform* shows absorption maxima at about 245 nm and 327 nm; absorbance at about 245 nm, about 0.87 and at about 327 nm, about 0.52.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *light petroleum (60° to 80°)* and 1 volume of *ethyl acetate*.

Test solution. Dissolve 0.25 g of the substance under examination in 100 ml of *ethanol (95 per cent)*.

Reference solution. A 0.25 per cent w/v solution of *gugulipid RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air until the odour of the solvent is no longer detectable and examine in ultraviolet light at 254 nm and 365 nm, spray with a 10 per cent v/v solution of *sulphuric acid* in *methanol*. Heat the plate at 100° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 0.75 g of the substance under examination, add 50 ml of *acetonitrile* and warm on a boiling water-bath for 10 minutes. Cool and add sufficient *acetonitrile* to produce 100.0 ml.

Reference solution. A solution containing 0.02 per cent w/v of *gugulsterones (Z and E)* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times are about 0.69 for gugulsterone E and 1.0 for gugulsterone Z and the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject alternately the test solution and the reference solution. Calculate the contents of gugulsterones (Z and E).

Storage. Store protected from light at a temperature not exceeding 30°.

Gugulipid Tablets

Gugulipid Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of gugulsterones (*Z* and *E*). The tablets may be coated.

Identification

Extract a quantity of the powdered tablets containing about 20 mg of gugulsterones (*Z* and *E*) with two quantities, each of 15 ml, of *ethyl acetate*, combine the extracts, filter and evaporate to dryness. The residue complies with the following tests.

A. When examined in the range 230 nm to 360 nm (2.4.7), a 0.005 per cent w/v solution in *chloroform* shows absorption maxima at about 245 nm and 327 nm; absorbance at about 245 nm, about 0.87 and at about 327 nm, about 0.52.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 3 volumes of *light petroleum (60° to 80°)* and 1 volume of *ethyl acetate*.

Test solution. A 0.25 per cent w/v solution of the substance under examination in *ethanol (95 per cent)*.

Reference solution. A 0.25 per cent w/v solution of *gugulipid RS* in *ethanol (95 per cent)*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and spray with 50 per cent w/v solution of *sulphuric acid*. The principal spots in the chromatogram obtained with the test solution correspond to those in the chromatogram obtained with the reference solution.

Tests

Disintegration (2.5.1). 60 minutes.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.13).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of gugulsterones (Z and E) and extract with five quantities, each of 20 ml, of *acetonitrile*, with the aid of heat. Combine the extracts and concentrate to 50.0 ml. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm and use the filtrate.

Reference solution. A solution containing 0.02 per cent w/v of *gugulsterones (Z and E)* in *acetonitrile*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilyl silica gel (5 µm),
- mobile phase: a filtered and degassed mixture of 45 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 242 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative retention times are about 0.69 for gugulsterone E and 1.0 for gugulsterone Z and the relative standard deviation for replicate injections is not more than 2.0 per cent.

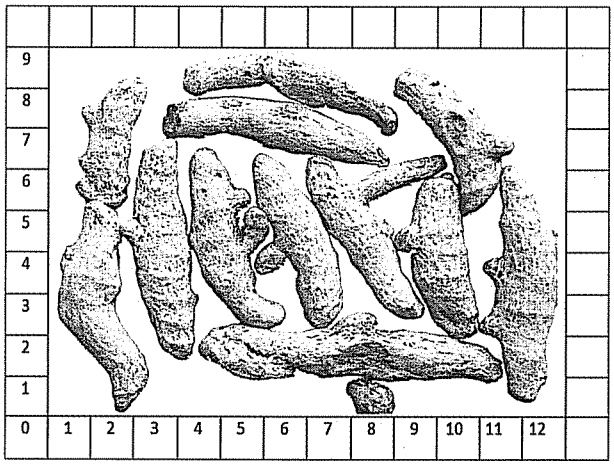
Inject the test solution and the reference solution. Calculate the contents of gugulsterones (Z and E) in the tablets.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent amount of gugulsterones (*Z* and *E*).

Haridra

Haldi; Turmeric; *Curcuma longa*



Haridra consists of the dried rhizomes of *Curcuma longa* Linn. (Fam. Zingiberaceae).

Haridra contains not less than 1.5 per cent w/w of curcumin, calculated on the dried basis.

Description. Externally yellowish to yellowish brown with root scars and annulations. Odour, aromatic; taste, warmly aromatic and bitter.

Identification

A. *Macroscopic* — Rhizome oblong, conical or cylindrical to elongate, finger-like; internally orange yellow. Texture hard and heavy; fracture short.

B. *Microscopic* — Ground tissue of parenchyma cells; cells filled with gelatinized starch grains and yellow pigment. Fibrovascular bundles and oil cells scattered through out ground tissue.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 94 volumes of *chloroform*, 5 volumes of *ethanol* and 1 volume *glacial acetic acid*.

Test solution. Extract 1 g of the coarsely powdered substance under examination with 5 ml *methanol* for 10 minutes with slight warming. Filter and use the filtrate.

Reference solution. Reflux 1 g of coarsely powdered *haridra RS* with 5 ml *methanol* for 15 minutes, cool and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light 254 nm, 365 nm and also under day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 6.0 per cent.

Water-soluble extractive (2.6.3). Not less than 12.0 per cent by Method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 12.0 per cent, determined on 0.2 g.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 1 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes cool and filter. Reflux the residue further with 5 x 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution. A 0.01 per cent w/v solution of *curcumin RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with silicagel consisting of porous spherical particles with chemically bonded nitrile group,
- mobile phase: a mixture of 35 volumes of tetrahydrofuran 65 volumes of a buffer solution prepared by dissolving 10 g of *citric acid* in 1000 ml of *water*, adjusting the pH to 3.0 with *dilute ammonia solution*,
- flow rate. 1.2 ml per minute,
- spectrophotometer set at 430 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of curcumin.

Storage. Store protected from moisture.

Haridra Dry Extract

Haridra Dry Extract is a partially purified natural complex of diaryl heptanoid derivatives isolated by extracting Haridra *(Curcuma longa* L., Fam. Zingiberaceae), rhizomes with *acetone* or *ethanol* or any other suitable solvent and evaporation of solvent under vacuum..

Haridra Dry Extract contains not less than 95.0 per cent w/w and not more than 102.0 per cent w/w of the stated amount of total curcuminoids calculated on the dried basis, as the sum of curcumin, demethoxycurcumin and bisdemethoxycurcumin. It contains not less than 70.0 per cent w/w to not more than 80.0 per cent w/w of curcumin, not less than 15.0 per cent w/w to not more than 25.0 per cent w/w of demethoxycurcumin and not less than 2.5 per cent w/w to not more than 6.5 per cent w/w of bisdemethoxycurcumin.

Description. An orange yellow crystalline powder.

Identification

A. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 10 volumes of *chloroform*, 1.0 volume of *methanol*, 0.5 volume of *glacial acetic acid* and 0.5 volume of *formic acid.*

Test solution. Dissolve about 50 mg of the extract under examination with 100.0 ml of *methanol* and filter.

Reference solution. A solution containing 0.01 per cent w/v each of *bisdemethoxycurcumin RS, demethoxycurcumin RS, curcumin RS* in the *methanol.*

Apply to the plate 10 µl of each of solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a *anisaldehyde- sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine the plate at 365 nm and day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Total curcuminoids — Determine by liquid chromatography (2.4.14).

Test Solution. Dissolve a quantity of the extract under examination containing about 50 mg curcumin in 100.0 ml of *tetrahydrofuran.* Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution. A solution containing 0.01 per cent w/v each of *bisdemethoxycurcumin RS, demethoxycurcumin RS, curcumin RS* in *tetrahydrofuran.* Dilute 5ml of this solution to 50 ml with the mobile phase.

Chromatographic system

- a stainless steel column, 25 cm x 0.46 mm packed with octadecylsilane bonded to porous silica (5µm),
- mobile phase: a mixture of 40 volumes of 0.1 per cent w/v solution of *citric acid* and 60 volumes of *tetrahydrofuran,*
- flow rate. 1.0 ml per minute,
- spectrophotometer set at 420 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent. The relative retention time with reference to curcumin for bisdemethoxycurcumin is about 1.3 and for demethoxycurcumin is about 1.14.

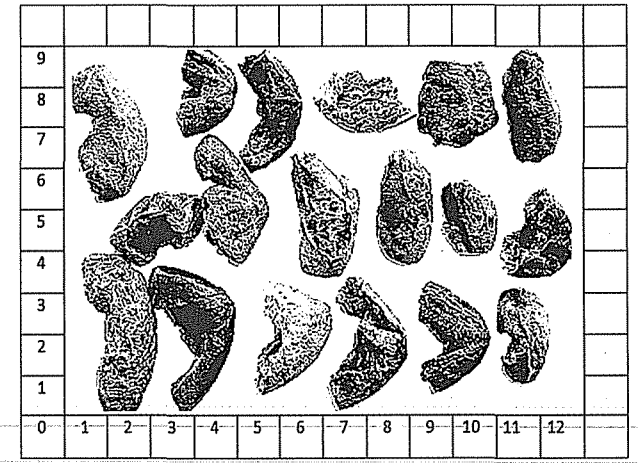
Inject the test solution and the reference solution.

Calculate the content of the bisdemethoxycurcumin, demethoxycurcumin and curcumin in extract.

Storage. Store protected from heat and moisture.

Haritaki

Harad; Chebulic myrobalan; *Terminalia chebula*



Haritaki consists of pericarp of the dried fruit of *Terminalia chebula* Retz. (Fam. Combretaceae).

Haritaki contains not less than 5 per cent w/w of chebulagic acid and not less than 12.5 per cent w/w of chebulinic acid, calculated on the dried basis.

Description. It has a shine on its external part and has longitudinal ridges. The colour varies from yellowish brown to light black. It has a astringent taste and is also slightly bitter

Identification

Test C may be omitted if tests A, B and D are carried out and test D may be omitted if tests A, B and C are carried out.

A. *Macroscopic* — The fruit is 2 to 3 cm in length and 1 to 2 cm in diameter with hard stony appearance. Externally it is shining and is adorned with longitudinal ridges. Color of the fruit rind varies from yellowish brown, uniform brown to light black. Internally the fruit is light yellow.

B. *Microscopic* — Epicarp has thick walls covered with cuticle. The mesocarp has many stone cells of various sizes and shapes which forms a reticulum. Large quantity of tannin is present in the mesocarp. Simple starch granules are present in plenty.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 35 volumes of *toulene*, 50 volumes of *acetone*, 15 volumes of *glacial acetic acid* and 5 volumes of *formic acid*.

Test solution. To 1 g of the coarsely powdered substance being examined, add 50-75 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combined all the filtrates and concentrate under vacuum to 100 ml.

Reference solution. To 0.1 g of the *haritaki RS*, add 50-75 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with 10 per cent w/v *ferric chloride solution* in *water*. Heat the plate at 100° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

D. In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with reference solution (a).

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 35.0 per cent.

Water-soluble extractive (2.6.3). Not less than 50 per cent by Method I.

Total ash (2.3.19). Not more than 6.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm)

Loss on drying (2.4.19). Not more than 12 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 0.5 g of coarsely powdered sample, add 50 ml of *water*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with *water* and filter. Dilute 10.0 ml of the solution to 25.0 ml with *water*.

Reference solution (a). Weigh 0.5 g of *haritaki RS*, add 50 ml of *water*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with *water* and filter. Dilute 10.0 ml of the solution to 25.0 ml with *water*.

Reference solution (b). A 0.1 per cent w/v solution of *chebulagic acid RS* in *water*.

Reference solution (c). A 0.1 per cent w/v solution of *chebulinic acid RS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm).
- mobile phase: A.a buffer solution pH 2.5 prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and make upto 1000 ml with *water*,

B. *acetonitrile*,

- flow rate. 1.5 ml per minute
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
18	65	35
25	45	55
28	45	55
35	95	5

Inject the reference solution (b) and (c). The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of *Chebulagic acid* and *Chebulinic acid.*

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Haritaki Extract

Haritaki extract is obtained by extracting Haritaki (*Terminalia chebula* Retz., Fam. Combretaceae) from the dried fruit pericarp with *ethanol* or any other suitable solvent.

Haritaki extract contains not less than 90 per cent w/w and not more than 120 percent w/w of the stated amount of chebulinic acid and chebulagic acid.

Description. Light yellowish to yellowish brown powder with odour, characteristic; taste, bitter.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 6 volumes of *ethyl acetate*, 2 volumes of *formic* acid, 2 volumes of *toluene* and 1 volume of *methanol.*

Test solution. Dissolve 0.5 g of extract under examination with 100 ml *methanol* and filter.

Reference solution. A. 0.1 per cent w/v solution of *chebulagic acid RS and* 0.1 per cent w/v solution of *chebulinic acid RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultra-violet light at 254 nm. Spray the plate with *ferric chloride reagent.* Heat the plate at 110° for 10 minutes and examine the plates at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

B. In the assay, the peaks due to c hebulinic acid and chebulagic acid in the chromatogram obtained with test solution corresponds to the peak obtained with reference solutions.

Tests

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 2.0 g by drying in an oven at 105° for 3 hours.

Total ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, (Method B) 20 ppm.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.5 g of the extract or quantity equivalent to 100 mg of polyphenols in water, make up to 100 ml and filter.

Reference solution (a). 0.01 per cent w/v solution of *chebulagic acid RS* in *water.*

Reference solution (b). 0.01 per cent w/v solution of *chebulinic acid RS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 500 ml of *water,* add 0.5 ml of *orthophosphoric acid* and dilute to 1000 ml with water,
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	95	5
18	75	25
25	65	35
28	65	35
35	95	5

Inject reference solution (a) and (b). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of *chebulinic acid* and *chebuliagic acid* .

Usual strength. 15 per cent w/w.

Storage. Store protected from heat and moisture.

Haritaki Aqueous Extract

Haritaki Aqueous Extract is obtained from the dried fruit pericarp of Haritaki (*Terminalia chebula* Retz., Fam. Combretaceae) by extraction with water.

Haritaki Aqueous Extract contains not less than 90.0 per cent w/w and not more than 120.0 percent w/w of the stated amount of total polyphenols (sum of chebulagic acid, chebulic acid, gallic acid, corlagin, 1,3,6 trigalloyl glucose and ellagic acid).

Description. Brown to brown powder; with characteristic odour and bitter taste.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 6 volumes of *ethyl acetate*, 2 volumes of *formic* acid, 2 volumes of *toluene* and 1 volume of *methanol.*

Test solution. Dissolve 0.5 g of extract under examination with 100 ml *methanol* and filter.

Reference solution. A 0.1 per cent w/v solution of *chebulagic acid RS* and 0.1 per cent w/v solution of *gallic acid* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultra-violet light at 254 nm. Spray the plate with *ferric chloride reagent* . Heat the plate at 110° for 10 minutes and examine the plates under 365nm and under day light. The chromatographic profile of the test solution is similar to that of the reference solution.

B. In the assay the peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the chebulinic acid and gallic acid.

Tests

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 2.0 g by drying in an oven at 105° for 3 hours.

Total ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, (Method B) 20 ppm.

Microbial contamination (2.2.9). Complies with the microbial contamination test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 0.5 g of the extract containing 100 mg of polyphenols in 100 ml of water,and filter.

Reference solution (a). 0.01 per cent w/v solution of *chebulagic acid RS* in *water.*

Reference solution (b). 0.01 per cent w/v solution of *gallic acid RS* in *water.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase:A.a buffer solution prepared by mixtures dissolving 0.136 g of *Potassium di-hydrogen orthophosphate* in 500 ml of *water,* add 0.5 ml of *orthophosphoric acid* and dilute to 1000 ml with water.

B. *acetonitrile*

- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min)	mobile phase A (per cent v/v)	mobile phase B (per cent v/v)
0	95	5
18	75	25
25	65	35
28	65	35
35	95	5

Inject the reference solutions (a) and (b). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of Total polyphenols by summing the peak areas of *chebuliagic acid, ellagic acid, 1,3,6 tri galloyl glucose, corilagin,* gallic acid and *chebulic acid* using chebulagic acid. The relative retention times of various polyphenols with reference to gallic acid and chebulagic acid are as follows.

Chebulic acid	0.80	0.25
Gallic acid	1.00	0.32
Corlagin	2.57	0.81
1, 3, 6 Trigalloyl glucose	2.81	0.89
Chebulagic acid	3.17	1.00
Ellagic acid	3.49	1.10
Chebulinic acid	3.64	1.15

Usual strength. 40 per cent w/w.

Storage. Store protected from heat and moisture.

Hydrogenated Castor Oil

Castor Wax; Opalwax

Hydrogenated Castor Oil is refined, bleached, hydrogenated and deodorised castor oil. It consists mainly of the triglyceride of hydroxystearic acid.

Description. A white to yellow powder of uniform consistency and texture. It may have a hard, waxy consistency.

Tests

Melting range (2.4.21). 85° to 88°, determined by Method II.

Free fatty acids. Weigh accurately about 20 g, melt on a water-bath, add 75 ml of hot *ethanol (95 per cent)*, previously neutralised to *phenolphthalein solution* with *0.1 M sodium hydroxide*, swirl, add 1 ml of *phenolphthalein solution* and titrate with *0.1 M sodium hydroxide*, swirling vigorously until the solution remains faintly pink after being shaken for 60 seconds; not more than 11.0 ml of *0.1 M sodium hydroxide* is required.

Heavy metals (2.3.13). 2.0 g complies with the limit test for heavy metals, Method B (10 ppm).

Acetyl value (2.3.22). Not less than 143.

Acid value (2.3.23). Not more than 2.0.

Hydroxyl value (2.3.27). 154 to 162.

Iodine value (2.3.28). Not more than 5.0.

Peroxide value (2.3.35). Not more than 5.0.

Saponification value (2.3.37). 176 to 182.

Storage. Store at a temperature not exceeding 30°. Avoid exposure to excessive heat.

Ipecac Tincture

Ipecac Tincture obtained from roots and rhizomes of *Cephaelis ipecacuanha* A. Rich (Fam. Rubiaceae).

Ipecac Tincture contains not less than 90 per cent w/w and not more than 110 per cent w/w of alkaloids of Ipecac calculated as emetine.

Description. A yellowish brown liquid.

Identification

Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 2 volumes of *ammonia*, 15 volumes of *methanol*, 18 volumes of *ethyl acetate* and 65 volumes of *toluene*.

Test solution. Shake 2.0 ml of tincture under examination with 2 ml of water and 0.1 ml of *ammonia*. Add 10 ml of *ether* and shake. Separate the ether layer, dry it over about 2 g of *anhydrous sodium sulphate* & filter.

Reference solution. Dissolve 2.5 mg of *emetine hydrochloride RS* in *methanol* and dilute to 10 ml with the same solvent.

Apply to the plate 10 µl of each solution as bands of 10 mm by 2 mm. Allow the mobile phase to rise to 10 cm. Dry the plate in air. Spray the plate with 0.5 per cent w/v solution of *iodine* in *ethanol* (95 per cent v/v) solution. Heat the plate at 60° for 10 min and examine the plate in ultraviolet light at 365 nm and in day light. An intense yellow, fluorescent zone of emetine is observed in chromatogram of test solution corresponding to intense yellow fluorescent zone of emetine in chromatogram of reference solution.

Tests

Ethanol. Not less than 24.0 per cent and not more than 28.0 per cent.

Determine by gas chromatography (2.4.13).

Test solution. A 0.8 per cent v/v solution of the sample under examination and 02 per cent v/v of *acteonitrile* as internal standard.

Reference solution. A solution in water containing 0.2 per cent v/v of *ethanol* and 2 per cent v/v of *acetonitrile* (internal standard).

Chromatographic system
- a stainless steel column 1.8 m x 3 mm, packed with copolymer of ethylvinylbenzene and divinylbenzene (100 to 150 mesh),
- temperature:
  column. 120°,
  inlet port. 210°,
- a flame ionization detector at 210°,
- flow rate. Adjust the carrier flow so that acetonitrile, internal standard, elutes in 5 to 10 minutes.

Calculate the percentage v/v of ethanol.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Transfer 5 ml of sample into a 50 ml volumetric flask. Dilute to volume with 0.01M *hydrochloric acid* and mix.

Reference solution. A 0.010 per cent *emetine hydrochloride heptahydrate RS* and 0.010 per cent of *cephaeline* in 0.01M *hydrochloric acid*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecyl silane chemically bonded to porous silica (5 µm).
- mobile phase: a mixture of 50 volumes of *methanol* and 50 volumes of buffer prepared by dissolving 2.0 g of *1-heptane sulfonate* in 500 volumes *water*,
- column temperature: 50°
- flow rate. Adjust the flow rate so that the retention time of *emetine* is about 14 minutes,
- spectrophotometer set at 283 nm,
- injection volume 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of cephaeline and emetine as total alkaloids in the sample.

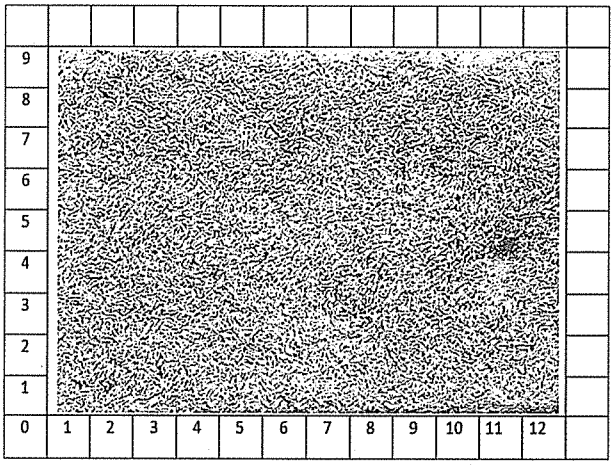
1 mg of *emetine hydrochloride* contains 0.868 mg of *emetine* and correction factor for *cephaeline* is 0.971.

Usual strengths. 0.1 per cent w/w; 0.2 per cent w/w.

Storage. Store protected from light and moisture.

Ispaghula Husk

Isapgol Husk; *Plantago ovata*



Ispaghula Husk consists of the epidermis and collapsed adjacent layers removed from the dried ripe seeds of *Plantago ovata* Forssk.(Fam. Plantaginaceae).

Description. Pale buff, brittle flakes, more or less lanceolate, up to 2 mm long and 1 mm wide at the centre, much broken into smaller fragments; many of the flakes having a small, brownish, oval spot, about 0.8 to 1.0 mm long, in the centre; the material swells rapidly in water, forming a stiff mucilage.

Identification

When mounted in *cresol* and examined under a microscope, the particles are found to be transparent and angular, the edges straight or curved and sometimes rolled. They are composed of polygonal prismatic cells with four to six straight or slightly curved walls; the cells vary in size in different parts of the seed coat, from about 25 mm to 60 mm at the summit of the seed, that is, near and over the brown spot, to 25 mm to 100 mm for the remainder of the epidermis except at the edges of the seed, where the cells are smaller, about 45 mm to 70 mm. When mounted in *ethanol (95 per cent)* and irrigated with *water*, the mucilage in the outer part of the epidermal cells swells rapidly and goes into solution, while the two inner layers of mucilage are more resistant and swell to form rounded papillae. When mounted in *0.005 M iodine*, occasional simple and two- to four-compound starch granules, about 2 mm to 10 mm, can be seen in some of the cells. Occasional fragments of thick-walled, reddish brown endosperm, cells with pitted walls and elongated fragments of grey embryo may be present.

Swelling power. Transfer 1 g to a 100-ml stoppered cylinder containing 90 ml of *water*, shake well for 30 seconds and allow to stand 24 hours, shaking gently on three occasions during this period. Add sufficient *water* to produce 100 ml, mix gently for 30 seconds, avoiding the entrapment of air, allow to stand for 5 hours and measure the volume of mucilage. Repeat the determination three times. The average of four determinations is not less than 40 ml.

Total ash (2.3.19). Not more than 4.5 per cent, determined on 1 g.

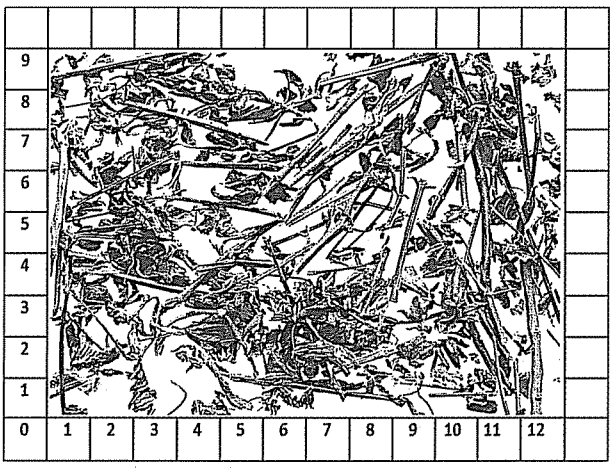
Acid-insoluble ash (2.3.19). Not more than 0.45 per cent.

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 0.5 g by drying in an oven at 105° for 5 hours.

Storage. Store protected from moisture and from attack by insects and rodents.

Kalmegh

Andrographis paniculata



Kalmegh consists of the dried aerial parts, mainly stems and leaves, of *Andrographis paniculata* Nees. (Fam. Acanthaceae).

Kalmegh contains not less than 1.0 per cent w/w of andrographolide, calculated on the dried basis.

Description. Taste, intensely bitter.

Identification

A. *Macroscopic* — Mixture of crisp, dark green-coloured broken leaves and quadrangular stems; leaves brittle. Stem fracture short, fibrous.

B. *Microscopic* — Stems quadrangular with collenchyma strands at angles and on side; small acicular crystals of calcium oxalate present in pith and cortex. Trichomes 1-3 celled, glandular hair disc-shaped and multicellular.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 7 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. Reflux 1 g of coarsely powdered substance under examination with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 10 ml.

Reference solution. Reflux 0.5 g of *kalmegh RS* with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *methanolic sulphuric acid* (20 per cent, v/v). Heat the plate at 120° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 3.0 per cent.

Water-soluble extractive (2.6.3). Not less than 12.0 per cent by Method I.

Total ash (2.3.19). Not more than 15 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2.5 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 50.0 ml.

Reference solution. A 0.1 per cent w/v solution of *andrographolide RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 65 volumes of *methanol* and 35 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 223 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of andrographolide.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Kalmegh Dry Extract

Kalmegh Dry Extract is obtained by extracting Kalmegh with *aqueous ethanol* or *ethanol* or any other suitable solvent.

Kalmegh Dry Extract contains not less than 90.0 per cent w/w and not more than 120.0 per cent w/w of the labelled amount of andrographolides (sum of andrographolide, neo-andrographolide and andrograpanin) The content of 14-deoxy-11,12-didehydroandrogapholide content shall not be more than one sixth of the amount of andrographolide.

Description. A light green to dark green powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 7 volumes of *chloroform* and 1 volume of *methanol*.

Test solution. Dissolve 200 mg of the extract under examination with 50 ml *methanol* and filter.

Reference solution. A 0.02 per cent w/v solution of *andrographolide RS* in *methanol*.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm and also

under day light. Spray the plate with *methanolic sulphuric acid* (20 per cent) and heat at 120° for 10 minutes. The chromatogram obtained with test solution shows a band corresponding to the band obtained using reference solution indicating the presence of andrographolide.

Tests

Acid insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss of drying (2.4.19). Not more than 5.0 per cent.

Microbial contamination (2.2.9). Complies with the microbial contamination test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 250 mg of the extract under examination or quantity equivalent to 20 mg of andrographolide in *methanol* by gently heating, make it up to 100.0 ml and filter.

Reference solution (a). A 0.02 per cent w/v solution of *andrographolide RS* in *methanol*.

Reference solution (b). A 0.005 per cent w/v solution of *14-deoxy-11,12-didehydroandrographolide RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 0.136 g of *potassium dihydrogen orthophosphate* in 500 ml of *water* and 0.5 ml of *orthophosphoric acid*, dilute to 1000 ml with *water*,
  B. *acetonitrile*,
- flow rate. 1.5 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 223 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase (per cent v/v)	Mobile phase (per cent v/v)
0	95	5
8	55	45
25	20	80
30	95	5

Inject reference solution (b). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent. The relative retention time with respect to andrographolide, for neo-andrographolide is about 1.2 and for andrograpanin is about 1.6.

Inject the test solution and reference solution (a).

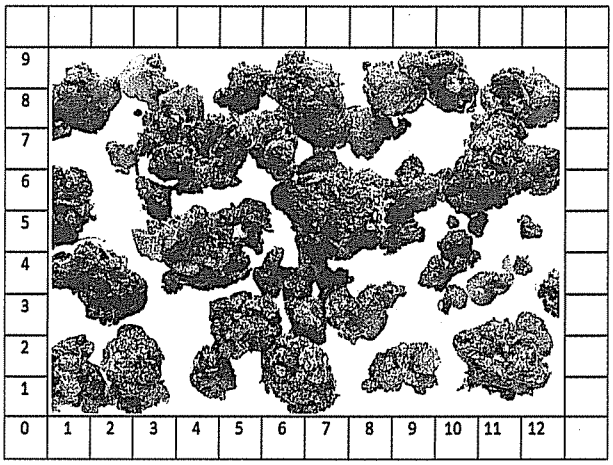
Calculate the content of 14-deoxy-11, 12-didehydroandrographolide and andrographolides in the extract.

Calculate andrographolide by summing the peak areas of andrographolide, neo-andrographolide, and andrograpanin.

Storage. Store protected from heat and moisture.

Kunduru

Sallaki Gum; Gum of *Boswellia serrata*



Kunduru is the gum-resin from *Boswellia serrata* Roxb. (Fam. Burseraceae).

Kunduru contains not less than 1.0 per cent w/w of total 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid, calculated on the dried basis.

Description. Translucent, brittle, whitish yellow substance, in roundish, club-shaped, pear-shaped, or irregular tears.

Identification

A. Macroscopic — Fracture dull. Slightly sticky to touch; odour, balsamic; taste slightly mucilaginous, bitter and aromatic.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 7 volumes of *hexane* and 3 volumes of *ethyl acetate*.

Test solution. Reflux 1 g of coarsely powdered substance under examination with 50 ml *methanol* on a boiling water-bath for 30 minutes, cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 10 ml of *methanol*.

Reference solution. Reflux 1 g of coarsely powdered *kunduru RS* with 50 ml *methanol* on a boiling water-bath for 30 minutes,

cool and filter. Evaporate the filtrate to dryness and dissolve the residue in 10 ml of *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light 254 nm and 365 nm, spray with *methanolic sulphuric acid* (10 per cent, v/v). Heat the plate at 110° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 35 per cent.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 12.0 per cent, determined on 0.2 g.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution (a). A 0.01 per cent w/v solution of *11-keto-β-boswellic acid RS* in *methanol.*

Reference solution (b). A 0.05 per cent w/v solution of *acetyl-11-keto-β-boswellic acid RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 90 volumes of *methanol* and 10 volumes of a mixture containing 5 ml of *acetonitrile* and 95 ml of *water*, adjusting the pH to 2.8 with *dilute orthophosphoric acid,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 247 nm,
- injection volume. 20 µl.

Inject the reference solution (a) and (b). The test is not valid unless the relative retention times are about 0.65 for 11-keto-β-boswellic acid and 1.0 for acetyl-11-keto-β-boswellic acid and the relative standard deviation for the replicate injections is not more than 2.0 per cent.

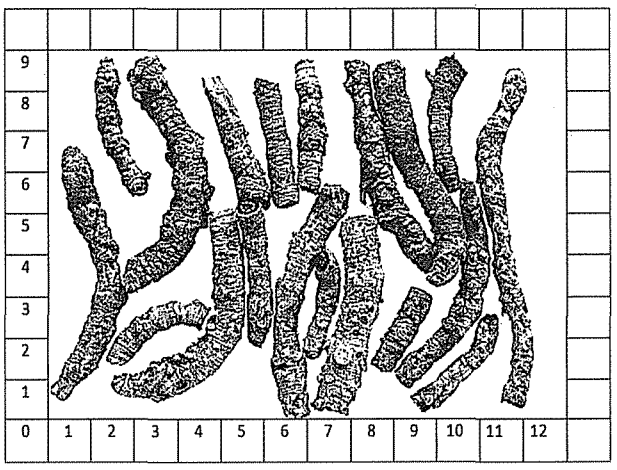
Inject the test solution.

Calculate the sum of the contents of 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Kutki

Picrorhiza kurroa



Kutki consists of dried roots of *Picrorhiza kurroa* Royle ex Benth.(Fam. Scrophulariaceae).

Kutki contains not less than 5 per cent w/w of kutkin, calculated on the dried basis.

Description. Rhizomes are sub cylindrical, straight or slightly curved, externally greyish with wrinkled surfaces, circular scars of roots and bud scales, with cork exposed at places.

Identification

A. *Macroscopic* — 3-6 cm long and about 1 cm thick, sub-cylindrical, straight, grayish brown, wrinkled roots. Odour is pleasant and tastes bitter.

B. *Microscopic* — There is well-developed periderm. About 7-10 layers of cork cells are seen. Cells of phelloderm loosely arranged.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 7.5 volumes of *ethyl acetate,* 2.2 volumes of *methanol* and 0.1 volume *glacial acetic acid.*

Test solution. Reflux 1 g of coarsely powdered substance under examination with 50 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol,* cool and filter. Combine both the filtrates and concentrate under vacuum to dryness. Extract the dried residue with 50 ml of

methanol at 50° for 10 minutes, filter the solution and use the filtrate for analysis.

Reference solution. Reflux 5 g of *kutki RS* with 50 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol*, cool and filter. Combine both the filtrates and concentrate under vacuum to dryness. Extract the dried residue with 10 ml of *methanol* at 50° for 10 minutes, filter the solution and use the filtrate for analysis.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° for 10 minutes and examin in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 15.0 per cent.

Water-soluble extractive (2.6.3). Not less than 25.0 per cent by method I.

Total ash (2.3.19). Not more than 6.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 5 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 25.0 ml of *methanol*, filter.

Reference solution. A 0.1 per cent w/v solution of *kutkin RS* in *methanol*.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 83 volumes of 1 per cent v/v *orthophosphoric acid* in *water* and 17 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

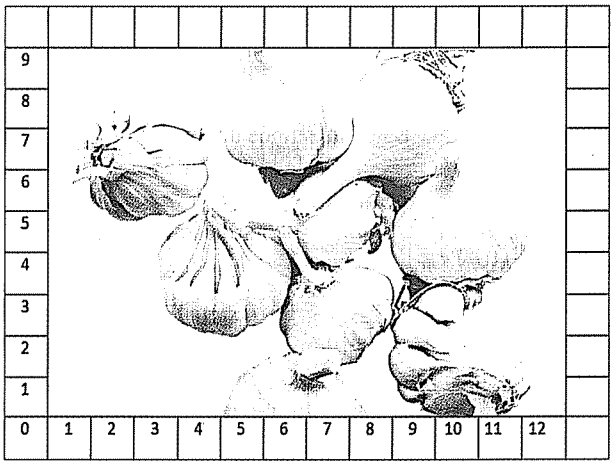
Inject the test solution and reference solution.

Calculate the content of kutkin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Lasuna

Garlic; *Allium sativum bulb*



Lasuna consists of the fresh or dried compound bulbs of *Allium sativum* Linn. (Fam. Liliaceae).

Lasuna contains not less than 0.2 per cent w/w of alliin, calculated on the dried basis.

Description. Bulbs made up of cloves and is wrapped in a white papery sheath with pungent taste and odour

Identification

A. *Macroscopic* — Each bulb has several cloves which are arranged in concentric rings and enclosed in a shining white or pinkish papery envelope. The cloves are attached to a flat, circular hard disc with numerous thin wiry roots from its underside and short, cylindrical out growth from the upper surface. Each clove is ovoid and further covered by papery sheath with a tail like structure at one and opposite to its attachment. The cloves in the outer ring are loose and white in colour where as cloves in the inner ring are adherent and pale pinkish in colour. Each clove covered with a white scale leaf and a pinkish white epidermis, easily separated from the solid portion. In the middle of the bulb a hollow, cylindrical, linear remnant of the scape is seen.

B. *Microscopic* — The protective leaf contains an epidermis enclosing a mesophyll free from chlorophyll. The outer epidermis consists of lignified sclereid cells of thick, pitted walls, elongated, covered with thin cuticle. The cortical cells

are thick walled, non lignified, tending to collapse on maturity, isodiametric and contain purple pigments. The vascular bundles consist of lignified spiral and annular vessels. The storage leaves show an outer epidermis of thin, delicate cells of variable shape. Stomata are present on the outer epidermis only at extreme tip near the base of the foliage leaves.The mesophyll consists of swollen storage parenchyma cells filled with fine granular reserve material.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 3 volumes of *butyl alcohol*, 1 volume of *n-propyl alcohol*, 1 volume of *glacial acetic acid* and 1 volume of *water*.

Test solution. Reflux 1 g of substance under examination with 20 ml of *methanol (50 per cent)* for 10 minutes, cool and filter. Reflux the residue with another 20 ml of *methanol (50 per cent)*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Reference solution. Reflux 1 g of *lasuna RS* with 20 ml of *methanol (50 per cent)* for 10 minutes, cool and filter. Reflux the residue with another 20 ml of *methanol (50 per cent)*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with 0.2 per cent w/v solution of *ninhydrin* in mixture of 95 volumes of *butyl alcohol* and 5 volumes of *2 M acetic acid*. Heat the plate at 100° to 105° for about 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

D. Transfer about 10 g of garlic bulbs that have been cut into small pieces to a beaker, add 10 ml of *1 M sodium hydroxide* and 10 ml of *water*, heat the beaker in a boiling water for 10 minutes, cool and filter. To 2 ml of this filtrate add few drops of freshly prepared solution of sodium nitroprusside. Appearance of a red or orange red colour indicates the presence of sulphur containing compounds in the sample.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Total ash (2.3.19). Not more than 5.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 65.0 per cent for fresh bulbs, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 2 g of the freshly peeled substance under examination. Add 15 ml of hot *water* and grind in a porcelain mortor and filter. Grind the residue further with 2 × 15 ml of hot *water* and filter. Combine all the filtrates and make up to volume 50 ml with distilled *water*.

Reference solution. A 0.004 per cent w/v solution of *alliin RS* in *water*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 0.1 per cent v/v *phosphoric acid* prepared by diluting 1ml of *phosphoric acid* to 1000 ml with *water*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

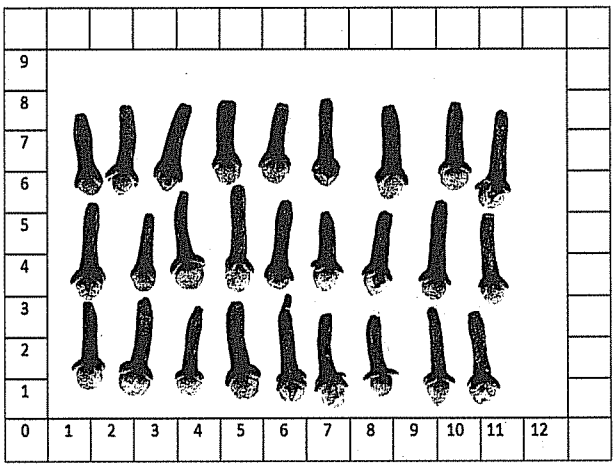
Inject the test and reference solution.

Calculate the content of alliin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Lavang

Syzigium aromaticum



Lavang consists of dried flower buds of *Syzigium aromaticum* (L.) Merr. & L.M. Perry. (Fam.Myrtaceae).

Lavang contains not less than 7.0 per cent w/w of eugenol, calculated on the dry basis.

Description. Aromatic odour and spicy taste.

Identification

A. *Macroscopic*—The flower bud is reddish brown in colour and consist of quadrangular stalked portion, the hypanthium, surrounded by four lobes of sepals.

B. *Microscopic*—Epidermis followed by thick cuticle,characteristic zone of bilateral vascular bundles, numerous schylogenous oil glands present beneath the epidermis. Central collumella and air spaces or lacuna in parenchyma regeion.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethylacetate*.

Test solution. Reflux 2.0 g of the coarsely powdered substance under examination with 50 ml *dichloromethane* for 15 minutes, cool and filter. Reflux the residue further for two times with 50ml *dichloromethane*, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. Reflux 1.0 g of the coarsely powdered Lavang RS with 25 ml *dichloromethane* for 15 minutes, cool and filter. Reflux the residue further for two times with 50 ml *dichloromethane*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with a *vanillin sulphuric acid reagent*. Heat the plate at 105° for 5 minutes and examine the plate at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1).Not more than 2.0 per cent.

Ethanol soluble extractive (2.6.2). Not less than 7.0 per cent.

Water soluble extractive (2.6.3). Not less than 20.0 per cent by Method I.

Total ash (2.3.19). Not more than 7.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0g complies with the limit test for heavy metals, Method B (20ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by gas chromatography (2.4.14).

Test solution. A 0.5 per cent w/v of substance under examination in *methanol*.

Reference solution. A 0.04 per cent w/v of *eugenol RS* in *methanol*.

Chromatographic system
- a stainless steel 30m×0.53mm capillary column
- temperature:
  column.70° for 1minute, then increased to 100° at a rate of 10° per minute, then increased to 200° at a rate of 20° per minute and maintained at this temperature for 2 minutes,
  inlet port. 220°,
  detector. 250°,
- flow rate 1.5 ml per minute of the carrier gas.

Inject reference solution and the test solution.

Calculate the eugenol content in the substance under examination using the ratios of the area of peak corresponding to eugenol to the area of the peak obtained with test solution and the reference solution.

Storage. Store protected from light, moisture and against attack by rodents and insects.

Malt Extract

Malt Extract is a product obtained by extracting malted grains of cereals (barley, cholam or wheat) with water at a suitable temperature and evaporation of the strained liquid until a viscous product is obtained. It may be mixed with 10 per cent by weight of Glycerin.

Malt Extract contains nitrogen equivalent to not less than 4.0 per cent w/w of protein.

Description. A sweet, viscous, light brown liquid; odour, pleasant and characteristic.

Tests

Refractive index (2.4.27). 1.489 to 1.498, determined at 20°.

Arsenic (2.3.10). Dissolve 10.0 g in 10 ml of *water*, add 10 ml of *brominated hydrochloric acid*, allow to stand for 5 minutes and remove the excess of bromine with a few drops of *stannous chloride solution AsT*. The resulting solution complies with the limit test for arsenic (1 ppm).

Lipase. To 95 ml of *water* add 6.5 ml of *triacetin* and 0.2 ml of a 0.1 per cent w/v solution of *bromocresol purple*, neutralise with *0.5 M sodium hydroxide* and add sufficient *water* to produce 110 ml. Place 50 ml of this solution in each of two large test-tubes (20 cm × 3 cm) A and B kept in a water-bath at 30° ± 1°. Insert in each tube a rubber stopper having two holes, one for the tip of a burette and the other for a short

glass tube through which passes a thread operating a glass stirring coil. Stir the contents of the tube until they attain the temperature of the water-bath. Prepare a solution of 5.0 g of the substance under examination in 10 ml of *water*. To tube A add 1 ml of this solution; to tube B add 1 ml of this solution after previous boiling. Adjust and maintain the pH of the two tubes to between 6.2 and 6.4 by the dropwise addition of *0.05 M sodium hydroxide*, stirring frequently. After 6 hours, the difference between the amounts of *0.05 M sodium hydroxide* added to the tubes is not more than 1.0 ml.

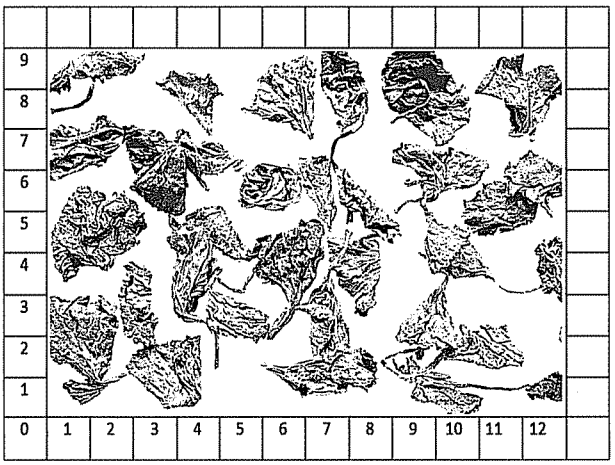
Assay. Weigh accurately about 5.0 g into a 200-ml long-necked flask and carry out the determination of nitrogen, Method A (2.3.30), using *0.05 M sulphuric acid* instead of *0.1 M sulphuric acid*.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.001401 g of N and multiply the result by 6.25 to obtain the protein content.

Storage. Store protected from moisture.

Mandukaparni

Gotu Kola; *Centella asiatica*



Mandukaparni consists of the dried aerial parts of *Centella asiatica* (Linn.) Urban. (Fam. Umbelliferae).

Mandukaparni contains not less than 0.5 per cent w/w of asiaticoside, calculated on the dried basis.

Description. A green to greenish yellow in colour, taste, slightly bitter and sweet.

Identification

A. *Macroscopic* — A slender trailing herb with rooted nodes and internodes. Leaves with elongated petioles and sheathing leaf bases; lamina reniform with crenate margin.

B. *Microscopic* — Animocytic stomata on both surfaces, more on lower surface; petiole epidermis has calcium oxalate prisms; vascular bundles seven arranged in 'U' shape, parenchyma cells contain simple and compound starch granules.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *chloroform*, 32 volumes of *glacial acetic acid*, 12 volumes of *methanol* and 8 volumes of *water*.

Test solution. Reflux 2 g of coarsely powdered substance under examination with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 10 ml under reduced pressure.

Reference solution. Reflux 1 g of *mandukaparni RS* with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 6.0 per cent.

Water-soluble extractive (2.6.3). Not less than 15.0 per cent by Method I.

Total ash (2.3.19). Not more than 24 per cent.

Acid-insoluble ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 3 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution. A 0.1 per cent w/v solution of *asiaticoside RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octylsilane bonded to porous silica (10 µm),
- mobile phase: 25 volumes of *acetonitrile* and 75 volumes of *water,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

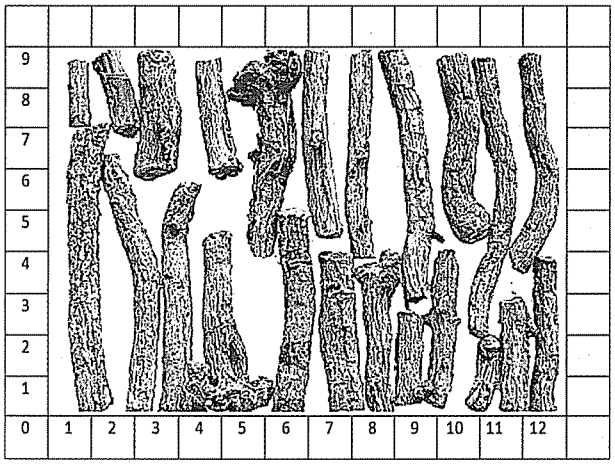
Inject the reference solution and the test solution.

Calculate the content of asiaticoside.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Manjistha

Indian Madder; *Rubia cordifolia*



Manjistha consists of dried stem of *Rubia cordifolia* Linn.*sensu* Hook.f. (Fam. Rubiaceae).

Manjistha contains not less than 0.02 per cent w/w of rubiadin, calculated on the dried basis.

Description. A cylindrical, slightly flattened, wiry pieces of brown to purple coloured root with mild bitter taste.

Identification

A. *Microscopic* — Exfoliating cork, consisting of 4-12 or more layered radially arranged, thin walled cells.

B. *Macroscopic* — Stem, slender, cylindrical, wiry and about 0.5 cm thick. It is brown to purple coloured and with longitudinal cracks.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 7 volumes of *toluene,* 25 volumes of *ethyl acetate* and 0.5 volumes *glacial acetic acid.*

Test solution. To 4 g of coarsely powdered substance under examination, add 100 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 50 ml *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 100 ml.

Reference solution. To 1 g of coarsely powdered manjistha, add 100 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 50 ml *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *anisaldehyde sulphuric acid reagent.* Heat the plate at 100° for 5 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 4.0 per cent.

Water-soluble extractive (2.6.3). Not less than 20.0 per cent by method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 0.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 5 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 4 g of coarsely powdered substance under examination, add 100 ml of *methanol,* reflux on a water-bath 15 minutes, cool and filter. Reflux the residue 2 × 50 ml *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 100.0 ml.

Reference solution. A 0.002 per cent w/v solution of *rubiadin RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution pH 2.5 prepared by dissolving 0.136 g of *potassium di-hydrogen*

orthophosphate in 900 ml of *water*, add 0.5 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*,

B. *acetonitrile*,

- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
5	50	50
15	20	80
30	20	80
35	50	50
40	65	35

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

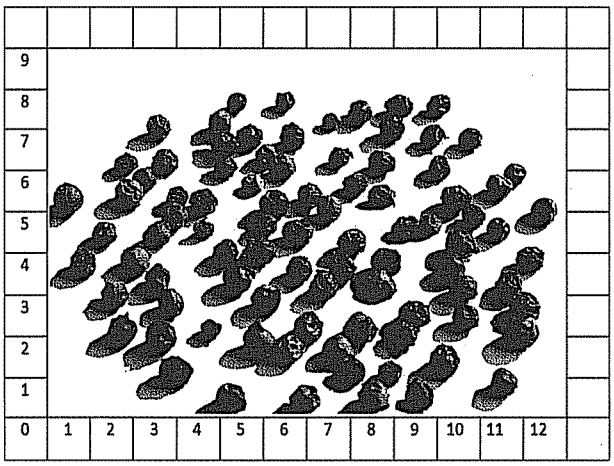
Inject the test solution and reference solution.

Calculate the content of rubiadin.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Maricha

Black pepper; pepper; *Piper nigrum*



Maricha consists of the unripe fruits of *Piper nigrum* Linn. (Fam. Piperaceae).

Maricha contains not less than 2.5 per cent w/w of piperine, calculated on the dried basis.

Description. Fruits are globular or oblong. They have a blackish brown cover, with raised reticulated wrinkles. The odour is aromatic and the taste is strong and pungent.

Identification

A. *Macroscopic* — The fruits are globular or oblong, 4-6 mm in diameter. The outer cover is blackish brown, with raised reticulated wrinkles. One seeded, seeds are white and hollow.

B. *Microscopic* — The fruit has a well differentiated pericarp, testa and perisperm. Isolated, tangentially elongated oil cells are in the outer region of the mesocarp. Endocarp has beaker shaped stone cells and numerous polyhedral masses of starch grains. Testa has a single layer of yellow colored cells.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *benzene*, 30 volumes of *ethyl acetate* and 10 volumes of *diethyl ether*.

Test solution. To 2 g of the coarsely powdered substance under examination, add 50 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Reference solution. To 2 g of the *maricha RS*, add 50 ml of *methanol* and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *vanillin sulphuric acid reagent*. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 6.0 per cent.

Water-soluble extractive (2.6.3). Not less than 6.0 per cent by method I.

Total ash (2.3.19). Not more than 7.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of coarsely powdered substance under examination, add 50 ml of *methanol*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool

and dilute to 100.0 ml with *methanol* and filter. Dilute further if necessary.

Reference solution. A 0.01 per cent w/v solution of *piperine RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase.A. a buffer solution pH 2.5 prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 500 ml of *water*, add 0.5 ml of *orthophosphoric acid* and dilute to 1000 ml with *water*,
- flow rate. 1.5 ml per minute,

  B.*acetonitrile,*
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 345 nm,
- injection volume. 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per centv/v)
0	95	5
18	55	45
25	20	80

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution. The relative retention time of piperine is 1.

Calculate the content of piperine.

Storage. Store protected from moisture and against attack by insects and rodents.

Mentha Oil

Mentha

Mentha Oil is the volatile oil distilled with steam from various species of *Mentha* (Fam. Labiatae) and rectified if necessary.

Mentha Oil contains not less than 50.0 per cent w/w of total *menthol*, $C_{10}H_{20}O$.

Description. A colourless or yellowish, clear liquid; odour, characteristic and pleasant.

Tests

Acidity or alkalinity. A solution of 1 ml in 3.5 ml of *ethanol (70 per cent)* is neutral to *litmus.*

Weight per ml (2.4.29). 0.892 g to 0.910 g.

Specific optical rotation (2.4.22). –18.0° to –33.0°.

Assay. Place about 10.0 g in an acetylation flask, add 10 ml of *acetic anhydride* and 1 g of *anhydrous sodium acetate*, attach a reflux condenser and boil for 2 hours. Cool, add 30 ml of *water* and warm on a water-bath for 15 minutes with occasional shaking. Transfer the contents of the flask to a separating funnel, reject the water layer and wash the remaining oil with *water* until the last washing no longer shows acid reaction. Dry the resulting oil by shaking with 2 g of *anhydrous sodium sulphate*, allow to stand for 30 minutes and filter through a dry filter paper. Weigh accurately about 1.5 g of the dry acetylated oil, add 3 ml of *ethanol (95 per cent)* and 0.1 ml of *phenolphthalein solution* and dropwise, *0.5 M ethanolic potassium hydroxide* until the solution acquires a faint pink colour. Add a further 20.0 ml of the alkali, attach a reflux condenser and boil for 1 hour on a water-bath. Cool, add 1 ml of *phenolphthalein solution* and titrate the excess of alkali with *0.5 M hydrochloric acid.* Repeat the operation with the same quantities of the same reagents in the same manner without the oil and calculate the amount of total menthol from the following expression.

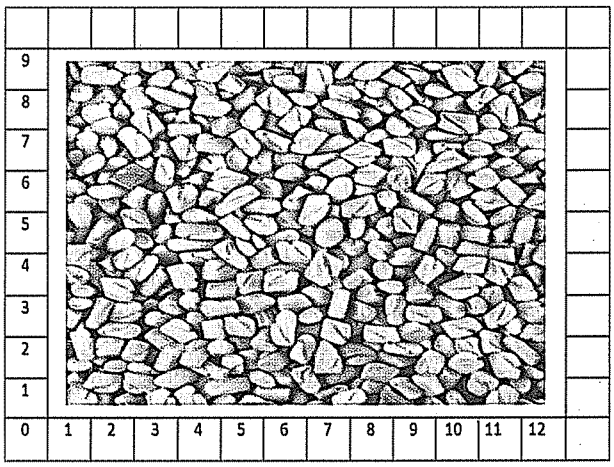
$$\text{Total menthol (per cent)} = \frac{(a-b)\times 7.813}{S-(a-b)\times 0.021}$$

where, S is the amount, in g, of the acetylated sample taken, a is the amount, in ml, of *0.5 M hydrochloric acid* consumed in the blank test, and b is the amount, in ml, of *0.5 M hydrochloric acid* consumed in saponification of the acetylated oil.

Storage. Store protected from light and moisture.

Methi

Trigonella foenum-graecum



Methi is dried, ripe seeds of *Trigonella foenum-graecum* L. (Fam.Fabaceae).

Methi contains not less than 0.1 per cent w/w of trigonelline on dry basis.

Description. The seed is hard, flattened, brown or reddish-brown and more or less rhomboidal with rounded edges. The widest surfaces are marked by a groove that divides the seed into 2 unequal parts. The smaller part contains the radicle; the larger part contains the cotyledons. They have a strong aromatic characteristic odour.

Identification

A. *Macroscopic* — Seed is rhomboidal with rounded edges. It is 3 mm to 5 mm long, 2 mm to 3 mm wide and 1.5 mm to 2 mm thick. The widest surfaces are marked by a groove that divides the seed into two unequal parts. The smaller part contains the radicle; the larger part contains the cotyledons.

B. Reduce to a powder. The powder is yellowish-brown. Examine under a microscope using *chloral hydrate solution*. The powder shows fragments of the testa in sectional view with thick cuticle covering lageniform epidermal cells, with an underlying hypodermis of large cells, narrower at the upper end and constricted in the middle, with bar-like thickenings of the radial walls; yellowish-brown fragments of the epidermis in surface view, composed of small, polygonal cells with thickened and pitted walls, frequently associated with the hypodermal cells, circular in outline with thickened and closely beaded walls; fragments of the hypodermis viewed from below, composed of polygonal cells whose bar-like thickenings extend to the upper and lower walls; parenchyma of the testa with elongated, rectangular cells with slightly thickened and beaded walls; fragments of endosperm with irregularly thickened, sometimes elongated cells, containing mucilage.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 30 volumes of *water* and 70 volumes of *methanol*.

Test solution. To 1.0 g of the powdered substance under examination, add 5.0 ml of the *methanol*. Heat on a water-bath at 65° for 5 minutes, cool and filter.

Reference solution. Dissolve 3 mg of *trigonelline hydrochloride* in 1.0 ml of *methanol*.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *potassium iodobismuthate solution*. Heat the plate at 105° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution. It also shows in its upper half, a broad light brownish-yellow zone (triglycerides).

Tests

Swelling Power. Not less than 6.0, determined on the powdered substance under examination, determined by the following method.

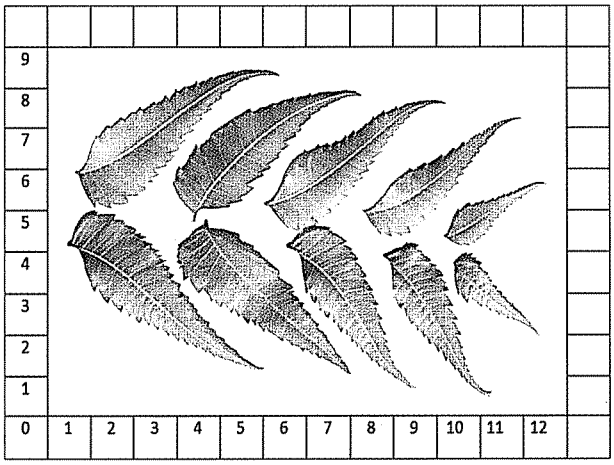
Transfer 1g to a 100 ml stoppered cylinder containing 90 ml of *water*, shake well for 30 seconds and allow to stand 24 hours, shaking gently on three occasions during this period. Add sufficient water to produce 100 ml, mix gently for 30 seconds, avoiding the entrapment of air, allow to stand for 5 hours and measure the volume of mucilage. Repeat the determination three times. The average of four determinations is not less than 40 ml.

Ash (2.3.19). Not more than 5.0 per cent.

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 1 g by drying in an oven at 105°.

Neem

Azadirachta indica



Neem consist of dried leaves of *Azadirachta indica* A.Juss. (Fam. Meliaceae).

Neem leaves contain not less than 1.0 per cent w/w of the stated amount of rutin, calculated on the dry basis.

Description. Characteristic odour and bitter taste.

Identification

A. *Macroscopic*—The leaves dark green, the petioles are short.The shape of mature leaflets is more assymetrical and their margins are dentate with the exception of the base of their basiscopal half, which is very strongly reduced and cuneate or wedge-shaped leaves.

B. *Microscopic*—Upper and lower epidermis, exhibiting two layers of palisade cells below the upper epidermis. The spongy parenchyma with intercellular spaces abundant on the border line of palisade cells. Midrib shows numerous collenchymatous cells below upper and lower epidermis. A characteristic zone of vascular bundles is present.

C. Determine by thin-layer chromatography (2.4.17) coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 25 volumes of *ethyl acetate*, 15 volumes of *n-butanol* , 5 volumes of *formic acid* and 5 volumes of *water*.

Test solution. Reflux 5.0 g of the coarsely powdered substance under examination with 100 ml of n-hexane for 2 hours, cool and filter. Reflux the residue further for 1hour with 100 ml of methanol, cool and filter. Concentrate under vacuum to 25ml.

Reference solution. Reflux 2.5g of the coarsely powdered drug with 100 ml n-hexane for 2 hours, cool and filter. Reflux the residue further for 1hour with 100 ml of methanol, cool and filter. Concentrate under vacuum to 10 ml.

Apply to the plate 10µl of each solution as bands 10mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254. Spray the plate with *anisaldehyde sulphuric acid reagent*. Heat the plate at 105° for 5 minutes and examine the plate at 365 nm and in day light. The chromatographic profile of test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol soluble extractive (2.6.2). Not less than 7.0 per cent.

Water soluble extractive (2.6.3). Not less than 19.0 per cent by Method I.

Total ash (2.3.19). Not more than 12.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20ppm).

Loss on drying (2.4.19).Not more than 12.0 per cent, determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5.0 g of the coarsely powdered substance under examination with 100 ml of *n-hexane* for 2 hours, cool and filter. Reflux the residue further for 1 hour with 100 ml of *methanol*, cool and filter. Concentrate under vacuum to 25ml.

Reference solution. A 0.05 percent w/v of *rutin RS* in *methanol*.

Chromatographic system

- stainless steel column 25cm×4.6mm packed with octadecylsilane bonded to porous silica(5µm),
- mobile phase:30 volumes of *acetonitrile* and 70 volumes of 0.5 per cent *formic acid* prepared by diluting 5ml of *formic acid* to 1000ml with *water*.
- flow rate, 1ml per minute,
- spectrophotometer set at 340nm,
- injectiion volume.20 µl.

Inject the reference solution .The test is not valid unless the relative standard deviation for the replicate injections is not more than 2 per cent.

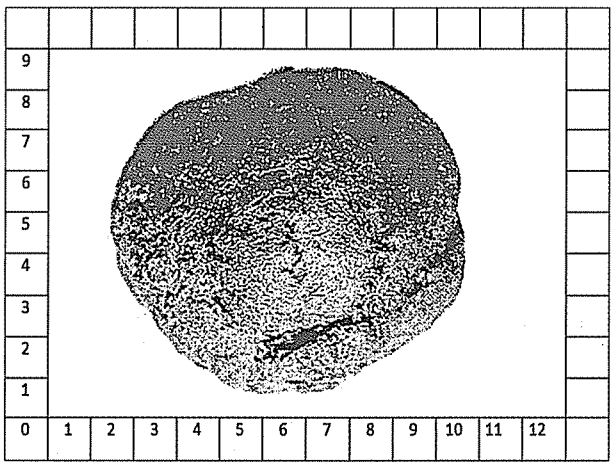
Inject the reference solution and the test solution.

Calculate the content of rutin.

Storage. Store protected from heat, moisture and against attack by rodents and insects.

Opium

Raw Opium; *Papaver somniferum*



Opium is the air-dried latex obtained by incision from the unripe capsules of *Papaver somniferum* Linn (Fam. Papaveraceae).

Opium contains not less than 10.0 per cent w/w of morphine, $C_{17}H_{19}NO_3$, and not less than 2.0 per cent w/w of codeine, $C_{18}H_{21}NO_3$, both calculated on the dried basis.

Description. Masses of various sizes which tend to be soft and shiny and, after drying, hard and brittle; usually in somewhat irregularly shaped masses (natural opium) or moulded into masses of more uniform size and shape (manipulated opium); colour, blackish brown; odour, strong and characteristic.

Identification

Strip off any covering, cut the substance under examination into thin slices, if necessary, dry at about 60° for 48 hours and reduce to a powder.

A. When examined under a microscope, a suspension in a 2 per cent w/v solution of *potassium hydroxide* appears as granules of latex agglomerated in irregular masses and light brown elongated filaments. Some fragments of vessels and rather elongated, refringent crystals are also visible, as well as

a smaller number of round pollen grains and fragments of elongated fibres. Hairs of various lengths with sharp points and a few grains of starch introduced during the handling of the latex may be present. Fragments of epicarp consisting of polygonal cells with thick walls defining a stellate lumen may also be present.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A freshly prepared mixture of 20 volumes of *acetone*, 20 volumes of *toluene*, 3 volumes of *ethanol (95 per cent)* and 1 volume of *strong ammonia solution*.

Test solution. Triturate 0.1 g of the powdered substance with 5 ml of *ethanol (70 per cent)*, add 3 ml of *ethanol (70 per cent)*, transfer to a 25-ml conical flask and heat in a water-bath at 50° to 60° for 30 minutes, with stirring. Cool, filter, wash the filter with *ethanol (70 per cent)* and dilute the filtrate to 10 ml with the same solvent.

Reference solution. Dissolve 2 mg of *papaverine hydrochloride RS*, 12 mg of *codeine phosphate RS*, 12 mg of *noscapine hydrochloride RS*, and 25 mg of *morphine hydrochloride RS* in *ethanol (70 per cent)* and dilute to 25 ml with the same solvent.

Apply to the plate 20 µl of each solution as 20 mm bands. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool and spray with *potassium iodobismuthate solution* and then with a 0.4 per cent w/v solution of *sulphuric acid*. The chromatogram obtained with the reference solution shows in the lower part an orange-red or red band (morphine), above it a similarly coloured band (codeine) and in the upper part an orange-red or red band (papaverine) and above it a similarly coloured band (noscapine). The chromatogram obtained with the test solution shows orange-red or red bands corresponding to those in the chromatogram obtained with the reference solution. The chromatogram obtained with the test solution may also show a dark red band (thebaine) situated between those due to codeine and to papaverine.

C. To 1 g of the powdered substance add 5 ml of *water*, shake for 5 minutes, filter and add to the filtrate 0.25 ml of *ferric chloride solution*; a red colour develops which does not disappear on the addition of 0.5 ml of *2 M hydrochloric acid*.

Tests

Thebaine. Not more than 3 per cent, calculated on the dried basis.

Determine by liquid chromatography (2.4.14).

Test solution. Use the test solution prepared in the assay.

Reference solution. Dissolve 25 mg of *thebaine* in sufficient mobile phase to produce 25.0 ml and dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic system as described in the Assay.

The test is not valid unless the capacity factor for thebaine is at least 3.0 and the number of theoretical plates is at least 3000. Calculate the percentage content of thebaine from the expression given in the assay.

Total ash (2.3.19). Not more than 6.0 per cent, determined on 0.5 g.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.25 g cut into thin slices, by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Suspend about 1.0 g, accurately weighed substance under examination, cut into thin slices, in 50 ml of *ethanol (50 per cent)*, mix with the aid of ultrasound for 1 hour, allow to cool, dilute to 100.0 ml with the same solvent and allow to stand. To 10.0 ml of the supernatant liquid add 5 ml of *ammonia buffer pH 9.5*, dilute to 25.0 ml with *water*, mix, transfer 20.0 ml of the solution to a column (about 15 cm × 30 mm) containing 15 g of *kieselguhr for column chromatography* and allow to stand for 15 minutes. Elute with two quantities, each of 40 ml, of a mixture of 85 volumes of *dichloromethane* and 15 volumes of *2-propanol*, evaporate the eluate to dryness at 40° at a pressure of 2 kPa, transfer the residue to a 25-ml volumetric flask with the aid of the mobile phase and dilute to volume with the same solvent.

Reference solution. Weigh accurately 0.1 g of *morphine hydrochloride* and 25 mg of *codeine*, dissolve in sufficient of the mobile phase to produce 25.0 ml and dilute 10.0 ml of this solution to 100.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm), fitted with a guard column 4 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 1.0 g of *sodium heptanesulphonate* in 420 ml of *water*, adjusting to pH 3.2 with *phosphoric acid* that has been diluted to contain 0.49 per cent w/v solution of H_3PO_4 (about 5 ml) and adding 180 ml of *acetonitrile*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm.

Inject suitable volumes of each solution. The assay is not valid unless the resolution between the peaks corresponding to morphine and codeine is at least 2.5. If necessary, adjust the volume of *acetonitrile* in the mobile phase. Inject the reference solution six times. The assay is not valid unless the relative standard deviation of the peak area for morphine is not more than 1.0 per cent.

Inject the test solution and the reference solution.

Calculate the percentage content of each alkaloid from the expression

$$\frac{w_1 \times A_2 \times 625 \times 100}{w_2 \times A_1 \times 5(100-h)}$$

where, w_1 = weight, in g, of the alkaloid used to prepare the reference solution,

w_2 = weight, in g, of the substance under examination used to prepare the test solution,

A_1 = area of the peak corresponding to the alkaloid in the chromatogram obtained with the reference solution,

A_2 = area of the peak corresponding to the alkaloid in the chromatogram obtained with the test solution,

h = percentage loss on drying.

For calculation, 1 mg of *morphine hydrochloride* may be taken as equivalent to 0.759 mg of morphine and 1 mg of *codeine phosphate* may be taken as equivalent to 0.943 mg of codeine.

Storage. Store protected from light and moisture.

Opium Powder

Opium Powder is Opium dried at a temperature not exceeding 70°, reduced to a fine or moderately fine powder and adjusted by the addition of Lactose, suitably coloured with Caramel, or other suitable diluent to contain about 10 per cent of morphine and 2 per cent of codeine.

Opium Powder contains not less than 9.5 per cent w/w and not more than 10.5 per cent w/w of morphine, $C_{17}H_{19}NO_3$, and not less than 1.9 per cent w/w and not more than 2.1 per cent w/w of codeine, $C_{18}H_{21}NO_3$, both calculated on the dried basis.

Description. A light brown powder consisting of yellowish brown or brownish red particles; odour, characteristic.

Identification

A. When examined under a microscope, the residue obtained after extraction with *water*, appears as granules of latex agglomerated in irregular masses and light brown elongated filaments. Some fragments of vessels and rather elongated, refringent crystals are also visible, as well as a smaller number of round pollen grains and fragments of elongated fibres. Hairs of various lengths with sharp points and a few grains of starch introduced during the handling of the latex may be present. Fragments of epicarp consisting of polygonal cells with thick walls defining a stellate lumen may also be present and if powdered cocoa husk is present, the following: brown colour of the fragments; narrow spiral vessels about 10 to 20 mm wide, in groups of from one to six, traversing a spongy parenchyma of thin-walled cells about 40 to 60 mm in either direction and united by arm-like projections enclosing almost circular intercellular spaces; fragments of the sclerenchymatous layer, consisting of thick-walled lignified brown, rectangular to polyhedral cells in a single layer, individual cells about 5 to 10 µm wide and 10 to 30 µm long; fragments of mucilage staining in *ruthenium red solution*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 20 volumes of *acetone*, 20 volumes of *toluene*, 3 volumes of *ethanol (95 per cent)* and 1 volume of *strong ammonia solution*.

Test solution. Triturate 0.10 g of the powdered substance with 5 ml of *ethanol (70 per cent)*, add 3 ml of *ethanol (70 per cent)*, transfer to a 25-ml conical flask and heat in a water-bath at 50° to 60° for 30 minutes, with stirring. Cool, filter, wash the filter with *ethanol (70 per cent)* and dilute the filtrate to 10 ml with the same solvent.

Reference solution. Dissolve 2 mg of *papaverine hydrochloride RS*, 12 mg of *codeine phosphate RS*, 12 mg of *noscapine hydrochloride RS*, and 25 mg of *morphine hydrochloride RS* in *ethanol (70 per cent)* and dilute to 25 ml with the same solvent.

Apply to the plate 20 µl of each solution as 20 mm bands. After development, dry the plate at 100° to 105° for 15 minutes, allow to cool and spray with *potassium iodobismuthate solution* and then with a 0.4 per cent w/v solution of *sulphuric acid*. The chromatogram obtained with the reference solution shows in the lower part an orange-red or red band (morphine), above it a similarly coloured band (codeine) and in the upper part an orange-red or red band (papaverine) and above it a similarly coloured band (noscapine). The chromatogram obtained with the test solution shows orange-red or red bands corresponding to those in the chromatogram obtained with the reference solution. The chromatogram obtained with the test solution may also show a dark red band (thebaine) situated between those due to codeine and to papaverine.

C. To 1 g of the powdered substance add 5 ml of *water*, shake for 5 minutes, filter and add to the filtrate 0.25 ml of *ferric chloride solution*; a red colour develops which does not disappear on the addition of 0.5 ml of *2 M hydrochloric acid*.

Tests

Thebaine. Not more than 3.0 per cent, calculated on the dried basis.

Determine by liquid chromatography (2.4.14).

Test solution. Use the test solution prepared in the Assay.

Reference solution. Dissolve 25 mg of *thebaine* in sufficient mobile phase to produce 25.0 ml and dilute 10.0 ml of this solution to 100.0 ml with the mobile phase.

Chromatographic condition as described in the Assay.

The test is not valid unless the capacity factor for thebaine is at least 3.0 and the number of theoretical plates is at least 3000. Calculate the percentage content of thebaine from the expression given in the Assay.

Total ash (2.3.19). Not more than 6.0 per cent, determined on 0.5 g.

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 0.25 g by drying in an oven at 105° for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Suspend about 1.0 g, accurately weighed, of the substance under examination, cut into thin slices, in 50 ml of *ethanol (50 per cent),* mix with the aid of ultrasound for 1 hour, allow to cool, dilute to 100.0 ml with the same solvent and allow to stand. To 10.0 ml of the supernatant liquid add 5 ml of *ammonia buffer pH 9.5*, dilute to 25.0 ml with *water*, mix, transfer 20.0 ml of the solution to a column (about 15 cm × 30 mm) containing 15 g of *kieselguhr for column chromatography* and allow to stand for 15 minutes. Elute with two quantities, each of 40 ml, of a mixture of 85 volumes of *dichloromethane* and 15 volumes of *2-propanol*, evaporate the eluate to dryness at 40° at a pressure of 2 kPa, transfer the residue to a 25-ml volumetric flask with the aid of the mobile phase and dilute to volume with the same solvent.

Reference solution. Weigh accurately 0.1 g of *morphine hydrochloride RS* and 25 mg of *codeine phosphate RS*, dissolve in sufficient of the mobile phase to produce 25.0 ml and dilute 10.0 ml of this solution to 100.0 ml with the same solvent.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (5 µm), fitted with a guard column 4 cm x 4.6 mm packed with octylsilane bonded to porous silica (5 µm),
- mobile phase: 1.0 g of *sodium heptanesulphonate* in 420 ml of *water*, adjusting to pH 3.2 with *phosphoric acid* that has been diluted to contain 0.49 per cent w/v solution of H_3PO_4 (about 5 ml) and adding 180 ml of *acetonitrile,*
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm.

Inject suitable volume of reference solution. The test is not valid unless the resolution between the peaks corresponding to morphine and codeine is at least 2.5. If necessary, adjust the volume of *acetonitrile* in the mobile phase. The relative standard deviation of the peak area for morphine is not more than 1.0 per cent.

Inject the test solution and the reference solution.

Calculate the percentage content of each alkaloid from the expression

$$\frac{w_1 \times A_2 \times 625 \times 100}{w_2 \times A_1 \times 5(100-h)}$$

where, w_1 = weight, in g, of the alkaloid used to prepare the reference solution,

w_2 = weight, in g, of the substance under examination used to prepare the test solution,

A_1 = area of the peak corresponding to the alkaloid in the chromatogram obtained with the reference solution,

A_2 = area of the peak corresponding to the alkaloid in the chromatogram obtained with the test solution,h

h = percentage loss on drying.

For calculation, 1 mg of *morphine hydrochloride* may be taken as equivalent to 0.759 mg of morphine and 1 mg of *codeine phosphate* may be taken as equivalent to 0.943 mg of codeine.

Storage. Store protected from light and moisture.

Papain

Carica papaya

Papain is an enzyme or a mixture of enzymes obtained from the juice of the unripe fruit of *Carica papaya* Linn. (Fam. Caricaceae). It may contain a suitable diluent such as Lactose.

Papain contains not less than the minimum protease activity determined under the conditions of the Assay.

Description. A white to light brown, amorphous or slightly granular powder; odour, characteristic.

Tests

Microbial contamination (2.2.9). 1.0 g is free from *Escherichia coli* and 10.0 g is free from *salmonellae.*

Loss on drying (2.4.19). Not more than 7.0 per cent, determined on 1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa for 4 hours.

Assay. Weigh accurately about 0.5 g, triturate with 10 ml of *cysteine hydrochloride solution* and dilute to 100.0 ml with *water*. To 30 ml of *water* in each of two flasks add 15.0 ml of *casein solution* and maintain at 60° by heating on a *water-bath*. To the first flask add 5.0 ml of the solution of the substance under examination, and to the second flask add 5.0 ml of the same solution, previously boiled for 2 minutes and cooled. Maintain the solutions at 60° for 30 minutes, cool

rapidly to room temperature and add to each flask 0.75 ml of *phenolphthalein solution* and 10 ml of *formaldehyde solution*, previously neutralised to *phenolphthalein solution*. Titrate both solutions with *0.1 M sodium hydroxide* to the same definite pink colour; the difference between the two titrations is not less than 4.5 ml.

Storage. Store protected from light and moisture.

Labelling. The label states the name of any added substance.

Peppermint Oil

Peppermint Oil is obtained by steam distillation from the aerial parts of the flowering plant of *Mentha piperita* L. and *M. arvensis* var. *piperascens* (Fam. Labiatae).

Peppermint Oil contains not less than 4.5 per cent w/w and not more than 10.0 per cent w/w of esters, calculated as menthyl acetate, $C_{12}H_{22}O_2$, not less than 44.0 per cent w/w of free alcohols, calculated as menthol, $C_{10}H_{20}O$, and not less than 15.0 per cent w/w and not more than 32.0 per cent w/w of ketones, calculated as menthone, $C_{10}H_{18}O$.

Description. A colourless, pale yellow or pale greenish yellow liquid; odour, characteristic; taste, characteristic followed by sensation of cold.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G F254*.

Mobile phase. A mixture of 95 volumes of *toluene* and 5 volumes of *ethyl acetate*.

Test solution. Dissolve 1 g of the oil under examination in 100 ml of *toluene*.

Reference solution. Dissolve 50 mg of *(–)menthol RS*, 20 µl of *cineole RS*, 10 mg of *thymol RS* and 10 µl of *menthyl acetate RS* in sufficient *toluene* to produce 10 ml.

Apply to the plate 20 µl of test solution and 10 µl of reference solution as bands 20 mm by 3 mm. After development, dry the plate in air until the odour of solvent is no longer detectable and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution there are no quenching bands at Rf values slightly lower than that of the faint band due to thymol in the chromatogram obtained with reference solution (carvone and pulegone). Spray the plate with *anisaldehyde solution* and examine in daylight after heating at 105° for 5 minutes. The chromatogram obtained with reference solution shows, in order of increasing Rf value, an intense blue to violet band (menthol) in the lower third, a violet-blue to brown band (cineole), a pink band (thymol) and a violet-blue band (menthyl acetate). The chromatogram obtained with test solution shows an intense band corresponding to menthol, a band corresponding to cineole, a violet-blue band corresponding to menthyl acetate in the middle of the chromatogram and at a slightly lower Rf value a greenish band (menthone). The chromatogram obtained with test solution does not show intense greyish green or faint bluish grey bands at Rf values between those of cineole and thymol in the chromatogram obtained with reference solution (carvone, pulegone, isomenthone). The chromatogram obtained with test solution shows an intense reddish violet band near the solvent front (hydrocarbons) and a brownish yellow band (menthofuran) at a slightly lower Rf value; other less intensely coloured bands may also be seen.

Tests

Acidity. To 2 g add 0.25 ml of *phenolphthalein solution*; not more than 0.1 ml of *0.5 M ethanolic potassium hydroxide* is required to change the colour of the solution.

Optical rotation (2.4.22). –10° to –30°.

Refractive index (2.4.27). 1.460 to 1.467.

Weight per ml (2.4.29). 0.900 g to 0.916 g.

Dimethyl sulphide. Distil 25 ml, collect the first 1 ml of the distillate and carefully superimpose it on 5 ml of a 6.5 per cent w/v solution of *mercuric chloride*; no white film is produced within 1 minute at the interface of the two liquids.

Fixed oils and resinified volatile oils. Allow 0.05 ml to fall on a filter paper. The oil evaporates completely within 24 hours without leaving a translucent or greasy mark.

Assay. *For esters* — To 2 g in a borosilicate glass flask add 2 ml of *ethanol (90 per cent)* and 0.25 ml of *phenolphthalein solution*, neutralise with *0.5 M ethanolic potassium hydroxide*, add an additional 25.0 ml and a little pumice powder or a few pieces of porous pot and heat under a reflux condenser on a *water*-bath for 30 minutes. Add 1 ml of *phenolphthalein solution* and immediately titrate with *0.5 M hydrochloric acid*. Repeat the operation without the substance under examination. The difference between the titrations represents the volume of alkali required to saponify the esters.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.09915 g of esters, calculated as menthyl acetate, $C_{12}H_{22}O_2$

For free alcohols — To 1 g in a dry, 150-ml acetylation flask, add 3 ml of a mixture of 3 volumes of *pyridine* and 1 volume of *acetic anhydride*. Determine the weight of the acetylation mixture to the nearest mg, keeping the flask closed while weighing. Boil under a reflux condenser in a *water*-bath for 3 hours, maintaining the *water* level 2 to 3 cm above the level of the liquid in the flask throughout. Remove the flask from the *water*-bath and add 50 ml of *water* through the condenser,

remove the condenser and wash the walls of the flask with 10 ml of *water*. Allow to stand for 15 minutes and titrate with *0.5 M sodium hydroxide* using 1 ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance under examination. The difference between the titrations represents the volume of sodium hydroxide required.

1 ml of *0.5 M sodium hydroxide* is equivalent to 0.07815 g of free alcohols, calculated as menthol, $C_{10}H_{20}O$. If the quantities of acetic anhydride in pyridine used in the two determinations differ by more than 5 mg, adjust the volume of alkali used in the second titration by multiplying with a/b where a is the weight, in g, of acetic anhydride in pyridine used in the first determination and b is the weight, in g, of acetic anhydride in pyridine used in the second test.

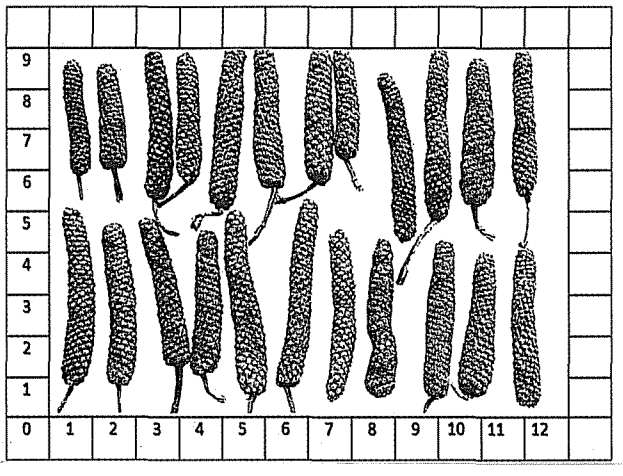
For ketones — To 2 g add 25 ml of a 5.0 per cent w/v solution of *hydroxylamine hydrochloride* in *ethanol (95 per cent)*, heat on a *water*-bath for 1 hour, allow to cool, add about 1 mg of *methyl orange* and titrate with *0.5 M ethanolic potassium hydroxide* until an orange-yellow colour is obtained. Repeat the heating for further periods of 1 hour until, after cooling, not more than 0.1 ml of *0.5 M ethanolic potassium hydroxide* is required to neutralise the solution.

1 ml of *0.5 M ethanolic potassium hydroxide* is equivalent to 0.07710 g of ketones, calculated as menthone, $C_{10}H_{18}O$.

Storage. Store protected from light and moisture, in well-filled containers.

Pippali, Large

Long Pepper; Catkins (Big); *Piper retrofractum*



Pippali, Large consists of the fruiting spikes of *Piper retrofractum* Vahl.(Syn. P. *latifolium* Hunter, (Fam. Piperaceae).

Pippali, Large contains not less than 1.0 per cent w/w of piperine, calculated on the dried basis.

Description. The spikes are blacking green to green in colour, cylindrical, erect and blunt. It has pungent taste and the odour is aromatic and characteristic. The spikes are 2 to 4 cm long and 0.4-0.7 cm in diameter.

Identification

A. *Macroscopic* — The spikes are blackish green to green in colour, surface rough. The spikes bear bracts and numerous small fruits sunk in solid spike.

B. *Microscopic* — The penduncle is circular in outline, trichomes are absent, epidermis is a single layer of tangentially elongated cells. Vascular traces are in five to six bundles. Pith is star shaped. Endosperm cells are larger, cells are packed with abundant starch grains and oil droplets. Crystals are present in some of the cells, they appear unequal in size. The epicarp is a single layer of thick walled cells having greenish content. Endocarp is wavy in outline. Mostly, endocarp and seed coat are fused together to form a deep zone with hyaline content in outer layer and orange red (brown) inner region.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *benzene*, 30 volumes of *ethyl acetate* and 10 volumes of *diethyl ether*.

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Reference solution. Reflux 0.4 g of the coarsely powdered *pippali, Big RS* with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *vanillin sulphuric acid reagent*.. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 8.0 per cent.

Water-soluble extractive (2.6.3). Not less than 10.0 per cent by Method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of coarsely powdered substance under examination, add 50 ml of *methanol*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with *methanol* and filter. Dilute further if necessary.

Reference solution. A 0.01 per cent w/v solution of *piperine RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A.a buffer solution prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 900 ml of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*, B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
18	55	45
25	20	80
30	95	5

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

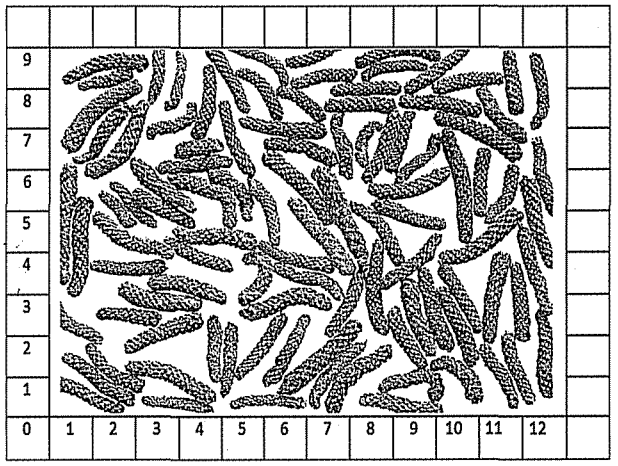
Inject the test solution and the reference solution.

Calculate the content of piperine.

Storage. Store protected from heat, moisture and against attach by insects and rodents.

Pippali, Small

Small Pepper; Catkins (small); *Piper longum*



Pippali, small consists of the fruiting spike of *Piper longum* Linn. (Fam. Piperaceae).

Pippali, small contains not less than 0.4 per cent w/w of piperine, calculated on the dried basis.

Description. The spikes are greenish black to black in colour, cylindrical, erect and blunt. It has pungent taste and the odour is aromatic and characteristic. The spikes are 1.0 to 1.9 cm long and 0.2 to 0.3 cm in diameter.

Identification

A. *Macroscopic* — The spikes are greenish black to black in colour, surface rough. The spikes bear bracts and numerous small fruits sunk in solid spike.

B. *Microscopic* — The peduncle has uniseriate septate trichomes, epidermis is a single layer of tangentially elongated cells. The penduncle vascular traces are in 2-3 bundles, pith is dumbbell shaped. Cells are small, packed with abundant strach grains and oil droplets. Crystal are present in some of the cells, they appear unequal in size. The epicarp is a single layer of thick walled cells having greenish content. Endocarp is wavy in outline. Mostly, endocarp and seed coat are fused together to form a deep zone with hyaline content in outer layer and orange red (brown) inner region.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 60 volumes of *benzene*, 30 volumes of *ethyl acetate* and 10 volumes of *diethyl ether.*

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 50 ml.

Reference solution. Reflux 0.4 g of the coarsely powdered *pippali, small RS* with 50-75 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *vanillin sulphuric acid reagent.*. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 8.0 per cent.

Water-soluble extractive (2.6.3). Not less than 10.0 per cent by Method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh 2 g of coarsely powdered substance under examination, add 50 ml of *methanol*, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with *methanol* and filter. Dilute further if necessary.

Reference solution. A 0.01 per cent w/v solution of *piperine RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a buffer solution prepared by dissolving 0.136 g of *potassium di-hydrogen orthophosphate* in 900 ml of *water*, adjust the pH to 2.5 with *orthophosphoric acid* and dilute to 1000 ml with *water*,

  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 270 nm,
- injection volume. 20 µl.

Time (min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
18	55	45
25	20	80
30	95	5

Inject the reference solution. The relative standard deviation for the replicate injections is not more than 2.0 per cent.

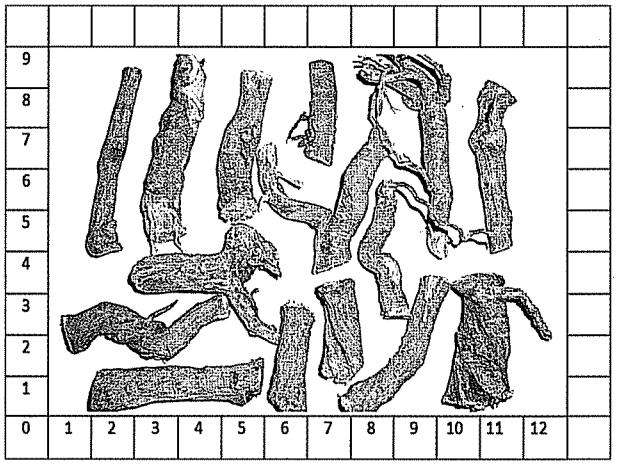
Inject the test solution and the reference solution. The relative retention time of piperine is 1.

Calculate the content of piperine.

Storage. Store protected from heat, moisture and against attach by insects and rodents.

Punarnava

Hogweed; *Boerhaavia diffusa*



Punarnava consists of the dried root of *Boerhaavia diffusa* Linn. (syn. *B. reperis* Linn) (Fam. Nyctaginaceae).

Punarnava contains not less than 0.005 per cent w/w of boeravinone B, calculated on the dried basis.

Description. A greyish-brown in colour, short and fibrous fracture and no distinct odour with bitter taste.

Identification

A. *Macroscopic* — Stout, tapering, somewhat knotty and twisted roots upto 30 cm or more long and 0.5-1.5 cm thick often crowned with stem bases, greyish-brown, surface is rough due to minute, irregular, longitudinal striations and root scars.

B. *Microscopic* — Transverse section of root shows outermost layer of cork which consists of thin walled

tangentially elongated cells with brownish walls followed by thin walled 1-2 layered cork cambium. Cortex many layered and composed of thin walled cells. Secondary cortex 2-4 layered and parenchymtous. Concentric bands of xylem tissues alternating with parenchymatous tissues. Vessels are radial with reticulate thickening. Simple and compound starch grains with centric hilum and raphide crystals of calcium oxalate in cortex region. Fibres are aseptate and spindle shaped with pointed ends.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 35 volumes of *chloroform,* 6 volumes of *methanol and* 1 volume of *glacial acetic acid.*

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Reference solution. Reflux 2 g of the *punarnava RS* with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *anisaldehyde sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 0.5 per cent.

Water-soluble extractive (2.6.3). Not less than 9.0 per cent by method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 10.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 25 ml. Dilute to 25.0 ml with *methanol.*

Reference solution. A 0.002 per cent w/v solution of *boeravinone B RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A.a gradient mixtures of *water,*
  B. *acetonitrile,*
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

Time (min)	Mobile phase A *(per cent v/v)*	Mobile phase B *(per cent v/v)*
0	90	10
25	10	90
35	90	10

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

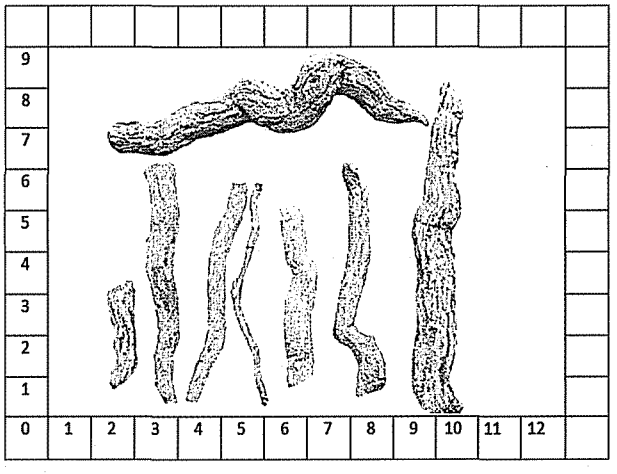
Inject the test solution and reference solution.

Calculate the content of boeravinone B.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Sarpagandha

Rauwolfia serpentina Root



Sarpagandha consists of the dried roots of *Rauwolfia serpentina Bentham* ex Kurz (Fam Apocynaceae).

Sarpagandha contains not less than 0.15 per cent of reserpine and ajmalicine, calculated on the dried basis.

Description. Taste bitter, odour indistinct.

Identification

A. *Macroscopic* — Roots are sub cylindrical to tapering, tortuous or curved, rarely branched. Occurs as segments usually from 5 to 15 cm in length and 3 to 20 mm in diameter. Externally grayish yellow to brown, wood pale yellow. Roots tough with longitudinal marking and slightly wrinkled surface. When scraped, bark separates readily from the wood. Fracture is short and irregular.

B. *Microscopic* — In transverse section, cork cells in 2 to 8 alternating bands of radically narrow and broader cells, thin, lignified up to 75 µm in tangential width, broader cells up to about 90 µm in radial length, phelloderm, tangentially elongated to isodimetric parenchyma cells containing starch and short latex cells with brown resinous matter; secondary cortex consists of parenchyma cells, heavily packed with starch grains secondary phloem contains phloem parenchyma and sieve elements, parenchyma contains starch and angular crystals of calcium oxalate 3 to 20 µm in length. Xylem is about 4/5 of the diameter of the root, wood is transversed by medullay rays 1 to 5 cells in width. Xylem consists of vessels, tracheids, wood parenchyma and wood fibers. Xylem vessels are elongated up to 350 µm in length and 50 µm in width and contains simple or bordered pits; tracheids lignified, pitted; wood parenchyma with moderately thick, lignified and pitted walls containing starch; wood fibres highly thickened with pointed ends, stone cells absent.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *chloroform* and 30 volumes of *acetone*.

Test solution. To 250 mg of the coarsely powdered substance under examination, add 5 ml *methanol*, shake for 10 minutes, and filter. Wash the residue with 5 ml of *methanol* and add the washing to the filtrate.

Reference solution. To 250 mg of *sarpagandha RS*, add 5 ml *methanol*, shake for 10 minutes, and filter. Wash the residue with 5 ml of *methanol* and add the washing to the filtrate.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° for 5 minutes and examine the plate in day light. The chromatogram obtained with test solution shows two pinkish-violet bands corresponding to the bands in the chromatogram obtained with reference solution. A dark brown band may also appear at the line of application in the chromatogram obtained with test solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent

Ethanol-soluble extractive (2.6.2). Not less than 2.0 per cent.

Water-soluble extractive (2.6.3). Not less than 5.0 per cent.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19): Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination limits (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2 g of the coarsely powdered substance under examination with 50 ml of *ethanol (95 per cent)*, on a water bath for 15 minutes, cool and filter. Reflux the residue further with *ethanol (95 per cent)*, till the last extract turns colorless, cool and filter. Combine all the filterates and concentrate to 100.0 ml.

Reference solution (a). A solution containing a 0.002 per cent w/v *reserpine RS* and 0.002 per cent w/v *ajmalicine RS* in *ethanol (95 per cent)*.

Reference solution (b). A 0.002 per cent w/v *reserpine RS* in *ethanol (95 per cent)* for peak identification.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a buffer solution prepared by dissolving 6.80 g of *potassium dihydrogen phosphate* in 1000 ml *water*, adjust the pH to 3.0 with *dilute orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 10 µl.

Inject the reference solution (a). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent. Inject reference solution (b) for peak identification.

Inject the test solution and reference solution (a).

Calculate the content of reserpine and ajmalicine.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Sarpagandha Powder

Rauwolfia serpentina Powder

Sarpagandha powder is obtained by *Rauwolfia serpentina* Bentham ex Kurz roots (Fam. Apocynaceae) reduced to a fine powder.

It contains not less than 0.15 per cent w/w and not more than 0.2 per cent w/w of reserpine-ajmalicine alkaloid calculated on dried basis.

Description. Taste bitter, odour indistinct.

Identification

Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 70 volumes of *chloroform* and 30 volumes of *acetone*.

Test solution. To 0.25 g of the substance under examination, add 5 ml *methanol*, shake for 10 minutes, and filter. Wash the residue with 5 ml of *methanol* and add the washing to the filtrate.

Reference solution. To 0.25 g of *Rauwolfia serpentina* powder RS, add 5 ml *methanol*, shake for 10 minutes, and filter. Wash the residue with 5 ml of *methanol* and add the washing to the filtrate.

Apply to the plate 20 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 12 cm. Dry the plate in air and examine in ultraviolet light at 365 nm. Spray the plate with *anisaldehyde sulphuric acid reagent*. Heat the plate at 100° for 5 minutes and examine the plate at 365 nm. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Total ash (2.3.19). Not more than 5.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 gm complies with the limit test for heavy metals, method B (20 ppm).

Loss on drying (2.4.19). Not more than 5.0 per cent determined on 1 gm by drying in an oven at 105°.

Microbial contamination limits (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2.0 g of the substance under examination with 50 ml of *ethanol (95 per cent)*, on a water bath for 15 minutes, cool and filter. Reflux the residue further with *ethanol (95 per cent)*, till the last extract turns colourless, cool and filter. Combine all the filterates and concentrate to 100.0 ml.

Reference solution (a). A solution containing a 0.002 per cent w/v *reserpine* RS and 0.002 per cent w/v *ajmalicine RS* in *ethanol (95 per cent)*.

Reference solution (b). A 0.002 per cent w/v *reserpine RS* in *ethanol (95 per cent)* for peak identification.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a buffer solution prepared by dissolving 6.80 g of *potassium dihydrogen phosphate* in 1000 ml *water*, adjust the pH to 3.0 with *dilute orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 10 µl.

Inject the reference solution (a). The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent. Inject reference solution (b) for peak identification.

Inject the reference solution and the test solution.

Calculate the content of reserpine and ajmalicine.

Usual strength. 0.175 per cent w/w.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Sarpagandha Tablets

Rauwolfia serpentina Tablets

Sarpagandha Tablets contain not less than 85.0 per cent w/w and not more than 115.0 per cent w/w of stated amount of the alkaloids contents mainly of reserpine together with ajmalicine.

Identification

In the assay the principal peak in chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution.

Tests

Other tests. Comply with the tests stated under tablets.

Loss on drying (2.4.19). Not more than 8.0 per cent, determined in 1.0 gm by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 1 g of *rauwolfia serpentina* into a 200 ml flat bottom flask. Add 50 ml *of ethanol (95 per cent)*, reflux on a water bath for 30 minutes, cool and filter. Reflux the residue further with *ethanol*

(*95 per cent*) till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution. A 0.001 per cent w/v *Reserpine RS* and 0.001% w/v *Ajmalicin RS* in *ethanol*(95 per cent).

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecyl silane chemically bonded to porous silica (5µ),
- mobile phase: a mixture of 35 volumes of *acetonitrile* and 65 volumes of a buffer (pH 3.0), prepared by dissolving 6.80 g of *potassium dihydrogen* phosphate in 1000 ml water, pH of which is adjusted to 3.0 with dilute ortho*phosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume.10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the refernce solution and the test solution.

Calculate the content of ajmalicin and reserpine.

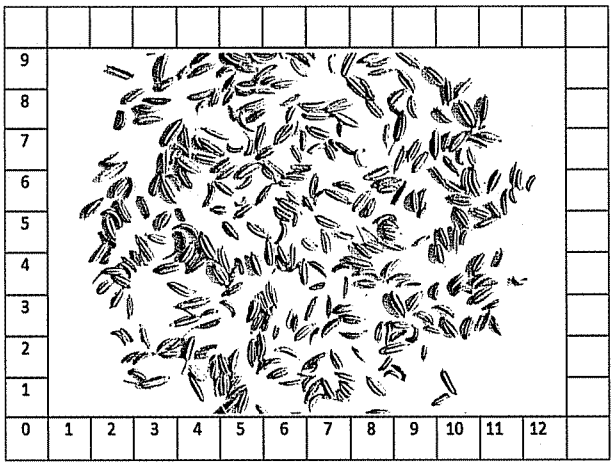
Usual strengths. 0.1 mg; 0.25 mg.

Storage. Store protected from light at a temperature not exceeding 30°.

Labelling. The quantity of active ingredient is stated in terms of reserpine-ajmalicine content in labelled amount.

Saunf

Fennel; *Foeniculum vulgare*



Saunf consists of the dried fruits of *Foeniculum vulgare* Mill. (Fam. Apiaceae).

Saunf contains not less than 0.60 per cent of anethole, calculated on the dried basis.

Description. The fruits are oval-oblong, greenish brown to yellowish brown in colour. They have an aromatic characteristic odour and the taste is sweet aromatic.

Identification

A. *Macroscopic* — Fruit cylindrical to oval with pedicel attached, consists of mericarps. Each mericap is 10 mm long and 4 mm broad, five sided with a wider commissural surface, tapered at apex and base, crowned with a conical stylepod, glabrous, greenish or yellowish brown with five ridges.

B. *Microscopic* — Transverse section of fruit shows pericarp with outer epidermis of quadrangular to polygonal cells with smooth cuticle. Vittae 4 dorsal and 2 commissural extending with length of each mericarp, having brown cells and volatile oil in cavity. Mesocarp with reticulate lignified parenchyma. Endocarp cells thin walled arranged parallel to one another in groups of 5-7. Endosperm consists of thick walled, cellulosic parenchyma containing fixed oil cells and rosette crystals of calcium oxalate.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254*.

Mobile phase. A mixture of 93 volumes of *toluene* and 7 volumes of *ethyl acetate*.

Test solution. To 1 g of the coarsely powdered substance under examination, add 25 ml of *dichloromethane,* reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 25 ml of *dichloromethane*, cool and filter. Combine all the filtrates and evaporate under vacuo to 25 ml.

Reference solution. To 2 g of the *saunf RS*, add 40 ml of *methanol,* reflux for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *methanol* twice, cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with a *anisaldehyde sulphuric acid reagent.* Heat at 110° for 10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 5.0 per cent.

Water-soluble extractive (2.6.3). Not less than 14.0 per cent by Method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 10 per cent, determined on 5.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Weigh 2.0 g of the coarsely powdered substance under examination, add 50 ml of *methanol,* reflux on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 100 ml. Dilute to 100.0 ml with *methanol.*

Reference solution. A 0.01 per cent w/v solution of *anethole RS* in *methanol.*

Chromatographic system

- a capillary column 30 m x 0.25 m, packed with DB1,
- temperature:
  oven 90° to 260° @10° per minute (initially and finally hold for 5 minutes respectively),
  injector 240°,
  detector 280°,
- flow rate. 0.8 ml per minute,
- split flow 20 ml per minute.

Inject 1 µl of the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 10.0 per cent.

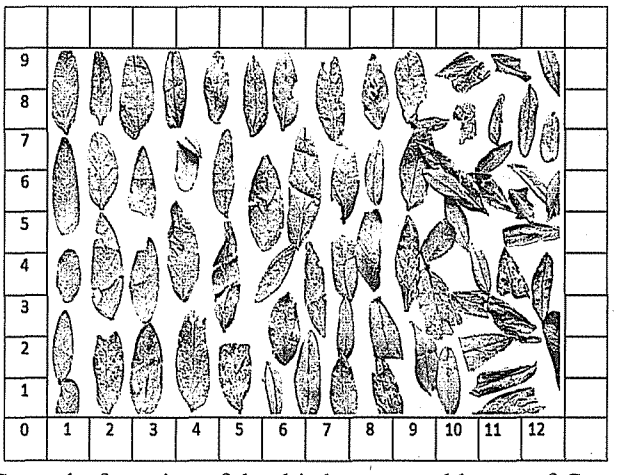
Inject 1 µl of the test solution and the reference solution.

Calculate the content of anethole.

Storage. Store protected from light in well-filled containers, at a temperature not exceeding 30°.

Senna Leaf

Cassia leaf; *Cassia angustifolia*



Senna leaf consists of the dried compound leaves of *Cassia angustifolia* or *Cassia senna* Vahal. (Fam.Leguminosae).

Senna leaf contains not less than 1.0 percent w/w of sennosides A and B, calculated on the dried basis.

Description. Pale yellowish green coloured leaflets with mucilaginous and faint odour.

Identification

A. *Macroscopic* — Leaflets, 2.5 to 8 cm long and 5-15 mm wide at centre, pale yellowish green, elongated lanceolate, slightly asymmetric at base; margins entire, flat, apex acute with a sharp spine; both surface smooth with sparce trichomes; odour, faint but distinctive; taste, mucilaginous and disagreeable but not distinctly bitter.

B. *Microscopic* — Transverse section shows outer single layered mucilaginous epidermal cells. Unicellular hairs presents. Stomata paracytic, numerous on both surface. Mesophyll consists of upper and lower palisade layers with spongy layer in between , primatic crystals of calcium oxalate present on larger veins.

C. In the Assay, the chromatogram obtained with test solution corresponds to the chromatogram obtained with reference solution.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 40 volumes of *n- propyl alcohol,* 40 volumes of *ethyl acetate,* 29 volumes of *water* and 1 volume of *glacial acetic acid.*

Test solution. Take 1 g of the dried leaves powder substance under examination. Add 25 ml of *methanol,* reflux for 10 minutes, cool and filter. Reflux the residue with another 20 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate to 10 ml.

Reference solution. Boil 0.5 g of dried *senna leaves RS* powder with 25 ml of *methanol* under reflux for 10 minutes, cool and filter. Boil under reflux the residue with another 20 ml of *methanol,* cool and filter. Combine all the filtrates and evaporate to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with 20 per cent v/v of *nitric acid solution.* Heat the plate at 100° to 105° for about 10 minutes and immediately examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 1.0 per cent.

Total ash (2.3.19). Not more than 14.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.5 per cent.

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately 1.0 g of the coarsely powdered substance in a round bottom flask, add about 10 ml of 1 per cent v/v *acetic acid* and 25 ml of *methanol* and reflux on a water bath for about 30 minutes. Cool to room temperature; make up the volume up to 50.0 ml with *methanol* and filter.

Reference solution. A 0.004 per cent w/v solution of *sennosides RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixtures of 82 volumes of 1 per cent v/v *acetic acid* in *water* and 18 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

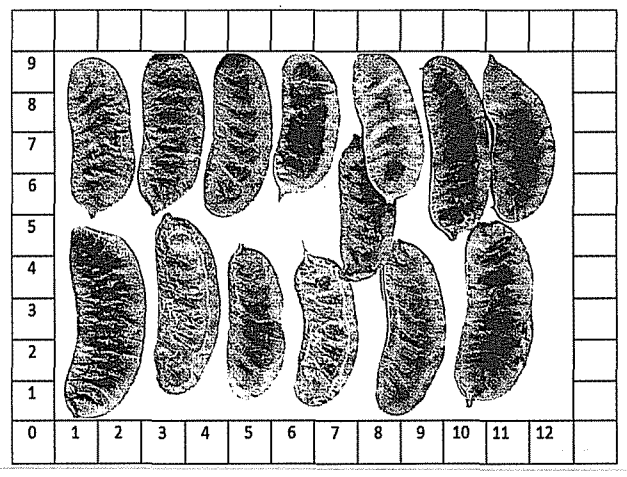
Inject the reference solution and the test solution.

Calculate the content of sennoside A and B.

Storage. Store protected from light and moisture.

Senna Pods

Senna Fruit; Pods of cassia; *Cassia angustifolia*



Senna pods consist of the dried compound Pods of *Cassia angustifolia* or *Cassia senna* Vahal. (Fam.Leguminosae).

Senna Pods contains not less than 1.0 per cent w/w of sennosides A and B, claculated on the dried basis.

Description. Pale yellowish green coloured pods with slight odour. Leaflets with mucilaginous and faint odour.

Identification

A. *Macroscopic* — Flattened reniform pods, brownish yellow at the edges, dark brown in the central area about 40 to 50 mm long and about at least 20 mm wide. At one end is a stylar point and at the other a short stalk. The pods contains 5 to 7 flattened and obovate seeds, green to pale brown, with a continuous network of prominent ridges on the tests and in complate wavy transverse ridges on the testa. Leaflets, 2.5 to 8 cm long and 5-15 mm wide at centre, pale yellowish green, elongated lanceolate, slightly asymmetric at base; margins entire, flat, apex acute with a sharp spine; both surface smooth with sparce trichomes; odour, faint but distinctive; taste, mucilaginous and disagreeable but not distinctly bitter.

B. *Microscopic* — The pods present an epicarp with strongly cuticularised isodiametric cells, occasional anomocytic or paracytic stomata, and very few conical, unicellular and warty trichomes. Hypodermis with collenchymatous cells, mesocarp with parenchymatous tissue, a layer of prism s of calcium oxalate and containing vascular bundles incompletely surrounded by fibres with a crystals sheth of calcium oxalate prisms, endocarp consisting of thick-walled and inter lacing fibres. The seeds present a sub epidermal layers of palisade cells with thick outer walls, endosperm composed of polyhedral cells with mucilaginous wall.

Reduce to moderately fine powder examine microscopically using chloral hydrate solution. The powder consists of epicarp with polygonal cells and a small number of warty trichomes and occasional anomocytic stomata, fibres in two crossed layers, accomparied by a crystal sheath of calcium oxalate prism, characteristic palisade cells in the seeds and stratified cells in the endosperm, cluster and prisms of calcium oxalate

C. In the Assay, the chromatogram obtained with test solution corresponds to the chromatogram obtained with reference solution.

D. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 40 volumes of *n- propyl alcohol*, 40 volumes of *ethyl acetate,* 29 volumes of *water* and 1 volume of *glacial acetic acid.*

Test solution. Take 1 g of the dried pods powder substance under examination. Add 25 ml of *methanol*, reflux for 10 minutes, cool and filter. Reflux the residue with another 20 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 10 ml.

Reference solution. Boil 0.5 g of dried *senna pods RS* powder with 25 ml of *methanol* under reflux for 10 minutes, cool and filter. Boil under reflux the residue with another 20 ml of

methanol, cool and filter. Combine all the filtrates and evaporate to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with 20 per cent v/v of *nitric acid solution*. Heat the plate at 100° to 105° for about 10 minutes and immediately examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 1.0 per cent.

Total ash (2.3.19). Not more than 14.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.5 per cent.

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately 1.0 g of the coarsely powdered substance in a round bottom flask, add about 10 ml of 1 per cent v/v *acetic acid* and 25 ml of *methanol* and reflux on a water bath for about 30 minutes. Cool to room temperature; make up the volume up to 50 ml with *methanol* and filter.

Reference solution. A 0.004 per cent w/v solution of *sennosides RS* in *methanol*.

Chromatographic system:

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase. a mixtures of 82 volumes of 1 per cent v/v *acetic acid* in *water* and 18 volumes of *acetonitrile*.
- flow rate. 1 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of sennoside A and B.

Storage. Store protected from light and moisture.

Senna Dry Extract

Senna Dry Extract is produced from Senna leaves or pods of *Cassia angustifolia* (Tinnevelly Senna) or *Cassia acutifilia* (Cassia Senna) as calcium salts.

Senna Dry Extract contains not less than 90.0 per cent w/w and not more than 110.0 per cent w/w of stated amount of *sennoside A* and *sennoside B*, calculated on the dried basis.

Description. A light brown to dark brown powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 40 volumes of *n-propyl alcohol*, 40 volumes of *ethylacetate*, 30 volumes of *water* and 1 volume of *glacial acetic acid*.

Test solution. Shake well 0.1 g of the extract under examination with 5 ml of a mixture of equal volumes of *methanol* and *water* for 5 minutes, heat to 60°, cool and allow to settle. Use the supernatant liquid.

Reference solution. Dissolve 10 mg of *senna dry extract RS* in 1 ml of a mixture of equal volumes of *methanol* and *water*.

Apply to the plate, 10 µl of each solution as bands of 10 mm by 2 mm. Allow the mobile phase to rise 10 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with 20 per cent v/v of *nitric acid solution*. Heat the plate at 110° for 10 minutes and examine the plate in day light. Allow to cool and spray with a 5 per cent w/v solution of *potassium hydroxide* in *ethanol* (50 per cent v/v) until the zones appear. The chromatographic profile of the test solution is similar to that of the reference solution.

B. To about 25 mg of the extract add 50 ml of *water* and 2 ml of *hydrochloric acid*. Heat in a water-bath for 15 minutes. Cool and shake with 40 ml of *ether*. Separate ether layer, dry over *anhydrous sodium sulphate* and evaporate 5 ml to dryness. To the cooled residue add 5 ml of *dilute ammonia*. A yellow or orange colour develops. Heat on a water-bath for 2 minutes. A reddish-violet colour develops.

Tests

pH (2.4.24). 5.5 to 7.5, determined in a 10 per cent solution in *water*.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 gm by drying in an oven at 105°.

Microbial contamination (2.2.9). Total viable aerobic count not more than 10^4 micro organisms per gm, fungi not more than 10^2 micro organisms per g and *Escherichia coli* and *salmonellae* absent.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.3 per cent v/v solution of *acetic acid* with the pH adjusted to 5.9 with *1 M sodium hydroxide*.

Test solution. Dissolve an accurately weighed quantity of substance under examination containing about 10 mg of sennosides in 50.0 ml of the solvent mixture.

Reference solution. Dissolve an accurately weighed quantity of *calcium sennosides RS* containing about 10 mg of sennosides in 50.0 ml of the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 83 volumes of a 1 per cent v/v solution of *glacial acetic acid* and 17 volumes of *acetonitrile,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

Calculate the content of sennosides A and B, as sennoside B in the extract.

Storage. Store protected from light, in air-tight containers.

Senna Tablets

Senna Tablets contain not less than 85.0 per cent w/w and not more than 115.0 per cent w/w of the stated amount of sennosides A and B, calculated as sennoside B.

Identification

In the assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Other tests. Comply with the tests stated under tablets.

Loss on drying (2.4.19). Not more than 6.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. A 0.3 per cent v/v solution of *acetic acid,* with the pH adjusted to 5.9 with *1 M sodium hydroxide.*

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing about 10 mg of sennosides, dissolve in about 40 ml of solvent mixture and mix with the aid of ultrasound for 15 minutes. Dilute to 50 ml with the solvent mixture and filter.

Reference solution. A 0.02 per cent w/v solution of *calcium sennoside RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane chemically bonded to porous silica (5 µm),
- mobile phase: a mixture of 83 volumes of a 1 per cent v/v solution of *glacial acetic acid* and 17 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 350 nm,
- injection volume. 10 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution.

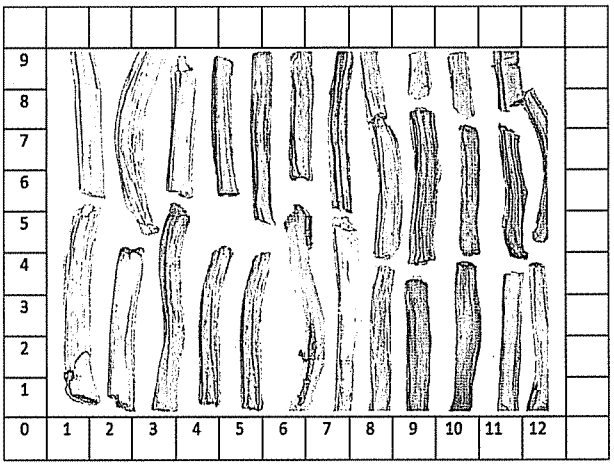
Calculate the content of sennosides A and B, as sennoside B in the tablets.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The quantity of active ingredient is stated in terms of total sennoside A and B, expressed as the equivalent content of sennoside B.

Shatavari

Asparagus racemosus root



Shatavari consists of the tuberous roots of *Asparagus racemosus* Willd. (Fam. Liliaceae).

Shatavari contains not less than 0.1 per cent of shatavarin IV, calculated on the dried basis.

Description. The tuberous root bits are dirty white in color, longitudinally wrinkled with yellow hard central core. It is starchy and slightly bitter followed by sweet taste.

Identification

A. *Macroscopic* — The tuberous roots are borne in a compact bunch and are fleshy, and spindle shaped. They are marketed in pieces 5-15 cm in length and 2 cm in thickness. They are silvery white or ash-colored externally and white internally, more or less smooth when fresh, developing longitudinal wrinkles when dry.

B. *Microscopic* — The inner parenchymatous zone of cortex is composed of 18-24 layers in upper and 42-47 layers in the middle tuberous portion of the roots. The cells are thin - walled cellulosic, with circular to oral outlines and distinct inter cellular spaces. In some roots 3-4 layers of cortex immediately adjacent to the endodermis are modified into a sheath of stone cells round the endodermis. The number of vascular bundles is 30-35 in the upper levels and 35-45 in the middle tuberous portions of the roots.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 13 volumes of *chloroform*, 10 volumes of *methanol* and 2 volumes of *water*.

Test solution. Reflux 1 g of the coarsely powdered substance under examination with 30 ml of *methanol* for 30 minutes, cool and filter. Reflux the residue further with 2 × 30 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10.0 ml.

Reference solution. Reflux 1 g of *shatavari RS* with 30 ml of *methanol* for 30 minutes, cool and filter. Reflux the residue further with 2 × 30 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10.0 ml.

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *vanillin sulphuric acid reagent*. Heat the plate at 120° for 10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 15.0 per cent.

Water- soluble extractive (2.6.3). Not less than 20.0 per cent by Method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Perform the Assay by following method I or by method II. The result of the method II will be official in case of dispute.

Method I

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 6.5 volumes of *chloroform* and 3.5 volumes of *methanol.*

Test solution. Reflux 4 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 30 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colourless, cool and filter. Combine all the filtrates and concentrate to 50 ml.

Reference solution. A 0.008 per cent w/v solution of *shatavarin IV RS* in *methanol.*

Apply to the plate 5 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with a 10 per cent v/v solution of *sulphuric acid* in *methanol.* Heat the plate at 100° for 5 minutes, scan the plate in absorbance mode at 500 nm. Record the chromatograms and measure the responses for the analyte peak.

Calculate the content of shatavarin IV.

Method II

Determine by liquid chromatography (2.4.14).

Test solution. Reflux 5 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 30 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colourless, cool and filter. Combine all the filtrates and concentrate to 50.0 ml.

Reference solution. A 0.1 per cent w/v solution of *shatavarin IV RS* in *methanol.* Dilute suitably to prepare 0.0075- 0.075 per cent w/v solution.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 60 volumes of *acetonitrile* and 40 volumes of *water*,
- flow rate. 1 ml per minute,
- use evaporative light scattering detector,
- temperature
  evaporator 110°,
  nebulizer 90°,
  nebulizer gas nitrogen and gas flow 1 SLM,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the regression coefficient is not more than 0.9.

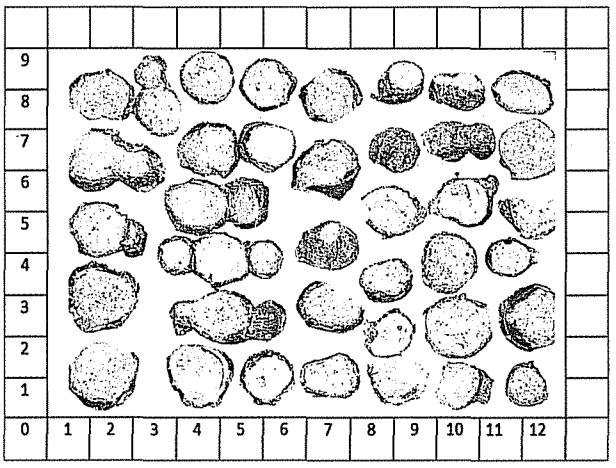
Inject the test solution and reference solution.

Calculate the content of shatavarin IV.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Shati

Hedychium spicatum



Shati consists of the dried rhizomes of *Hedychium spicatum* Buch.-Ham.ex Smith (Fam. Zingiberaceae).

Shati contains not less than 0.80 per cent w/w of p-methoxy cinnamic acid ethyl ester, calculated on the dried basis.

Description. A reddish-brown outer surface and white in side, short and uneven fracture and camphoraceous odour with aromatic and pungent taste.

Identification

A. *Macroscopic* — Tuberous rhizome having reddish brown, rough outer surface with round root scars or rootlets which remain attached at some places. Transversely sliced pieces of dried rhizome are spherical, flat, 1 cm in thickness and 2-3 cm in diameter having white and starchy surface.

B. *Microscopic* — Transverse section of rhizome shows outermost layer of cork having 2-6 layers of isodiametic, nonlignified and suberised cells which are radially arranged. In old rhizome, the cork cells are exfoliated or crushed. Cortex is a broad zone with 20-25 layers of thin walled parenchymatous cells. Cortex region is filled with abundant starch grains and numerous oleo-resin cells. Vascular bundles are closed and collateral and scattered throughout the ground tissues. Cambium has 4-5 rows of tangentially elongated cells. It forms a complete ring between the xylem and phloem groups. Starch grains tissue are simple, circular or oval in shape, found in ground cells.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *n-hexane* and 20 volumes of *acetone.*

Test solution. Reflux 1 g of the coarsely powdered substance under examination with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 25 ml.

Reference solution. Reflux 0.5 g of the *shati RS* with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol,* cool and filter. Combine all the filtrates and concentrate under vacuum to 12.5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *anisaldehyde sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 0.7 per cent.

Water-soluble extractive (2.6.3). Not less than 12.0 per cent by method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 3.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 14.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter. Combine all the filtrates and concentrate to a volume slightly less than 100 ml. Dilute to 100.0 ml with *methanol.*

Reference solution. A 0.01 per cent w/v solution of *p-methoxy cinnamic acid ethyl ester RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),

- mobile phase: 40 volumes of *water* and 60 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 310 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the content of p-methoxy cinnamic acid ethyl ester.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Shellac

Lac

Shellac consists of a resinous substance prepared from a secretion that encrusts the bodies of a scale insect, *Laccifer lacca Kerr* (Fam. Ciccidae).

Description. Lemon-yellow to brownish orange thin scales or hard, brittle masses; odourless or with a faint odour.

Identification

To 50 mg add a few drops of a mixture of 1 g of *ammonium molybdate* and 3 ml of *sulphuric acid*; a green colour is produced and it becomes lilac on standing for 5 minutes.

Tests

Acid value (2.3.23). 50 to 70, determined by the following method. Weigh accurately about 2.0 g and dissolve with the aid of gentle heat, in 50 ml of *ethanol (95 per cent)* previously neutralised to *ethanolic thymol blue solution*. Titrate with *0.1 M ethanolic potassium hydroxide* using *ethanolic thymol blue solution* as an external indicator. Calculate the acid value from the expression

$$5.61 \times a/w$$

where, a = number of ml of *0.1 M ethanolic potassium hydroxide* and
w = weight, in g, of the sample.

Ethanol-insoluble matter. Not more than 2.0 per cent, determined by the following method. Weigh accurately about 5.0 g in an extraction thimble, cover with *ethanol (95 per cent)* and allow to stand for 16 hours. Place in an apparatus for the continuous extraction of drugs, extract with *ethanol (95 per cent)* for 4 hours, dry the residue at 100° for 3 hours and weigh.

Colophony. Dissolve 2.0 g by shaking with 10 ml of ethanol, add slowly, with shaking 50 ml of *light petroleum (40° to 60°)*, wash with two successive portions, each of 50 ml, of *water*, filter the washed light petroleum solution, and evaporate to dryness; to the residue add 2 ml of a mixture of 1 volume of *liquified phenol* and 2 volumes of *carbon tetrachloride* and transfer to a cavity of a colour-reaction porcelain tile; fill an adjacent cavity with a mixture of 1 volume of *bromine* and 4 volumes of *carbon tetrachloride*, and cover both cavities with an inverted watch-glass; no purple or deep indigo-blue colour is produced in the liquid containing the residue.

Arsenic (2.3.10). Heat gently 5.0 g with 2 ml of *nitric acid* and 0.5 ml of *sulphuric acid* in a long-necked flask, until the first reaction has subsided, cool, add carefully and in small portions, 15 ml of *nitric acid* and 6 ml of *sulphuric acid* taking care to avoid excessive foaming, and continue heating, adding further small portions of *nitric acid*, if necessary, until white fumes are evolved and the solution becomes colourless or almost colourless. Cool, add carefully 10 ml of *water*, evaporate until white fumes are evolved, and repeat the addition of *water* and evaporation until all the nitric acid has been removed, cool, dilute to 50 ml with *water*, and add 10 ml of *stannated hydrochloric acid AsT*. The resulting solution complies with the limit test for arsenic (2 ppm). Use 0.5 ml of *arsenic standard solution (10 ppm As)* for the standard stain.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 1.0 per cent, determined on 0.5 g.

Storage. Store protected from moisture.

Starch

Starch consists of polysaccharide granules obtained from the caryopsis of maize or corn, *Zea mays* Linn.(Fam. Poceae), or of rice, *Oryza sativa* Linn., or of wheat, *Triticum aestivum* Linn.(Fam. Graminae) , or from the tuber of potato, *Solanum tuberosum* Linn (Fam. Solanaceae), or from the rhizomes of tapioca, *Manihot utilissima* Pohl.(Fam. Euphorbiaceae).

Description. A very fine, white or slightly yellowish powder or irregular white masses which are readily reducible to powder, creaks when pressed between the fingers; odourless and tasteless. The presence of granules showing cracks or edge irregularities is exceptional in starches other than wheat starch; wheat starch may contain granules with cracks on the edges.

Identification

A. *Corn or maize starch* — Polyhedral granules, 2 to 23 µm in size, or rounded granules, 25 to 32 µm in diameter. The central hilum consists of a distinct cavity or two- to five-rayed cleft; no concentric striations. Viewed between crossed nicol prisms, a distinct black cross is seen intersecting at the hilum.

Potato starch — Single granules, either irregular, ovoid or pear-shaped, 30 to 100 µm in size, or rounded, 10 to 35 µm in size; compound granules consisting of groups of two to four elements are rare. Eccentric hilum; clearly visible concentric striations. Viewed between crossed nicol prisms, a distinct black cross is seen intersecting at the hilum.

Rice starch — Polyhedral granules, 2 to 5 µm in size, either isolated or aggregated in ovoid masses, 10 to 20 µm in size. Central hilum poorly visible; no concentric striations. Viewed between crossed nicol prisms, a distinct black cross is seen intersecting at the hilum.

Tapioca starch — Principally simple granules, sub-spherical, muller-shaped or rounded polyhedral; smaller granules 5 to 10 µm, larger granules 20 to 35 µm in diameter; hilum, central, punctate, linear or triradiate; striations, faint, concentric; compound granules, few, of two to three unequal components.

Wheat starch — Large discoid or, more rarely, reniform granules, 10 to 45 µm in size; profile, elliptical and fusiform, slit along the main axis. Small rounded or polyhedral granules, 2 to 10 µm in size. Granules of intermediate size very rarely occur. Hilum and striations invisible or barely visible. Viewed between crossed nicol prisms, a distinct black cross is seen intersecting at the hilum.

B. Heat to boiling for 1 minute a suspension of 1 g in 50 ml of *water* and cool; a thin and cloudy mucilage is produced with all starches except potato starch which gives a thick and more transparent mucilage.

C. To 10 ml of the mucilage obtained in test B add 0.05 ml of *0.01 M iodine*; a dark blue colour is produced, which disappears on heating and reappears on cooling.

Tests

Acidity. Add 10.0 g to 100 ml of *ethanol (70 per cent)* previously neutralised to *phenolphthalein solution*, shake for 1 hour, filter and titrate 50 ml of the filtrate with *0.1 M sodium hydroxide*. Not more than 2.0 ml is required to change the colour of the solution.

Iron (2.3.14). Dissolve the residue obtained in the test for sulphated ash in 4 ml of *hydrochloric acid* with the aid of gentle heat, dilute with *water* to 50 ml and mix; 25 ml of the resulting solution complies with the limit test for iron (40 ppm).

Fluorescence. No fluorescence should be visible under screened ultra-violet light.

Oxidising substances. To 5.0 g add 10 ml of *water* and 1 ml of *acetic acid* and stir until a homogeneous suspension is obtained. Add 0.5 ml of a freshly prepared saturated solution of *potassium iodide*, mix and allow to stand for 5 minutes; no distinct brown or blue colour is observed.

Microbial Contamination (2.2.9). 1 g is free from *Escherichia coli* and *salmonellae*.

Sulphated ash (2.3.18). Not more than 0.6 per cent (for all starches except rice starch) and not more than 0.8 per cent (for rice starch), determined on 2.0 g.

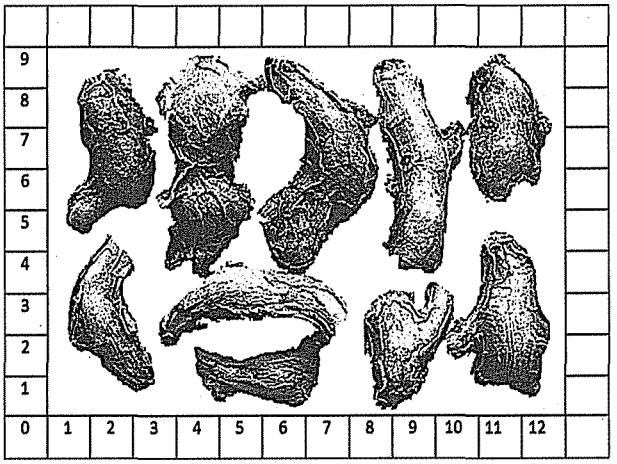
Loss on drying (2.4.19). Not more than 15.0 per cent (for all starches except potato starch) and not more than 20.0 per cent (for potato starch), determined on 0.2 g by drying in an oven at 105°.

Storage. Store protected from light and moisture.

Labelling. The label states the type of starch.

Sunthi

Saunth; Ginger; *Zingiber officinale*



Sunthi is the whole or cut scraped or unscraped, dried rhizomes of *Zingiber officinale* Roscoe. (Fam. Zingiberaceae).

Sunthi contains not less than 0.8 per cent w/w of total gingerols, calculated on the dried basis.

Description. Odour, agreeable and aromatic; taste, agreeable and pungent.

Identification

A. *Macroscopic* — Rhizome laterally compressed, bearing short, flattened, oblique branches; outer surface buff-coloured, longitudinally striate; inner surface pale yellow, starchy and fibrous. Fracture short with projecting fibers.

B. *Microscopic* — Fibro-vascular bundles and oleoresin cells with yellow pigment scattered in ground tissue. Starch grains abundant in parenchyma cells, mostly simple, sack shaped, spherical; hilum eccentric.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 30 volumes of *hexane* and 70 volumes of *diethyl ether.*

Test solution. Reflux 1 g of the coarsely powdered substance under examination with 25 ml of *methanol* for 15 minutes, cool and filter. Wash the residue with 10 ml of *methanol.* Combine all the filtrates and concentrate to 10 ml.

Reference solution. Reflux 0.5 g of coarsely powdered *sunthi RS* with 5 ml *methanol* for 15 minutes, cool and filter.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray the plate with *vanillin sulphuric acid reagent.* Heat the plate at 100° for 5-10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 2.0 per cent.

Water-soluble extractive (2.6.3). Not less than 10.0 per cent by Method I.

Total ash (2.3.19). Not more than 8.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 1.5 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Water (2.3.43). Not more than 12.0 per cent, determined on 0.2 g.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 3 g of the coarsely powdered substance under examination with 100 ml of *methanol* on a water–bath for 15 minutes cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 50.0 ml

Reference solution. A 0.1 per cent w/v solution of *6-gingerol RS* in *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 55 volumes of *acetonitrile* and 45 volumes of *water,*
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution.

Calculate the contents of total gingerols by summing the peak areas of 6-gingerol with all other peaks, which elute after 6-gingerol and have a peak area of at least 5 per cent of the peak area of 6-gingerol.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Sunthi Extract

Sunthi extract is obtained by extracting Sunthi (*Zingiber officinale* Roscoe, Fam. Zingiberaceae) dried rhizome of with ethanol or any other suitable solvent. The powdered extract may contain suitable excipients.

Sunthi extract contains not less than 90.0 per cent w/w and not more than 120 percent w/w of the stated amount of total gingerols and shogaols (sum of 6 gingerol, 8 gingerol, 10 gingerol and 6 shogaol). The content of 6 shogaol shall not be more than the content of 6 gingerol.

Description. Very light yellow to light brown powder or thick liquid with characteristic odour and pungent taste.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF 254.*

Mobile phase. A mixture of 30 volumes of *hexane* and 70 volumes of *diethyl ether.*

Test solution. Dissolve 0.5 g of extract under examination with 50 ml of *methanol* and filter.

Reference solution. 0.1 per cent w/v solution of *6 gingerol RS* in *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm. Spray the plate with *vanillin sulphuric acid.* Heat the plate at 100° for 10 minutes and examine the plate at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Water (2.3.43).Not more than 5.0 per cent, determined on 2.0 g when dried at 105° for 3 hours.

Total ash (2.3.19). Not more than 3 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, (Method B) 20 ppm.

Microbial contamination (2.2.9). Complies with the microbial contamination test

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 1g of the extract containing 100 mg of gingerols in methanol by heating, make up to 100 ml and filter.

Reference solution. A 0.1 per cent w/v solution of 6 *gingerol RS* in *methanol*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A mixture of 55 volumes of *Acetonitrile* and 45 volumes of *water*
- flow rate 1.3 ml per minute,
- spectrophotometer set at 278 nm,
- injection volume.20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of total *gingerols* by summing the peak areas of 6 gingerol, 8 gingerol, 10 gingerol and 6 shogaol. The relative retention time of 6 gingerol is 1.0, 8 gingerol is about 2.1, 10 gingerol is about 5.0 and 6 shogaol is about 2.6. The area of the peak of 6 gingerol in the sample chromatogram is more than the area of 6 shogaol indicating the content of 6 gingerol is more than the content of 6 shogaol.

Usual strengths. 5 per cent w/w; 20 per cent w/w.

Storage. Store protected from heat and moisture.

Tolu Balsam

Balsam of Tolu

Tolu Balsam is a solid or semi-solid, balsam obtained by incision from the trunk of *Myroxylon balsamum* (Linn.) Harms (Fam. Leguminosae).

Tolu Balsam contains not less than 35.0 per cent and not more than 50.0 per cent of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$, on the dry, ethanol-soluble matter.

Description. A soft, tenacious, brownish yellow or brown mass, when first collected; subsequently, becoming harder and finally brittle. Transparent in thin films; odour, aromatic and vanilla-like. Warmed and pressed between pieces of glass and examined with a lens, it exhibits crystals of cinnamic acid.

Identification

A. To a solution in *ethanol (90 per cent)* add *ferric chloride test solution*; a green colour is produced.

B. To 1 g add to 5 ml of water, heat to boiling, filter, add 30 mg of *potassium permanganate* and continue heating; the odour of benzaldehyde is produced.

Tests

Acidity. A solution in *ethanol (90 per cent)* is acidic to *litmus solution*.

Acid value (2.3.23). 97 to 160, calculated on the dry, ethanol-soluble matter, determined by the following method. Dissolve 5.0 g in 50 ml of boiling *ethanol (90 per cent)*, add 3 ml of *phenolphthalein solution* and titrate the hot solution with *1 M ethanolic potassium hydroxide*. When the colour becomes dark brown, attach to a reflux condenser, boil for a few minutes to break up the precipitate and complete the titration.

Ethanol-insoluble matter. Not more than 5 per cent, determined by the following method. Digest 2.5 g with 50 ml of *ethanol (90 per cent)*, filter through a sintered glass crucible, transfer the residue to the filter crucible with the aid of more *ethanol (90 per cent)*, wash with hot *ethanol (90 per cent)* until all soluble matter is removed and dry to constant weight at 100°.

Colophony. Add 5 g to 25 ml of *carbon disulphide*, warm gently on a water-bath under a reflux condenser, filter, evaporate the solution to dryness, dissolve the residue in 6 ml of *light petroleum (40° to 60°)* and shake with 10 ml of a 0.5 per cent w/v solution of *cupric acetate*; the light petroleum layer is not coloured green.

Ester value (2.3.26). 47 to 95, calculated on the dry, ethanol-soluble basis.

Saponification value (2.3.37). 170 to 230, calculated on the dry, ethanol-soluble basis.

Loss on drying (2.4.19). Not more than 4 per cent, determined on 2.0 g by drying in a thin layer in an oven at 60° over *phosphorus pentoxide* at a pressure not exceeding 2.7 kPa.

Assay. Weigh accurately about 2.0 g and boil with 25 ml of *dilute ethanolic potassium hydroxide solution* under a reflux condenser for 1 hour. Remove the ethanol and digest the residue with 50 ml of hot *water* until diffused. Cool the liquid, add 150 ml of *water* and 1.5 g of *magnesium sulphate* dissolved in 50 ml of *water*. Mix thoroughly and set aside for 10 minutes. Filter, wash the residue on the filter with 20 ml of water, acidify the combined filtrate and washings with *hydrochloric acid* and extract with successive quantities of 50, 40, 30, 30 and 30 ml of *ether*. Combine the ether extracts and discard the aqueous portion. Extract with successive

quantities of 20, 20, 10, 10 and 10 ml of *sodium bicarbonate solution*, washing each aqueous extract with the same 20 ml of *ether*. Discard the ether layers, acidify the combined aqueous extracts with *hydrochloric acid* and extract with successive quantities of 30, 20, 20 and 10 ml of *chloroform*, filtering each chloroform extract through a plug of cotton wool on which a layer of *anhydrous sodium sulphate* is placed. Evaporate the chloroform on a water-bath until about 10 ml remains and remove the remainder in a current of air stopping immediately when the last trace of solvent is removed. Dissolve the residue by warming with 10 ml of *ethanol (95 per cent)*, previously neutralised to *phenol red solution*, cool and titrate with *0.1 M sodium hydroxide* using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.01482 g of total balsamic acids, calculated as cinnamic acid, $C_9H_8O_2$.

Storage. Store protected from light and moisture. Avoid exposure to excessive heat.

Tragacanth

Tragacanth is the air-hardened gummy exudate, flowing naturally or obtained by incision, from the trunk and branches of *Astragalus gummifer* Labill. and certain other species of *Astragalus* (Fam. Fabaceae).

Description. Pale yellow, thin, flattened ribbons or brittle pieces; odourless and almost tasteless. On the addition of about 10 times its weight of *water*, it forms a mucilaginous gel.

It has the macroscopic and microscopic characteristics described under Identification tests A and B.

Identification

A. *Macroscopic* — Occurs as thin, flattened pieces, 30 mm long, 10 mm wide and up to 1 mm in thickness, more or less curved, marked on the surface by fine longitudinal striae and concentric transverse ridges; white, translucent, horny; fracture, short. May also be in the form of thicker, less brittle pieces, white to pale yellow and more opaque.

B. *Microscopic* — Reduce to powder. Examine under a microscope; the powder shows in the gummy mass numerous stratified cellular membranes which turn violet on the addition of *iodinated zinc chloride solution*. The mass includes starch granules, isolated or in small clusters, rounded or occasionally deformed, diameter 4 to 10 µm, and up to 20 µm, with a central hilum, visible in polarised light.

C. Moisten 0.5 g of the powdered material with 1 ml of *ethanol (95 per cent)* and add gradually, while shaking, 50 ml of *water* until a homogeneous mucilage is obtained. To 5 ml of the mucilage add 5 ml of *water* and 2 ml of *barium hydroxide solution*. A slightly flocculent precipitate is formed which, when heated for 10 minutes on a water-bath, gives an intense yellow colour.

D. Add 4 ml of a 0.5 per cent w/v dispersion in *water* to 0.5 ml of *hydrochloric acid* and heat on a water-bath for 30 minutes. To one half of the resulting liquid add 1.5 ml of *sodium hydroxide solution* and 3 ml of *alkaline cupric-tartrate solution* and heat on a water-bath; a reddish brown precipitate is formed. To the other half of the liquid, add a few drops of *barium chloride solution*; no precipitate is formed (freedom from agar).

Tests

Acacia and other soluble gums. To 20 ml of a 2.5 per cent w/v suspension of the powdered material prepared with freshly boiled *water* add 10 ml of *lead acetate solution*; a flocculent precipitate is formed. Filter and add to the filtrate 10 ml of *lead subacetate solution*; a slight cloudiness may appear, but there is no precipitate.

Karaya gum. Boil 1 g with 20 ml of *water* until a mucilage is formed, add 5 ml of *hydrochloric acid* and again boil for 5 minutes; no pink or red colour develops.

Sterculia. A. Shake 0.2 g of the powdered material with 10 ml of *ethanol (60 per cent)* in a 10-ml stoppered cylinder; any gel formed occupies not more than 1.5 ml.

B. Shake 1 g of the powdered material with 100 ml of *water* and titrate with *0.01 M sodium hydroxide*, using *methyl red solution* as indicator. Not more than 5.0 ml is required to change the colour of the solution.

Foreign matter. Not more than 1.0 per cent, determined by the following method. To 2.0 g of the powdered material in a 250-ml round-bottomed flask add 95 ml of *methanol*, swirl to moisten the powder and add 60 ml of *7 M hydrochloric acid*. Add a few glass beads and heat under a reflux condenser in a water-bath for 3 hours, shaking occasionally. Remove the glass beads and filter the hot suspension under reduced pressure through a sintered-glass crucible (porosity No. 1). Rinse the flask with a small quantity of *water*, passing the rinsings through the filter. Wash the residue on the filter with about 40 ml of *methanol* and dry to constant weight at 110°.

Arsenic (2.3.10). Mix 3.3 g with 3 g of *anhydrous sodium carbonate*, add 10 ml of *bromine solution* and mix thoroughly. Evaporate to dryness on a water-bath, gently ignite, and dissolve the cooled residue in 16 ml of *brominated hydrochloric acid AsT* and 45 ml of *water*. Remove the excess of bromine with 2 ml of *stannous chloride AsT*. The resulting solution complies with the limit test for arsenic (3 ppm).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method B (40 ppm).

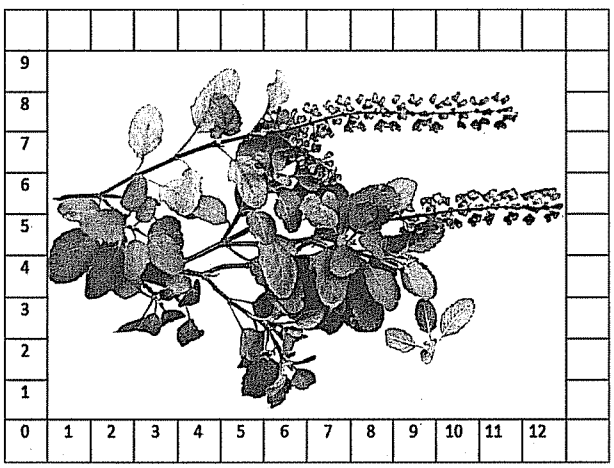
Total ash (2.3.19). Not more than 4.0 per cent, determined on 1.0 g.

Microbial contamination (2.2.9). 1 g is free from *Escherichia coli* and 10 g is free from *salmonellae*.

Storage. Store protected from moisture.

Tulasi

Basil; *Ocimum sanctum*



Tulasi consists of leaves of *Ocimum sanctum* Linn (Fam. Lamiaceae).

Tulasi contains not less than 0.40 per cent w/w of eugenol, calculated on the dried basis.

Description. Greyish-black in colour having characteristic odour with slightly pungent and aromatic taste.

Identification

A. *Macroscopic* — Leaves simple, elliptic, 2.7-7.5 cm long, 1-3 cm wide, with acute top, cuneate, obtuse to rounded base, margin entire, undulate or serrate, both surfaces thinly pubescent and dotted; petiole 0.2-3.0 cm long. Flowers are 5-7 mm in length. It has both male and female parts. Calyx: There are 5 sepals and it is greenish in colour. Corolla: There are 5 petals, bilabiate in shape and covered with scattered hairs. Petals whitish-purple.

B. *Microscopic* — Transverse section of leaf shows a pot shaped midrib. Upper epidermis consists of a layer of small, quadrangular transparent cells with thin walls and thin smooth cuticle. On tangential view, these cells are polygonal with straight or wavy walls. Lower epidermis consists of a layer of small, quadrangular transparent cells with thin walls and thin and thin smooth cuticle. Trichomes bent, consisting of 2-6 cells; glandular trichomes short, lamiaceae type, consisting of one stock cell and 2-4 cells with rounded heads. Palisade parenchyma consists of layer of long cylindrical cells containing chlorophyll; spongy parenchyma consists of polygonal cells with thin, straight or slightly wavy side walls. Vascular bundles collateral type. Stomata diacytic.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 97 volumes of *toluene* and 3 volumes of *ethyl acetate*.

Test solution. Reflux 2 g of the coarsely powdered substance under examination with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Reference solution. Reflux 1 g of *tulasi RS* with 25 ml of *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 25 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate under vacuum to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate 110° for 10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 3.0 per cent.

Water-soluble extractive (2.6.3). Not less than 10.0 per cent by method I.

Total ash (2.3.19). Not more than 15.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 5.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by gas chromatography (2.4.13).

Test solution. Reflux 0.5 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water-bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the extract turns colourless, cool and filter.

Combine all the filtrates and concentrate to a volume slightly less than 100 ml. Dilute to 100.0 ml with *methanol.*

Reference solution. A 0.004 per cent w/v solution of *eugenol RS* in *methanol.*

Chromatographic system
- a capillary column 30 m x 0.25 x 0.25 mm coated with100 per cent dimethylpolysiloxane
- temperature:
  oven 60° to 260° @10° per minute, (Initially and finally hold for 5 minutes respectively)
  Injector 240°,
  detector 280°,
- flow rate. 0.8 ml per minute,
- split flow 20 ml per minute.

Inject 1 µl of the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the refernce solution and the test solution.

Calculate the content of eugenol.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Tulasi Dry Extract

Tulasi Dry Extract is obtained from the Tulasi leaves (*Ocimum sanctum* Linn, Fam. Laminaceae) by extraction with *methanol* or other any suitable solvent and evaporation of solvent.

Tulasi Dry Extract contains not less than 90.0 per cent w/w and not more than 120.0 per cent w/w of the stated amount of the ursolic acid, calculated on the dried basis. It may contain suitable added substances.

Description. A pale green powder.

Identification

A. Determine by thin layer chromatography (2.4.17) coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 95 volumes of *chloroform* and 5 volume of *methanol.*

Test solution. Shakewell 0.2 g of the extract under examination with 10.0 ml *methanol,* filter.

Reference solution. A 0.02 per cent w/v solution of *ursolic acid RS* in the *methanol.*

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and spray with *anisaldehyde-sulphuric acid reagent.* Heat the plate at 110° for 10 minutes and examine in ultraviolet light at 365 nm and in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Ethanol-soluble extractive (2.6.2). Not less than 15.0 per cent.

Water-soluble extractive (2.6.3). Not less than 30.0 per cent by method I.

Total ash (2.3.19). Not more than 10.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 6.0 per cent determined on 1 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Shakewell about 0.5 g of the extract under examination in 100 ml of the *methanol.*

Reference solution. A 0.03 per cent w/v solution of *ursolic acid RS* in the *methanol.*

Chromatographic system
- a stainless steel column, 25 cm x 0.46 mm packed with octadescylsilane bonded to porous (0.5 µm),
- mobile phase: a mixture of 30 volumes of *methanol* and 70 volumes of *acetonitrile,*
- flow rate. 0.6 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

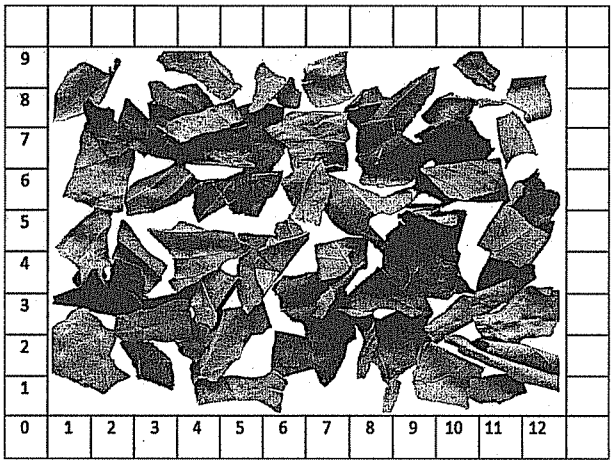
Calculate the content of the ursolic acid in extract.

Usual strengths. 2 per cent w/w; 3 per cent w/w.

Storage. Store in a container purged with Nitrogen under the refrigerated condition, protected from heat and moisture.

Vasaka

Adulasa; *Adhatoda vasica*



Vasaka consists of the dried mature leaves of *Adhatoda vasica* Nees. (Fam. Acanthaceae).

Vasaka contains not less than 0.6 per cent w/w of vasicine, calculated on the dried basis.

Description. Taste, bitter.

Identification

A. *Macroscopic* — Leaf pieces membranous, brittle, greyish-brown, a few pieces green coloured. Floral bracts leaf-like.

B. *Microscopic* — Stomata diacytic, more on the lower epidermis; glandular and non-glandular hair on both surfaces of leaf; elongated cystoliths present in palisade cells.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 8 volumes of *ethyl acetate*, 2 volumes of *methanol* and 0.2 volume of *strong ammonia solution*.

Test solution. Reflux 1 g of coarsely powdered substance under examination with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 10 ml.

Reference solution. Reflux 0.5 g of *vasaka RS* with 50 ml *methanol* for 15 minutes, cool and filter. Reflux the residue further with 2 × 50 ml of *methanol*, cool and filter. Combine all the filtrates and concentrate to 5 ml.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *dragendorff's reagent*. Heat the plate at 100° for 5-10 minutes and examine in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Foreign organic matter (2.6.1). Not more than 2.0 per cent.

Ethanol-soluble extractive (2.6.2). Not less than 3.0 per cent.

Water-soluble extractive (2.6.3). Not less than 22 per cent by Method I.

Total ash (2.3.19). Not more than 21.0 per cent.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss on drying (2.4.19). Not more than 12.0 per cent, determined on 5 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Reflux about 2 g of the coarsely powdered substance under examination with 50 ml of *methanol* on a water bath for 15 minutes, cool and filter. Reflux the residue further with *methanol* till the last extract turns colorless, cool and filter. Combine all the filtrates and concentrate to 100.0 ml.

Reference solution. Dissolve 25 mg of *vasicine hydrochloride RS* in 50 ml of *methanol*. Dilute 5.0 ml of this solution to 50.0 ml with *methanol*.

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 3 volumes of the solution prepared by dissolving 1 g of *sodium hexanesulphonate* in 1000 ml of *water*, 1 volume of *acetonitrile* and 20 volumes of *glacial acetic acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of vasicine.

Storage. Store protected from heat, moisture and against attack by insects and rodents.

Vasaka Extract

Vasaka extract is obtained by extracting Vasaka (*Adhatoda vasica* Nees, Fam. Acanthaceae) dried matured leaves with ethanol or any other suitable solvent. The powdered extract may contain suitable excipients.

Vasaka extract contains not less than 90.0 per cent w/w and not more than 120.0 percent w/w of the stated amount of vasicine.

Description. Light green to greenish brown powder; characteristic odour and bitter taste.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 8 volumes of *ethyl acetate*, 2 volumes of *methanol* and 1 volume of *strong ammonia*.

Test solution. Dissolve 1.0 g of extract under examination with 25 ml of *methanol* and filter.

Reference solution. A 0.1 per cent w/v solution of *vasicine hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm.Spray the plate with *Dragendorff reagent*.Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm.The chromatographic profile of the test solution is similar to that of the reference solution.

Tests

Loss of drying (2.4.19). Not more than 6.0 per cent, determined on 2.0 g when dried at 105° for 3 hours.

Total ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, (Method B) 20 ppm.

Microbial contamination (2.2.9). Complies with the microbial contamination test.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 1.5 g of the extract containing 20 mg of vasicine in 100 ml of *methanol* by gently heating, and filter.

Reference solution. A 0.025 per cent w/v solution of *vasicine hydrochloride RS* in *methanol*.

Chromatographic system

- a stainless steel column 250 cm x 4.6 mm packed with silica bonded with cyanopropyl groups (5 µm),
- *mobile phase*: A mixture of 92 volumes of buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen ortho phosphate* in 500 ml of *water* and 2 ml of *orthophosphoric acid*, dilute to 1000 ml with *water*, 5 volumes of *acetonitrile* and 3 volumes of *tetrahydrofuran*,
- flow rate. 1.0 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the refernce solution and the test solution.

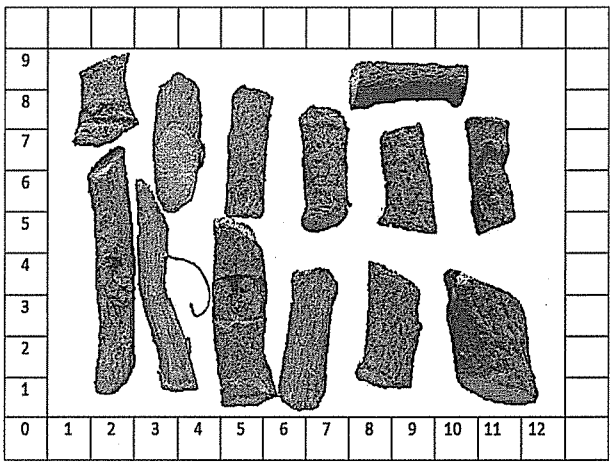
Calculate the content of *vasicine*.

Usual strengths. 1 per cent w/w; 1.5 per cent w/w.

Storage. Store protected from heat and moisture.

Yasti

Liquorice root; Mulethi; *Glycyrrhiza glabra*



Yasti consists of the dried, unpeeled roots and stolons of *Glycyrrhiza glabra* Linn. (Fam. Leguminosae).

Yasti contains not less than 3.0 per cent w/w of glycyrrhizinic acid.

Description. Odour, characteristic and slightly aromatic; taste, very sweet and faintly astringent; the bark is not bitter.

Identification

A. *Macroscopic* — Root with few branches, up to 1 m long and 0.5 to 3 cm in diameter. Bark, brownish-grey to brown with longitudinal striations, bearing traces of lateral roots. Stolons, cylindrical, 1 to 2 cm in diameter and up to several meters long, but may be cut into lengths of 10 to 15 cm; similar in external appearance to the root but with occasional small buds. Fracture of the root and stolon, granular and fibrous. Cork layer, thin; secondary phloem region, wide, light yellow with radial striations; xylem, compact, yellow, with radiate structure. The stolon has a central pith which is absent from the root.

B. *Microscopic* — Cork and phelloderm are narrow. Phloem consisting of bundles of thick-walled, yellow fibres with narrow

lumina surrounded by cells each containing a calcium oxalate prism, alternating in the external layers with areas of strongly hyaline keratenchyma; functional sieve tissue near the cambium. Medullary rays parenchymatous, widening towards the exterior, 3 to 12 cells wide. Xylem composed of radial rows of tracheids and vessels alternating with bundles of lignified fibres with crystal sheaths similar to those of the secondary phloem; vessels 30 µm to 150 µm in diameter with thick walls (5 µm to 10 µm) having reticulate thickenings or numerous bordered pits with slit-shaped openings associated with lignified xylem parenchyma. Medullary rays, 2 to 5 cells wide. Parenchymatous cells throughout containing simple, round, oval or fusiform starch granules 2 µm to 20 µm, mostly 5 µm to 12 µm, in diameter; parenchymatous pith present solely in the stolon.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 70 volumes of *butyl alcohol,* 20 volumes of *water* and 10 volumes of *acetic acid.*

Test solution. Add 10 ml of 70 per cent v/v *methanol* to 1 g of dried yasti powder, heat by shaking on a water bath for 5 minutes, cool and filter.

Reference solution. Add 10 ml of 70 per cent v/v *methanol* to 1 g of dried *yasti RS* powder, heat by shaking on a water bath for 5 minutes, cool and filter

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air and examine in ultraviolet light at 254 nm and 365 nm, spray with *anisaldehyde sulphuric acid reagent*. Heat the plate at 105° for 10 minutes and examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

D. Mix a small quantity, in powder, with 0.05 ml of *sulphuric acid*; the powder particles become orange-yellow and some fragments change, more slowly, to pinkish red.

Tests

Curcuma. When examined under a microscope, none of the fragments of the powder in *sulphuric acid* (see Identification test D) should immediately take on a carmine-red colour.

Water-soluble extractive (2.6.3). Not less than 20 per cent, determined by the following method. Mix 2.5 g of the finely powdered drug with 50 ml of *water* and allow to stand for 2 hours, shaking frequently. Filter, evaporate 10.0 g of the filtrate to dryness on a water-bath, dry the residue at 105° and weigh.

Acid-insoluble ash (2.3.19). Not more than 2.0 per cent.

Sulphated ash (2.3.18). Not more than 10.0 per cent.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Weigh accurately about 1.0 g of the coarsely powdered substance under examination in a 250-ml conical flask, add 100 ml of *0.1 M ammonia* and mix with the aid of ultrasound for 30 minutes. Centrifuge a part of the supernatant liquid and dilute 1.0 ml to 5.0 ml with *0.1 M ammonia*. Filter the solution through a membrane filter disc with an average pore diameter not greater than 1.0 µm and use the filtrate.

Reference solution. A 0.005 per cent w/v solution of *glycyrrhizinic acid RS* in *0.1 M ammonia*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

Inject the test solution and the reference solution and measure the responses for the analyte peak.

Calculate the content of glycyrrhizinic acid.

Storage. Store protected from light and moisture.

Yasti Dry Extract

Yasti Dry Extract is obtained by extracting Yasti with *water* or aqueous *ethanol* or any other suitable solvent.

Yasti Dry Extract contains not less than 90.0 per cent w/w and not more than 120.0 per cent w/w of the stated amount of glycyrrhizinic acid.

Description. A yellowish-brown to dark brown powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of *butanol,* 10 volumes of *acetic acid* and 20 volumes of *water*.

Test solution. Dissolve 200 mg of the extract under examination with 50 ml of *methanol* (70 per cent) and filter.

Reference solution. A 0.1 per cent w/v solution of *glycyrrhizin ammonical hydrate RS* in *methanol* (70 per cent).

Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air

and examine in ultraviolet light at 254 nm and 365 nm. Spray the plate with *anisaldehyde sulphuric acid reagent* and heat the plate at 105° for 10 minutes and examine the plate in day light. The chromatogram obtained with test solution shows a band corresponding to the band obtained with the reference solution indicating the presence of glycyrrhizin.

Tests

Acid insoluble ash (2.3.19). Not more than 2.0 per cent.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Loss of drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Microbial contamination (2.2.9). Complies with the microbial contamination tests.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 500 mg of the extract or a quantity containing 7 mg of glycyrrhizinic acid and dissolve in the mobile phase by mixing for 30 minutes with the aid of ultrasound and dilute to 100.0 ml with the mobile phase and filter.

Reference solution. A 0.01 per cent w/v solution of *glycyrrhizin ammonical hydrate RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 1 volume of *glacial acetic acid*, 67 volumes of *methanol* and 33 volumes of *0.2 M ammonium acetate* in *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 250 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of glycyrrhizinic acid in the extract.

Storage. Store protected from heat and moisture.

BLOOD AND BLOOD-RELATED PRODUCTS

Anti-A Blood Grouping Serum

Anti-A Blood Grouping Serum is a sterile, liquid or dried preparation containing the particular blood group antibodies derived from high-titered blood plasma or serum of human subjects, with or without stimulation by the injection of Blood Group Specific Substance A (or AB). It contains a suitable antimicrobial preservative. It is of two types polyclonal & monoclonal.

Production

The monoclonal antibody technique devised by Kohler and Milsten has proved useful in producing high titre and specific antibodies. Laboratory animals, usually mice are immunized for the production of monoclonal antibodies. After suitable immune response, mouse spleen cells containing antibody secreting lymphocytes are fused to neoplastic plasma cells of infinite reproductive capacity from a mouse (that is myeloma cells). The resulting hybridomas are screened for antibody with the required specificity and affinity. The antibody secreting clones may then be propagated in tissue culture or by inoculation into mice in which case the antibodies are collected as ascites. The clonal line produces a single antibody, there is no need for absorption or to remove heterospecific antibodies. All antibody molecules produced by a clone of hybridoma cells are identical in terms of antibody structure and antigen specificity. Once one antibody secreting clone of cells has been established, antibody with same specificity and reaction characteristics will be available indefinitely.

Identification

It agglutinates human red cells containing A-antigens that is blood groups A and AB (including subgroups A_1, A_2, A_1B, and A_2B but not necessarily weaker subgroups).

Tests

It complies with the test for potency, in parallel with, and not less than equivalent to, the Reference Blood Grouping Serum Anti-A, in agglutinating red blood cells from Group A_1 and Group A_2B donors. It complies with the tests for specificity with Group A_1, A_2B, B, and O cells and confirms the absence of contaminating antibodies reactive with M^g, Wr^a antigens as well as other antigens having an incidence of 1 per cent or greater in the general population (see under *Blood Grouping Serums Anti-D, Anti-C, Anti-E, Anti-c, rAnti-e*). It complies with the tests for avidity with Group A_1 and A_2B cells. All fresh or frozen red blood cell suspensions used for these tests are prepared under specified conditions and meet specified criteria. Anti-A Blood Grouping Serum may be artificially coloured blue.

Expiration date. The expiration date for liquid serum is not later than 1 year, and for dried serum not later than 5 years after the date of issue from manufacturer's cold storage (5°, 1 year; or 0°, 2 years), provided that the expiration date for dried serum is not later than 1 year after constitution.

Storage. Store at a temperature between 2° and 8°.

Labelling. The label states that the source material was not reactive for hepatitis B surface antigen, but that no known test method offers assurance that products derived from human blood will not transmit hepatitis in case of polyclonal. Label also states that it is for *in vitro* diagnostic use.

NOTE—The labeling is in black lettering imprinted on paper that is white or is coloured completely or in part to match the specified blue colour standard.

Anti-B Blood Grouping Serum

Anti-B Blood Grouping Serum is a sterile, liquid or dried preparation containing the particular blood group antibodies derived from high-titered blood plasma or serum of human subjects, with or without stimulation by the injection of Blood Group Specific Substance B (or AB). It contains a suitable antimicrobial preservative.

Production

The monoclonal antibody technique devised by Kohler and Milsten has proved useful in producing high titre and specific antibodies. Laboratory animals, usually mice are immunized for the production of monoclonal antibodies. After suitable immune response, mouse spleen cells containing antibody secreting lymphocytes are fused to neoplastic plasma cells of infinite reproductive capacity from a mouse (that is myeloma cells). The resulting hybridomas are screened for antibody with the required specificity and affinity. The antibody secreting clones may then be propagated in tissue culture or by inoculation into mice in which case the antibodies are collected as ascites. The clonal line produces a single antibody, there is no need for absorption or to remove heterospecific antibodies. All antibody molecules produced by a clone of hybridoma cells are identical in terms of antibody structure and antigen specificity. Once one antibody secreting clone of cells has been established, antibody with same specificity and reaction characteristics will be available indefinitely.

Identification

It agglutinates human red cells containing B-antigens, that is blood groups B and AB (including subgroups A_1B and A_2B).

Tests

It complies with the test for potency, in parallel with, and not less than equivalent to, the Reference Blood Grouping Serum

Anti-B, in agglutinating red blood cells from Group B donors. It complies with the tests for specificity with Group A_1, B, and O cells and confirms the absence of contaminating antibodies reactive with M^g, Wr^a antigens as well as other antigens having an incidence of 1.0 per cent or greater in the general population (see under *Blood Grouping Serums Anti-D, Anti-C, Anti-E, Anti-c, Anti-e*). It complies with the test for avidity with Group B cells. All fresh or frozen red blood cell suspensions used for these tests are prepared under specified conditions and meet specified criteria. Anti-B Blood Grouping Serum may be artificially coloured yellow.

Expiration date. The expiration date for liquid serum is not later than 1 year and for dried serum not later than 5 years after the date of issue from manufacturer's cold storage (5°, 1 year; or 0°, 2 years), provided that the expiration date for dried serum is not later than 1 year after constitution.

Storage. Store at a temperature between 2° and 8°.

Labelling. The label states that the source material was not reactive for hepatitis B surface antigen, but that no known test method offers assurance that products derived from human blood will not transmit hepatitis in case of polyclonal. Label also states that it is for *in vitro* diagnostic use.

NOTE—The labelling is in black lettering imprinted on paper that is white or is coloured completely or in part to match the specified yellow colour standard.

Anti-D (Rh_0) Immunoglobulin

Human Anti-D Immunoglobulin

Human anti-D immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. The preparation is intended for intramuscular administration. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. Human normal immunoglobulin may be added.

It complies with the monograph on Human Normal Immunoglobulin, except for the minimum number of donors and the minimum total protein content. For products prepared by a method that eliminates immunoglobulins with specificities other than anti-D, where authorised, the test for antibodies to hepatitis B surface antigen is not required.

Production

Human anti-D immunoglobulin is preferably obtained from the plasma of donors with a sufficient titre of previously acquired anti-D antibodies. Where necessary, in order to ensure an adequate supply of human anti-D immunoglobulin, it is obtained from plasma derived from donors immunised with D-positive erythrocytes that are compatible in relevant blood group systems in order to avoid formation of undesirable antibodies.

Erythrocyte donors

Erythrocyte donors comply with the requirements for donors prescribed in the monograph on Human Plasma for Fractionation.

Immunisation

Immunisation of the plasma donor is carried out under proper medical supervision. Recommendations concerning donor immunisation, including testing of erythrocyte donors, have been formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives*, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

Pooled plasma

To limit the potential B19 virus burden in plasma pools used for the manufacture of anti-D immunoglobulin, the plasma pool is tested for B19 virus using validated nucleic acid amplification techniques (2.8.1).

B19 virus DNA. Maximum 10^4 IU per ml.

A positive control with 10^4 IU of B19 virus DNA per ml and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

B19 virus DNA for NAT testing reference preparation is suitable for use as a positive control.

If Human Normal Immunoglobulin is added to the preparation, the plasma pool from which it is derived complies with the above requirement for B19 virus DNA.

Tests

Potency. Determine the assay of human anti-D immunoglobulin by Method A (2.8.2). The estimated potency is not less than 90.0 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 120.0 per cent of the estimated potency.

Method B or C (2.8.3) may be used for potency determination if a satisfactory correlation with the results obtained by Method A has been established for the particular product.

Storage. For the liquid preparation, store protected from light, in a plastic container. For the freeze-dried preparation, store protected from light, in a plastic container.

Labelling. The label states (1) for liquid preparations, the volume of the preparation in the container and the protein

content expressed in grams per litre; (2) for freeze-dried preparations, the quantity of protein in the container; (3) the route of administration; (4) for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added; (5) where applicable, that the preparation is suitable for use in the prophylaxis of hepatitis A infection; (6) where applicable, the anti-hepatitis A virus activity in International Units per ml; (7) where applicable, the name and amount of antimicrobial preservative in the preparation; (8) the number of International Units per container.

Anti-D Immunoglobulin for Intravenous Use

Anti-D Immunoglobulin Human for Intravenous Use

Anti-D Immunoglobulin for intravenous administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It contains specific antibodies against erythrocyte D-antigen and may also contain small quantities of other blood-group antibodies. Human normal immunoglobulin for intravenous use may be added.

It complies with the monograph on Human Normal Immunoglobulin for Intravenous Administration, except for the minimum number of donors, the minimum total protein content, the limit for osmolality and the limit for prekallikrein activator. For products prepared by a method that eliminates immunoglobulins with specificities other than anti-D where authorised, the test for antibodies to hepatitis B surface antigen is not required; a suitable test for Fc function is carried out.

Production

Polyclonal (human) anti Rh (D) serum

Human anti-D immunoglobulin is preferably obtained from the plasma of donors with a sufficient titre of previously acquired anti-D antibodies. Where necessary, in order to ensure an adequate supply of human anti-D immunoglobulin, it is obtained from plasma derived from donors immunised with D-positive erythrocytes that are compatible in relevant blood group systems in order to avoid formation of undesirable antibodies.

Erythrocyte donors

Laboratory tests are carried out for each donation to detect the following viral markers:

1. Antibodies against human immunodeficiency virus 1 (anti-HIV-1),
2. Antibodies against human immunodeficiency virus 2 (anti-HIV-2),
3. Antibodies against hepatitis C virus (anti-HCV).
4. Hepatitis B surface antigen (HBsAg),

Pending complete harmonisation of the laboratory tests to be carried out, the competent authority may require that a test for alanine aminotransferase (ALT) also be carried out.

The test methods used are of suitable sensitivity and specificity and comply with the regulations in force. If a repeat-reactive result is found in any of these tests, the donation is not accepted.

Immunisation

Immunisation of the plasma donor is carried out under proper medical supervision. Recommendations concerning donor immunisation, including testing of erythrocyte donors, have been formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives*, WHO Technical Report Series, No. 840, 1994 or subsequent revision).

Pooled plasma

To limit the potential B19 virus burden in plasma pools used for the manufacture of anti-D immunoglobulin, the plasma pool is tested for B19 virus using validated nucleic acid amplification techniques (2.8.1).

Identification

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and sample under examination in a dilution of 10 g per litre of protein. The main component of the sample corresponds to the IgG component of normal human serum.

Tests

B19 virus DNA. Maximum 10^4 IU per ml.

A positive control with 10^4 IU of B19 virus DNA per ml and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors.

B19 virus DNA for NAT testing RP is suitable for use as a positive control.

If Human normal immunoglobulin for intravenous administration is added to the preparation, the plasma pool from which it is derived complies with the above test for B19 virus DNA.

Assay. Determine by Method A of human anti-D immunoglobulin (2.8.2). The estimated potency is not less

than 90.0 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 120.0 per cent of the estimated potency.

Method B or C (2.8.3) may be used for potency determination if a satisfactory correlation with the results obtained by Method A has been established for the particular product.

Storage. For the liquid preparation, store protected from light, in a colourless glass container, at the temperature stated on the label. For the freeze-dried preparation, store protected from light, in colourless glass container, at a temperature not exceeding 25°.

Labelling. The label states (1) for liquid preparations, the volume of the preparation in the container and the protein content expressed in gram per litre; (2) for freeze-dried preparations, the quantity of protein in the container; (3) the amount of immunoglobulin in the container; (4) the route of administration, (5) for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added; (6) the distribution of subclasses of immunoglobulin G present in the preparation; (7) where applicable, the amount of albumin added as a stabilizer; (8) the maximum content of immunoglobulin A; (9) the number of International Units per container.

Anti-Human Globulin Serum

Anti-Human Globulin Serum is a sterile, liquid preparation of serum produced by immunizing lower animals such as rabbits or goats with human serum or plasma, or with selected human plasma proteins. It is free from agglutinins and from hemolysins to nonsensitized human red cells of all blood groups. It contains a suitable antimicrobial preservative.

Three varieties of Anti-Human Globulin Serum are recognized: (1) a general-purpose polyspecific reagent which, as a minimum, contains antibodies specific for immunoglobulin IgG, and at least the C3d component of human complement (for use in the direct antiglobulin test, it contains this Anti-C3d and Anti-IgG activity) and which may be artificially coloured green; (2) a reagent containing antibodies only against immunoglobulin IgG (not heavy chain specific) intended for use in the indirect antiglobulin test, and which may be artificially coloured green; and (3) reagents containing antibodies specific for individual or selected components of human complement, such as Anti-C3, and Anti-C3b-C3d-C4, or a single class of immunoglobulins, such as Anti-IgG (heavy chain specific), used only to identify plasma components coated on the surface of red blood cells. Anti-Human Globulin Serum containing Anti-IgG complies with the test for potency, in parallel with the Reference Anti-Human Globulin (Anti-IgG) Serum (at a 1:4 dilution) when tested with red cells suspended in isotonic saline sensitized with decreasing amounts of nonagglutinating Anti-D (Anti-Rh_o) serum, and with cells sensitized in the same manner with an immunoglobulin IgG Anti-Fy^a serum of similar potency. Anti-Human Globulin Serum containing one or more Anti-complement components complies with the tests for potency in giving a 2+ agglutination reaction (i.e., agglutinated cells dislodged into many small clumps of equal size) by the low-ionic sucrose or sucrose–trypsin procedures when tested as recommended in the labelling. Anti-Human Globulin Serum containing Anti-3Cd activity meets the requirements for stability, by potency testing of representative lots every 3 months during the dating period.

Expiration date. Its expiration date is not later than 1 year after the date of issue from manufacturer's cold storage (5°, 1 year; or 0°, 2 years).

Storage. Store at a temperature between 2° and 8°.

Labelling. The label states the animal source of the product, the specific antibody activities present; the application for which the reagent is intended; a cautionary statement that it does not contain antibodies to immunoglobulins or that it does not contain antibodies to complement components, wherever and whichever is applicable; and states that it is for *in vitro* diagnostic use.

NOTE—The lettering on the label of the general-purpose polyspecific reagent is black on a white background. The label of all other Anti-Human Globulin Serum containers is in white lettering on a black background.

Blood Grouping Serums Anti-D, Anti-C, Anti-E, Anti-c, Anti-e

Anti-Rh Blood Grouping Serums

Blood Grouping Serums Anti-D, Anti-C, Anti-E, Anti-c, Anti-e (Anti-Rh Group) are sterile, liquid or dried preparations derived from the blood plasma or serum of human subjects who have developed specific Rh antibodies. They are free from agglutinins for the A or B antigens and from alloantibodies other than those for which claims are made in the labelling. They contain a suitable antimicrobial preservative. Liquid serums are not artificially coloured. Two varieties of Anti-Rh Blood Grouping Serums are recognized, i.e., (1) saline agglutinating "complete" antiserums, which specifically agglutinate human red blood cells suspended in saline and (2) "blocking or incomplete" antiserums, which contain protein or other macromolecular substances, usually require the cells to be suspended in serum or plasma, and generally are for slide or rapid tube tests. The most commonly used of these

blood grouping serums are listed in Table 1 each reacting with the antigen(s) designated by the corresponding letter(s) with the alternative nomenclature indicated parenthetically.

Table - 1

Serum	Antigen(s) Reacting
Anti-D (Anti-Rh_o)	D (Rh_o)
Anti-C (Anti-rh′)	C (rh′)
Anti-E (Anti-rh″)	E (rh″)
Anti-CD (Anti-Rh_o′)	D (Rh_o) and C (rh′)
Anti-DE (Anti-Rh_o″)	D (Rh_o) and E (rh″)
Anti-CDE (Anti-Rh_o‴)	D (Rh_o), C (rh′), and E (rh′)
Anti-c (Anti-hr′)	c (hr′)
Anti-e (Anti-hr″)	e (hr″)

Each serum complies with the test for potency in the case of serums for saline tube test in parallel with, and not less than equivalent to, the Reference Blood Grouping Serum for Anti-D, Anti-C, or Anti-E, whichever is applicable, or, in the case of Anti-c and Anti-e for saline tube test which have no reference preparations, the test for minimum agglutination reactivity at a specified dilution; and in the case of serums for slide or rapid tube test in parallel with, and not less than equivalent to, the Reference Blood Grouping Serum for Anti-D, Anti-C, Anti-E, Anti-c, or Anti-e, whichever is applicable, in agglutinating as a minimum red blood cells from the donors indicated in Table 2 (which may be from Group A, B, AB, or O).

Each serum for slide or rapid tube test complies with the tests for avidity with the cells as indicated under tests for potency above.

Table - 2

Serum	Phenotype of Cells
Anti-D	cDe
Anti-C	Ccde
Anti-E	cdEe
Anti-CD	cDe, Ccde
Anti-DE	cDe, cdEe
Anti-CDE	cdEe, cDe, Ccde
Anti-c	CcDEe
Anti-e	cdEe

Each serum complies with the tests for specificity by the most sensitive method recommended by the manufacturer, in which not less than 4 positive and 4 negative phenotypes are included (see Table 3), and confirms the absence of contaminating antibodies reactive with M^g, Wr^a antigens as well as other antigens having an incidence of 1.0 per cent or greater in the general population, except where some of these confirmatory tests can not be done by the manufacturer, in which event such omissions are noted.

Table - 3

Serum	Cells
Anti-D	CcDe, cDe, Ccde, cdEe, A_1 cde, B cde, O cde, and where recommended for use by indirect antiglobulin technique, cde Bg(a+) cells from 3 different donors
Anti-C	cDe, Ccde, cdEe, C + rh_i neg. cells, A_1 cde, B cde, O cde
Anti-E	cDe, Ccde, cdEe, A_1 cde, B cde, O cde
Anti-CD	cDe, Ccde, cdEe, A_1 cde, B cde, O cde, and where recommended for detection of the G antigen, r^Gr
Anti-DE	cDe, Ccde, cdEe, A_1 cde, B cde, O cde
Anti-CDE	cDe, Ccde, cdEe, A_1 cde, B cde, O cde, and where recommended for detection of the G antigen, r^Gr
Anti-c	Ccde, A_1 CDe, B CDe, O CDe, and CDEe or CDE or CdE
Anti-e	cdEe, A_1 cDE, B cDE, O cDE, and CcDE or CDE or CdE

All fresh or frozen red blood cell suspensions used for these tests are prepared under specified conditions and meet specified criteria.

Monoclonal Rh (D) antibodies. Monoclonal antibodies are derived from hybridoma cell lines produced by fusing mouse antibody produced by B lymphocyte with mouse myeloma cells or are derived from a human B cell line through Epstein-Barr Virus (EBV) transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.

The type of monoclonal anti-D reagents are:

1. IgM anti-D monoclonal reagent,
2. Blend of IgM and IgG monoclonal antibodies reagent,
3. Blend of monoclonal IgM and polyclonal (human) IgG anti-D.

IgM anti-D monoclonal antibodies are highly specific and saline reacting equally well at room temperature and at 37°. They are good for slide test or immediate spin tube tests as well as routine Rh(D) typing in tube. IgM anti D are unreliable for detection of weak D (Du) by AHG test. Blend of IgM and

IgG (monoclonal) anti-D or blend of IgM (monoclonal) and polyclonal (human) IgG anti-D can be used for testing weak D (Du) antigen by AHG test. Mostly blended IgM and IgG (monoclonal) anti- Rh(D) or blended monoclonal IgM and polyclonal (human) IgG anti Rh (D) antibodies are used now in routine.

Expiration date. The expiration date for liquid serums is not later than 1 year and for dried serums not later than 5 years after date of issue from manufacturer's cold storage (5°, 1 year; or 0°, 2 years), provided that the expiration date for dried serums is not later than 1 year after constitution.

Storage. Store at a temperature between 2° and 8°.

Labelling. The label each to state that the source material was not reactive for hepatitis B surface antigen, but that no known test method offers assurance that products derived from human blood will not transmit hepatitis. Label each to state that it is for *in vitro* diagnostic use.

Concentrated Human Red Blood Corpuscles

Concentrate of Human Red Blood Cells; Packed Red Cells

Concentrated Red Blood Cells (RBCs) are units of whole blood with most of the plasma removed. Additive red cell preservative systems consist of a primary collection bag containing an anticoagulant preservative with at least two satellite bags integrally attached, one is empty and one contains an additive solution (AS).

Each 100 ml citrate phosphate dextrose (CPD) contains

Citric acid (monohydrate)	0.327 g
Sodium citrate (dihydrate)	2.63 g
Sodium acid phosphate (dihydrate)	0.251 g
Dextrose (monohydate)	2.55 g
Water for injection	100 ml

AS contains sodium chloride, dextrose, adenine and other substances that support red cell survival and function up to 42 days. The volume of AS in 350 ml is 49 ml and in 450 ml is 63 ml. AS is added to the red cells remaining in the primary bag after most of the plasma has been removed. This allows blood centres to use or recover a maximum amount of plasma, yet still prepare a red cell component with a final haematocrit between 55.0 per cent and 65.0 per cent, a level that facilitates excellent flow rates and allows easy administration.

Production

It may be prepared by centrifugation or undisturbed sedimentation for the separation of plasma and anticoagulant solution equivalent to not less than 40.0 per cent of the total volume of Whole Human Blood. A portion of the plasma, sufficient to ensure optimal cell preservation, shall be left. All surfaces that come in contact with the red cells and plasma shall be sterile and pyrogen-free and the entire processing of the blood shall be conducted in a sterile system or in a closed system by use of satellite bags. The final containers used for the Concentrated Human Red Blood Corpuscles shall be the original blood containers unless the method of processing requires a different container. Immediately after processing, the containers are stored at a temperature between 2° and 8° and not opened until immediately before transfusion. Human Red Blood Corpuscles may be stored for a period not longer than that for which the Whole Human Blood from which it is prepared. However, if the hermetic seal is broken during processing, the product must be used within 24 hours. From the tube of the blood bag sample is taken for compatibility and infections markers testing.

Concentrated Human Red Blood Corpuscles should be administered only with suitable equipment meant for the transfusion of blood and blood components.

Concentrated Human Red Blood Corpuscles contains not less than 15.5 per cent w/v of haemoglobin.

Description. A dark red fluid when prepared; after standing, the red corpuscles may form a sediment, leaving a small supernatant layer of yellowish plasma.

Tests

Sterility (2.2.11). Complies with the tests for sterility, determined by Method B.

Assay. Determine the haemoglobin content by photometric haemoglobinometry (2.8.12).

Storage. Store in containers which are made of colourless and transparent glass, or of a suitable plastic material, are sterile and sealed so as to exclude micro-organisms. Store at a temperature between 2° and 8°.

Labelling. The label states (1) the reference number of the Whole Human Blood from which the preparation was made; (2) the ABO and Rh groups of the Whole Human Blood; (3) the date of collection of the Whole Human Blood from which the preparation was made; (4) the storage conditions; (5) the date after which the preparation is not suitable for transfusion; (6) that the preparation should not be used if there is any visible evidence of haemolysis or other deterioration.

Cryoprecipitated Antihaemophilic Factor

Cryoprecipitated Antihaemophilic Factor is a sterile, frozen concentrate of human antihaemophilic factor prepared from

the Factor VIII-rich cryoprotein fraction of human venous plasma obtained from suitable whole-blood donors from a single unit of plasma derived from whole blood or by plasmapheresis, collected and processed in a closed system. It contains no preservative. It complies with the test for potency by comparison with the Standard Antihaemophilic Factor (Factor VIII) or with a working reference that has been calibrated with it, in having an average potency of not less than 80 Antihaemophilic Factor Units per container, made at intervals of not more than 1 month during the dating period.

Expiration date. The expiration date is not later than 1 year from the date of collection of source material.

Storage. Store in hermetic containers at a temperature of - 18° or lower.

Labelling. Label it to indicate (1) the ABO blood group designation and the identification number of the donor from whom the source material was obtained; (2) with the type and result of a serologic test for syphilis, or to indicate that it was non-reactive in such test; with the type and result of a test for hepatitis B surface antigen, or to indicate that it was non-reactive in such test; with a warning not to use it if there is evidence of breakage or thawing; with instructions to thaw it before use to a temperature between 20° and 37°, after which it is to be stored at room temperature and used as soon as possible but within 6 hours after thawing; (3) to state that it is to be used within 4 hours after the container is entered; (4) to state that it is for intravenous administration; (5) that a filter is to be used in the administration equipment.

Dried Human Antihaemophilic Fraction

Dried Factor VIII Fraction; Freeze-dried Human Coagulation Factor VIII

Dried Human Antihaemophilic Fraction is a preparation of antihaemophilic factor which is obtained from human plasma. It is rich in clotting factor VIII.

When the contents of a sealed container of Dried Human Antihaemophilic Fraction are dissolved in a volume of *water* equal to the volume of *water for injection* stated on the label, the resulting solution contains not less than 3.0 Units per ml, not less than 0.1 Unit per mg of protein, not more than 80.0 per cent of which is fibrinogen, and not more than 200 millimoles of sodium ions per litre.

Production

The plasma to be used for preparing Dried Human Antihaemophilic Fraction is obtained from blood of healthy human donors who are, as far as can be ascertained after clinical examination, laboratory tests on their blood and consideration of their medical history, free from detectable agents of infection transmissible by blood transfusion. The examinations and tests to be carried out are decided by the National Regulatory Authority. In particular, the blood must be tested with negative results for (a) evidence of syphilitic infection; (b) hepatitis B surface antigen and (c) HIV antibodies by suitably sensitive methods. The haemoglobin value of the donor's blood is not less than 12.5 per cent w/v.

The blood is withdrawn aseptically through a closed system of sterile tubing into a sterile container in which a suitable anticoagulant solution has been placed before sterilisation. During the withdrawal there is no interruption in the flow from the donor, and the container is gently agitated. Immediately after the withdrawal is completed, the blood is cooled to 4°; if the plasma is to be stored frozen it is separated from the cellular components by centrifugation and frozen to –30° or below, preferably within 12 hours of collection; if the plasma is not to be frozen it is separated from the cellular components by centrifugation as soon as possible and not later than 18 hours after collection, and fractionation begun without delay.

Dried Human Antihaemophilic Fraction may be prepared from human plasma so obtained by precipitation under controlled conditions of pH, ionic strength and temperature with organic solvents, or by freezing and thawing. The precipitate may be washed by extraction with suitable solvents, dissolved in a solution of *sodium citrate* adjusted to a pH of 6.8 to 7.2, which may also contain *sodium chloride*. The solution is sterilised by filtration through a membrane filter, distributed in sterile containers and dried from the frozen state. The air is removed or replaced by oxygen-free nitrogen and the containers are sealed so as to exclude micro-organisms. No antimicrobial preservative is added but an antiviral agent may be added provided that it can be demonstrated to have no deleterious effect on the final product in the amount present and to cause no adverse reaction in man. Heparin may be used.

For the following tests, where it is directed that a solution is to be used, dissolve the contents of a sealed container in a volume of the appropriate solvent equal to the volume of *water for injection* stated on the label.

Description. A white or pale yellow powder or friable solid.

Identification

A. Precipitation tests with a suitable range of species specific antisera give positive results for the presence of plasma proteins of human origin and negative results with antisera specific to plasma proteins of the other species.

B. A freshly prepared solution in *water* causes a reduction in clotting time when treated as directed under Assay.

Tests

pH (2.4.24). 6.8 to 7.4, determined on the reconstituted solution.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined by drying 0.5 g over *phosphorus pentoxide* at a pressure not exceeding 3 kPa for 24 hours.

Haemagglutinins, anti-A and anti-B. Dissolve in *water*. Dilute the solution with *saline solution* to produce a solution containing 3 Units per ml. Carry out the test for haemagglutinins anti-A and anti-B using a suitable indirect method such as that described below.

Prepare in duplicate serial dilutions of the preparation under examination in *saline solution*. To each dilution of one series add an equal volume of a 5.0 per cent v/v suspension of group A_1 red blood cells previously washed three times with *saline solution*. To each dilution of the other series add an equal volume of a 5.0 per cent v/v suspension of group B red blood cells previously washed three times with *saline solution*. Incubate the suspensions at 37° for 30 minutes and then wash the cells three times with *saline solution*. Leave the cells in contact with a polyvalent anti-human globulin reagent for 30 minutes. Without centrifuging, examine each suspension for agglutination under a microscope. The 1 in 64 dilutions do not show agglutination.

Hepatitis B surface antigen. Dissolve in *water*. Examine the solution by a suitably sensitive method such as radioimmunoassay. Hepatitis B surface antigen is not detected.

Abnormal toxicity (2.2.1). When dissolved in *water for injection*, complies with the test for abnormal toxicity, Method B, injecting into each mouse a volume containing 1.5 Units and into each guinea-pig a volume containing 15 Units.

Pyrogens (2.2.8). When dissolved in *water for injection*, complies with the test for pyrogens, using a volume containing 10 Units per kg of the rabbit's weight in rabbits that have not previously received blood products.

Sterility (2.2.11). Complies with the tests for sterility.

Assay

For potency — Carry out the biological assay of human antihaemophilic fraction described below. The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The fiducial limits of error are not less than 64.0 per cent and not more than 156.0 per cent of the stated potency.

Biological assay

The potency of human antihaemophilic fraction is determined by comparing the amount necessary to reduce the clotting time of a test mixture containing substances that cause clotting of blood with the amount of the Standard preparation necessary to produce the same effect under the conditions of the following method of assay.

Standard preparation

The Standard preparation is the 4th International Standard for Blood coagulation factor VIII:C, concentrate, human, established in 1989, consisting of an intermediate purity concentrate of human blood clotting factor VIII (supplied in ampoules containing 6.3 Units of clotting factor VIII), or another suitable preparation the potency of which has been determined in relation to the International Standard

The Unit is the specific antihaemophilic factor contained in such an amount of the Standard preparation as the Ministry of Health & Family Welfare, Govt. of India may from time to time indicate as the quantity exactly equivalent to the Unit accepted for international use.

Special reagents

Normal serum reagent. Collect normal human blood in a dry, sterile, glass bottle, shake continuously until coagulation is complete, incubate at 37° for 3 hours, maintain at 4° overnight, remove the serum, store at –20°, dry from the frozen state and keep in a vacuum desiccator over *phosphorus pentoxide*. Dissolve a quantity of the dried serum calculated to have been obtained from 1 ml of the serum in sufficient *imidazole buffer pH 7.4* to produce 10 ml and allow to stand at 4° for 16 to 24 hours.

Phospholipid. Wash a quantity of normal human or bovine brain freed from meninges and blood vessels and macerate in a suitable blender. Weigh 1,000 to 1,300 g of the macerate and measure its volume (*V*). Extract with three quantities, each of 4*V* ml, of *acetone*, filter by suction and dry the precipitate at 37° for 18 hours. Extract the dried precipitate with two quantities, each of 2*V* ml, of a mixture of two volumes of *light petroleum (boiling range 30° to 40°)* and 3 volumes of *light petroleum (boiling range 40° to 60°)*, filtering each extract through a filter paper previously washed with the light petroleum mixture. Combine the extracts and evaporate to dryness at 45° at a pressure not exceeding 0.7 kPa. Dissolve the residue in 0.2*V* ml of *ether* and allow to stand at 4° until a deposit forms. Centrifuge and evaporate the clear supernatant liquid under reduced pressure until the volume is about 100 ml per kg of the original macerate. Allow to stand at 4° until a precipitate forms (12 to 24 hours) and centrifuge. To the clear supernatant liquid add 5 times its volume of *acetone*, centrifuge, discard the supernatant liquid, dry the precipitate and store protected from light in a vacuum desiccator.

Phospholipid reagent. Suspend 0.125 g of *phospholipid* in 5 ml of *water*, shake and stir until a uniform suspension is obtained. Prepare a dilution with *saline solution* that will give minimum clotting time consistent with the largest clotting time

differences between consecutive dilutions of the Standard preparation and the preparation under examination. The concentration usually lies between 50 and 250 µg per ml. The diluted suspension may be kept at –20° for 6 weeks.

Clotting factor V solution. Prepare from fresh oxalated bovine plasma by fractionation at 4° with a saturated solution of *ammonium sulphate* prepared at 4°. Use the fraction precipitating between 38 per cent and 50 per cent saturation (which contains clotting factor V not significantly contaminated with clotting factor VIII), dialysed to remove *ammonium sulphate* and diluted with *saline solution* to give a solution containing between 10 per cent and 20 per cent of the amount of clotting factor V present in fresh normal human plasma.

Determine the clotting factor V content of the solution as follows. Prepare two dilutions in *imidazole buffer pH 7.4* to contain 1 volume of the solution under examination in 10 volumes and 20 volumes respectively. Test each dilution as follows. Mix 0.1 ml each of *substrate plasma deficient in clotting factor V*, the dilution under test, *thrombokinase extract* and *0.025M calcium chloride*. Record as the clotting time the interval between the addition of the *calcium chloride solution* and the first indication of fibrin formation, which may be observed visually or by mechanical means.

Similarly determine the clotting times, in duplicate, for four dilutions of pooled normal human plasma in *imidazole buffer pH 7.4* containing 1 volume in 10 volumes (equivalent to 100 per cent of clotting factor V), in 50 volumes (20 per cent), in 100 volumes (10 per cent), and in 1,000 volumes (1 per cent), respectively.

To calculate the result, plot the mean of the clotting times for each dilution of human plasma on double cycle log/log paper against the equivalent percentage of clotting factor V and read the percentage of clotting factor V for the two dilutions of *clotting factor V solution* by interpolation from the curve. The mean of the two results is taken as the percentage of clotting factor V in the solution.

Substrate plasma. Separate the plasma from 9 volumes of human or bovine blood collected in 1 volume of a 3.8 per cent w/v solution of *sodium citrate* or from 3.5 volumes of human or bovine blood collected in 1 volume of a solution containing 2.0 per cent w/v of *sodium acid citrate* and 2.5 per cent w/v of *dextrose*. In the former case, prepare the substrate plasma on the day of collection of the blood. In the latter case, the substrate plasma may be prepared up to 2 days after collection of the blood. Store at –20°.

Substrate plasma deficient in clotting factor V. Preferably use congenitally deficient plasma or, alternatively, prepare as follows. Separate the plasma from human blood collected in one-tenth its volume of a 1.34 per cent w/v solution of *sodium oxalate* and incubate at 37° for 24 to 36 hours. This plasma should have a clotting time, when tested by the assay method given under *clotting factor V solution*, of 70 to 100 seconds; if the clotting time is less than 70 seconds, incubate the plasma for a further 12 to 24 hours.

Storage. Store in small amounts, at -20° or below.

Suggested method

Dissolve the contents of the sealed container of the substance under examination in the volume of the liquid stated on the label and use immediately. Reconstitute the entire contents of one ampoule of the Standard preparation as stated on the label and use immediately.

To the reconstituted Standard preparation and the preparation under examination, add sufficient *imidazole buffer pH 7.4* to produce solutions containing between 0.5 and 2 Units per ml; these solutions are stable for 15 minutes at 20°. Using a mixture of 1 volume of a 3.8 per cent w/v solution of *sodium citrate* and 5 volumes of *saline solution* as the diluent, make from the solutions three successive 2-fold dilutions in the range 1 in 16 to 1 in 256 so that all the clotting times are between 17 and 35 seconds; the dilutions must be accurately made and used immediately.

Introduce into each of six glass incubation tubes (75 mm to 100 mm) 0.1 ml each of *clotting factor V solution, phospholipid reagent* and *normal serum reagent*. To the first tube add 0.1 ml of the highest dilution of the Standard preparation, place the tube in a water-bath at 37°, add 0.1 ml of *0.05M calcium chloride* and start a stop-watch. During the next minute add 0.1 ml of the second highest dilution of the standard to a second tube, place it in the water-bath, and add 0.1 ml of *0.05M calcium chloride* at exactly 1 minute by the stop-watch. Repeat the procedure with the lowest dilution of the standard and the highest to lowest dilutions of the preparation under examination so that the *calcium chloride solution* is added at 2, 3, 4 and 5 minutes by the stop-watch, respectively.

Place in a water-bath at 37°, twelve glass tubes each containing 0.2 ml of *0.025M calcium chloride* and a further tube containing about 3 ml of *substrate plasma*. At 14 minutes, 40 seconds by the stop-watch, transfer 0.1 ml of the mixture from the first incubation tube to one of the tubes containing 0.2 ml of *0.025M calcium chloride solution* and mix. At 15 minutes add 0.2 ml of the warmed substrate plasma and, using a second stop-watch, record as the clotting time the interval between the addition of the substrate plasma and the first indication of fibrin formation, which may be observed visually or by mechanical means. Repeat the procedure with the other incubation tubes at 1-minute intervals and carry out a second series of determinations at 21 to 26 minutes. The period of incubation should, if necessary, be adjusted so that the clotting times recorded in the corresponding tests in the two series of determinations do not differ by more than 5.0 per cent, showing

that a stable plateau of prothrombin activator formation has been reached.

Carry out a blank determination using in place of the preparation under examination, an equal quantity of a mixture of 1 volume of a 3.8 per cent w/v solution of *sodium citrate* and 5 volumes of *saline solution*. The result of the assay is not valid unless the clotting time in the blank determination is more than 40 seconds. Calculate the result of the assay by standard statistical methods.

For total protein. Dilute 1.0 ml to 10.0 ml with *saline solution*. Determine the assay described under Human Plasma using 5.0 ml of the dilution and beginning at the words "add 0.2 ml of a 7.5 per cent w/v solution....".

For fibrinogen. Dilute 1.0 ml of the solution to 10.0 ml with a *phosphate-saline buffer pH 6.5* and ionic strength 0.15. Clot 5.0 ml of the dilution with the minimum amount of *thrombin*, collect the clot and add three drops of a 30 per cent w/v solution of *copper sulphate* and 1 ml of *nitrogen-free sulphuric acid* and boil gently for 10 minutes; cool, add 1 g of *anhydrous sodium sulphate* and 10 mg of *selenium*, boil gently for 1 hour and cool. Transfer to an ammonia distillation apparatus, add 6 ml of a saturated solution of *sodium hydroxide* and pass steam through the flask; distil for seven minutes, collecting the distillate in a mixture of 5 ml of a saturated solution of *boric acid*, 5 ml of *water*, and 1 drop of a saturated solution of *methyl red* in *alcohol* containing 0.1 per cent of *methylene blue*, and titrate with *0.02 M hydrochloric acid*.

1 ml of *0.02M hydrochloric acid* is equivalent to 0.00175 g of fibrinogen.

For sodium ions. To 10.0 ml of the solution add sufficient *water* to produce 100 ml, dilute 10.0 ml to 500 ml with *water* and determine the content of sodium ions by Method B for flame photometry (2.4.4), measuring at about 589 nm and using *sodium solution FP* suitably diluted with *water* as the standard solution.

Storage. Store protected from light, in an atmosphere of nitrogen at a temperature below 8°. The containers are sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the ABO blood group designation of the source of blood; (2) the number of Units in the container; (3) that 1 Unit is approximately equivalent to the antihaemophilic activity of 1 ml of average normal plasma; (4) the concentration of protein in g per litre and of sodium ions in millimoles per litre of the solution constituted as directed; (5) the maximum fibrinogen content; (6) where applicable, the number of Units of heparin in the container; (7) the name and amount of any other added substance contained in it; (8) the volume of Water for Injections necessary to constitute the solution; (9) the instructions for constitution and that reconstitution may take upto 30 minutes; (10) that if the solution is not complete or if a gel forms on constitution, the preparation should not be used; (11) that the solution should be used as soon as possible and in any case within 3 hours of constitution and any unused solution should be discarded; (12) that the solution should be administered only with equipment that includes a filter; (13) the storage conditions.

Fibrin Sealant Kit

Fibrin Sealant Kit is essentially composed of two components, namely fibrinogen concentrate (component 1), a protein fraction containing human fibrinogen and a preparation containing human thrombin (component 2). A fibrin clot is rapidly formed when the two thawed or reconstituted components are mixed. Other ingredients (for example, human coagulation factor XIII, a fibrinolysis inhibitor or calcium ions) and stabilisers (for example, Human albumin solution may be added). No antimicrobial preservative is added.

Human constituents are obtained from plasma that complies with the requirements of the monograph on Human Plasma for Fractionation. No antibiotic is added to the plasma used.

When thawed or reconstituted as stated on the label, component 1 contains not less than 4.0 per cent of clottable protein; the thrombin activity of component 2 varies over a wide range (approximately 4-1000 IU per ml).

Production

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

Constituents or mixtures of constituents are passed through a bacteria-retentive filter and distributed aseptically into sterile containers. Containers of freeze-dried constituents are closed under vacuum or filled with oxygen-free nitrogen or other suitable inert gas before being closed. In either case, they are closed so as to exclude micro-organisms.

If the human coagulation factor XIII content in component 1 is greater than 10 Units per ml, the assay of coagulation factor XIII is carried out.

Description. Freeze-dried constituents are hygroscopic, white or pale yellow powders or friable solids. Frozen constituents are colourless or pale yellow, opaque solids. Liquid constituents are colourless or pale yellow.

For the freeze-dried or frozen constituents, reconstitute or thaw as stated on the label immediately before carrying out the Identification and the Tests, except those for solubility and water.

Component 1 (Fibrinogen Concentrate)

Identification

A. Complies with the limits of the assay of fibrinogen.

B. Complies with the limits of the assay of factor XIII (where applicable).

Tests

pH (2.4.24). 6.5 to 8.0.

Stability of solution. No gel formation appears at room temperature during 120 minutes following thawing or reconstitution.

Water. Determine by semi-microdetermination (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Assay

Fibrinogen (Clottable Protein)

The estimated content in mg of clottable protein is not less than 70.0 per cent and not more than 130.0 per cent of the content stated on the label.

Mix 0.2 ml of the reconstituted preparation with 2 ml of a suitable *buffer solution pH 6.6 to 7.4* containing sufficient *human thrombin* (approximately 3 IU per ml) and *calcium* (0.05mol per l). Maintain at 37° for 20 minutes, separate the precipitate by centrifugation (5000 g, 20 minutes), wash thoroughly with a 0.9 per cent solution of *sodium chloride* and determine the protein as nitrogen by sulphuric acid digestion (2.3.30). Calculate the protein content by multiplying the result by 6.0. If for a particular preparation this method cannot be applied, use another validated method for determination of fibrinogen.

Factor XIII

Where the label indicates that the human coagulation factor XIII activity is greater than 10 Units per ml, the estimated activity is not less than 80.0 per cent and not more than 120.0 per cent of the activity stated on the label.

Make at least 3 suitable dilutions of thawed or reconstituted component 1 and of human normal plasma (reference preparation) using as diluent coagulation factor XIII deficient plasma or another suitable diluent. Add to each dilution suitable amounts of the following reagents:

a. *activator reagent,* containing *bovine or human thrombin,* a suitable *buffer, calcium chloride* and a suitable inhibitor such as *Gly-Pro-Arg-Pro-Ala-NH_2* which inhibits clotting of the sample but does not prevent coagulation factor XIII activation by thrombin,

b. *detection reagent,* containing a suitable *factor XIIIa-specific peptide substrate, such as Leu-Gly-Pro-Gly-Glu-Ser-Lys-Val-Ile-Gly-NH_2 and glycine ethyl ester* as 2nd substrate in a suitable *buffer solution,*

c. *NADH reagent,* containing *glutamate dehydrogenase, α-ketoglutarate* and *NADH* in a suitable *buffer solution.*

After mixing, the absorbance changes (Ä*A* per min) are measured at a wavelength of 340 nm, after the linear phase of the reaction is reached.

1 Unit of factor XIII is equal to the activity of 1 ml of human normal plasma.

Calculate the activity of the test preparation by the usual statistical methods. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 125.0 per cent of the estimated activity.

Component 2 (Thrombin Preparation)

Identification

Complies with the limits of the assay of thrombin.

Tests

pH (2.4.24). 5.0 to 8.0.

Water. Determine by semi-microdetermination of water (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Assay

Thrombin

The estimated activity is not less than 80.0 per cent and not more than 125.0 per cent of the activity stated on the label.

If necessary, dilute the reconstituted preparation under examination to approximately 2-20 IU of thrombin per ml using as diluent a suitable buffer pH 7.3 to 7.5, such as *imidazole buffer solution pH 7.3* containing 1.0 per cent of *human albumin* or *bovine albumin.* To a suitable volume of the dilution, add a suitable volume of fibrinogen solution (0.1 per cent of clottable protein) warmed to 37° and start measurement

of the clotting time immediately. Repeat the procedure with each of at least 3 dilutions, in the range stated above, of a reference preparation of thrombin, calibrated in International Units. Calculate the activity of the test preparation by the usual statistical methods. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 125.0 per cent of the estimated activity.

Storage. Store protected from light.

Labelling. The label states (1) the amount of fibrinogen (mg of clottable protein), thrombin (International Units) per container, and coagulation factor XIII, if this is greater than 10 Units per ml; (2) where applicable, the name and volume of solvent to be used to reconstitute the components.

Human Albumin

Human Normal Albumin; Human Albumin Solution

Human Albumin is a sterile non-pyrogenic solution of the albumin component obtained from pooled human blood or from normal placentae frozen immediately after collection. It is obtained by fractionating source material such as blood, plasma, serum or placentae from healthy human donors and tested individually for the absence of hepatitis B surface antigen, HCV antibodies and HIV antibodies and complies with other tests and requirements prescribed by the appropriate national control authority. Source material obtained from donors who do not meet all the requirements stated may be used provided that it has been demonstrated to the national control authority that the process of fractionation will remove any known agent capable of adversely affecting the health of subjects treated with the preparation. It may be prepared from pooled source materials by precipitation with organic solvents under controlled conditions of pH, ionic strength and temperature or by chromatography or by any other method which does not affect the integrity of the product and has been shown to yield consistently a product containing not less than 95.0 per cent w/v of the total protein as albumin which is safe for intravenous injection. Residual organic solvent, if present, is removed by freeze-drying or other suitable treatment. The product is dissolved in sufficient water to obtain a suitable concentration, and a suitable stabilising agent is added to stabilise it to heat. It is prepared as a solution containing 15.0 to 25.0 per cent w/v of total protein or as an isotonic solution containing 4.0 to 5.0 per cent w/v of total protein. No antimicrobial agent is added at any stage during preparation and all processing steps are conducted in a manner to minimise risk of contamination from either micro-organisms or other deleterious matter. The solution is sterilised by filtration and distributed aseptically into containers which are then sealed so as to exclude micro-organisms. The solution is then heated to and maintained at 60° ± 0.5° for 10 hours so as to prevent the transmission of agents of infection transmissible by transfusion of blood or blood derivatives. Finally, the containers are stored for not less than 14 days at 30° to 32° or for not less than 4 weeks at 20° and examined visually. Those showing abnormalities such as abnormal colour, turbidity, microbial contamination, or presence of atypical particles must be discarded.

Albumin Solution should be tested in accordance with the requirements decided by the National Regulatory Authority; in particular, tests for the absence of hepatitis B surface antigen and HIV antibodies are carried out by suitably sensitive methods.

Human Albumin contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of protein. It contains not less than 95.0 per cent and not more than 105.0 per cent of the contents of Na and K stated on the label which are, in any case, not more than 160 millimoles of Na per litre and 2 millimoles of K per litre.

Description. A clear, slightly viscous liquid, ranging in colour from almost colourless to greenish-yellow or amber depending on protein concentration and the method of fractionation used.

Identification

A. It contains plasma proteins of human origin only as determined by precipitation tests with specific antisera.

B. Determine the cellulose acetate by electrophoresis (2.4.12), using *barbitone buffer pH 8.6*, ionic strength 0.1 and *human albumin for electrophoresis RS*; 96.0 per cent of the protein has the mobility of human albumin.

Tests

Acidity or alkalinity (2.4.24). Dilute with sufficient *saline solution* to produce a solution containing 1.0 per cent w/v of protein; pH of the resulting solution, 6.7 to 7.3.

Alkaline phosphatase. Not more than 0.1 Unit per g of protein, determined by the following method. Transfer a mixture of 0.5 ml of the substance under examination and 0.5 ml of *diethanolamine buffer pH 10.0* to a spectrophotometer cell maintained at a temperature of 37° ± 0.2° and add 0.1 ml of *nitrophenyl phosphate solution*. Using a continuously recording spectrophotometer record the absorbance of the solution at about 405 nm (2.4.7), over a period of at least 30 seconds from the time of addition of the *nitrophenyl phosphate solution*. Calculate the alkaline phosphatase activity at 37° in Units per g of protein from the expression 118.3x/P, where x is the rate of increase of absorbance per minute and P is the content of total protein in g per litre, as determined in the Assay.

Haem content. Dilute with sufficient *saline solution* to produce a solution containing 1.0 per cent w/v of protein; absorbance of the resulting solution at about 403 nm, not more than 0.15 (2.4.7).

Denatured protein. Equilibrate a column (60 to 75 cm x 2.5 to 3.0 cm) of a gel of a cross-linked dextran suitable for fractionation of proteins in the range of molecular weight from 5,000 to 3,50,000 with a lower molecular weight for complete exclusion of globulin proteins of molecular weight between 4,00,000 and 5,00,000 (Such as Sephadex G 150) at 20° to 25° with a *saline-phosphate solution* prepared by mixing 2 volumes of *saline solution* and 1 volume of *mixed phosphate buffer pH 7.0* with *azide*. Apply to the column 2.5 ml of normal human serum, previously clarified by centrifuging, and elute with the *saline-phosphate solution* at a rate of 20 ml per hour. Prepare a chromatogram by recording the absorbance (2.4.7), of the eluate at about 280 nm in relation to its volume. The chromatogram exhibits three well-defined peaks. Determine the volume, V, of the eluate from the entry of the sample into the column to the apex of the first peak.

Dilute the substance under examination with the *saline-phosphate solution* to contain about 5.0 per cent w/v of protein, apply 2.5 ml to the column and elute under the above conditions, collecting the eluate in 5-ml portions. Three peaks may appear in similar positions to those in the chromatogram obtained from the normal human serum but the relative peak heights may be different. To the fraction eluted between volume 0.85 V and 1.15 V, add for each 10 ml, 0.4 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 0.4 ml of a mixture of 1 part of *nitrogen-free sulphuric acid* and 30 parts of *water*, shake, centrifuge for 5 minutes and complete the Assay described under Human Plasma beginning at the words "decant the supernatant...". The weight of protein in the fraction of the eluate is not more than 3.5 per cent of the weight of protein in the volume of the substance under examination applied to the column.

Heat the substance under examination for 50 hours at 56.5° to 57.5° and repeat the chromatographic separation and the determination of the weight of protein in the fraction eluted between 0.85 V and 1.15 V. When expressed as a percentage of the weight of protein in the volume of the substance under examination applied to the column, it exceeds the percentage obtained before heating by not more than 1.5 per cent w/v.

Protein composition. Not less than 95.0 per cent w/v of the total protein as albumin, when determined by the following method. Carry out Method II for cellulose acetate electrophoresis (2.4.12), using one strip of cellulose for each solution.

Test solution. Dilute the substance under examination with *saline solution* to contain 2.0 per cent w/v of total protein.

Reference solution. Dilute *human albumin for electrophoresis RS* with *saline solution* to obtain a solution containing 2.0 per cent w/v of total protein.

Not more than 5.0 per cent of total protein is contained in bands other than the principal band in the strip obtained with test solution. The test is not valid if the proportion of the protein in the principal band is not within the limits stated in the leaflet supplied with *human albumin for electrophoresis RS*.

Stability. The contents of the final container remain unchanged, as determined by visual inspection, after heating at 57° for 50 hours, when compared to its control consisting of a sample from the same lot which has not undergone this heating.

Pyrogens (2.2.8). Complies with the test for pyrogens, using 3 ml per kg of the rabbit's weight, irrespective of the protein content, in rabbits that have not previously received blood products.

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity, using Method B and 0.5 ml of the solution for each mouse and 5 ml for each guinea-pig irrespective of the protein content.

Assay

For protein. Dilute to about 0.75 per cent w/v of total protein with *saline solution*. Take 2 ml of this solution in a round-bottomed centrifuge tube, add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture of 30 volumes of *water* and 1 volume of *nitrogen-free sulphuric acid*. Shake, centrifuge for 5 minutes, decant the supernatant liquid and let the inverted tube stand on a filter paper to drain the fluid. Carry out Method E for determination of nitrogen (2.3.30), on the residue thus obtained and multiply the result by 6.25 to obtain the protein content.

For sodium. Dilute to 0.01 per cent w/v of protein with *water* and determine by Method A for atomic absorption spectrophotometry (2.4.2), or by Method B for flame photometry (2.4.4), measuring at about 589 nm and using *sodium solution FP* suitably diluted with *water* as the standard solution.

For potassium. Dilute to 0.25 per cent w/v of protein with *water* and determine by Method A for atomic absorption spectrophotometry (2.4.2), or by Method B for flame photometry (2.4.4), measuring at about 767 nm and using *potassium solution FP* suitably diluted with *water* as the standard solution.

Human Albumin intended for administration to patients undergoing dialysis or to premature infants complies with the following additional test.

Aluminium (2.3.8). Not more than 200 µg of Al per litre. Determine by atomic absorption spectrophotometry (2.4.2), with a furnace as atomic generator and measuring at 309.3 nm and using as standard solutions a suitable range of dilutions in *water* of *aluminium standard solution* (10 ppm Al) further diluted, as necessary, with a solution containing 0.17 per cent w/v of *magnesium nitrate* and 0.05 per cent w/v of *octoxinol 10* in a solution of *nitric acid* containing 1 per cent w/v of *nitric acid*. Prepare suitable dilutions of the preparation under examination and *human albumin for aluminium validation RS* with *water*. Dilute the solutions, as necessary, with the *magnesium nitrate-octoxinol 10-nitric acid solution* used for dilution of the standard solution. The test is valid only if the aluminium content determined for *human albumin for aluminium validation RS* is within 20 per cent of the stated value.

NOTE — Wash all equipments with a solution containing 20.0 per cent w/v of nitric acid before use and use plastic containers only to prepare all solutions.

Storage. Store protected from light, at a temperature between 2° and 25°. Human Albumin stored at 2° to 8° may be expected to continue to meet the requirements of the monograph for 5 years from the date on which it was heated at 60° for 10 hours. Human Albumin stored at a temperature not exceeding 25° may be expected to continue to meet the requirements of the monograph for 3 years from the date on which it was heated at 60° for 10 hours.

Labelling. The label states (1) the volume in the container; (2) the total amount of protein in the container expressed in g per litre or as percentage; (3) the concentration of sodium and potassium ions expressed in millimoles per litre; (4) the names and concentrations of any stabilising agents and any other additives in the final solution; (5) the type of source material used to manufacture the product; (6) the words "Do not use if turbid"; (7) that the contents must not be used more than 4 hours after the container has been penetrated and any remnant portion must be discarded; (8) the storage conditions; (9) the date after which the solution is not intended to be used; (10) either that the preparation is suitable for administration to patients undergoing dialysis and to premature infants or that it is not intended for such purpose.

Human Coagulation Factor IX

Human Coagulation Factor IX is a plasma protein fraction containing coagulation factor IX, prepared by a method that effectively separates factor IX from other prothrombin complex factors (factors II, VII and X). It is obtained from human plasma that complies with the monograph on Human Plasma for Fractionation.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor IX per ml.

Production

The method of preparation is designed to maintain functional integrity of factor IX, to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 50 IU of factor IX per mg of total protein, before the addition of any protein stabiliser.

The factor IX fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be included. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

Consistency of the method

The consistency of the method of production is evaluated by suitable analytical procedures that are determined during process development and which normally include (1) assay of factor IX; (2) determination of activated coagulation factors; (3) determination of activities of factors II, VII and X which shall be shown to be not more than 5.0 per cent of the activity of factor IX.

Description. A white or pale yellow, hygroscopic powder or friable solid.

Reconstitute the preparation under examination as stated on the label, immediately before carrying out the Identification, Tests (except those for solubility and water) and Assay.

Identification

It complies with the limits of the Assay.

Tests

pH (2.4.24). 6.5 to 7.5.

Osmolality (2.4.23). Minimum 240 mosmol per kg.

Total protein. If necessary, dilute an accurately measured volume of the preparation under examination with a 0.9 per cent w/v solution of *sodium chloride*, to obtain a solution which may be expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of that solution, in a round-bottomed centrifuge tube, add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate*

and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 minutes decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (2.3.30) and calculate the amount of protein by multiplying the result by 6.25.

For some products, especially those without a protein stabiliser such as albumin, this method may not be applicable. Another validated method for protein determination must therefore be performed.

Activated coagulation factors (2.8.4). If necessary, dilute the preparation under examination to contain 20 IU of factor IX per ml. For each of the dilutions the coagulation time is not less than 150 seconds.

Heparin. If heparin has been added during preparation, determine the amount by the assay of heparin in coagulation factor concentrates (2.8.10). The preparation under examination contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor IX.

Water. Determine by semi-micro determination of water (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a volume equivalent to not less than 50 IU of factor IX.

Assay. Determine the assay of human blood coagulation factor IX (2.8.8).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 125.0 per cent of the estimated potency.

Storage. Store protected from light.

Labelling. The label states (1) the number of International Units of factor IX per container; (2) the amount of protein per container; (3) the name and quantity of any added substances including, where applicable, heparin; (4) the name and volume of the liquid to be used for reconstitution; (5) that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Human Coagulation Factor VII

Human Coagulation Factor VII is a plasma protein fraction that contains the single-chain glycoprotein factor VII and may also contain small amounts of the activated form, the two-chain derivative factor VIIa. It may also contain coagulation factors II, IX and X and protein C and protein seconds. It is obtained from human plasma that complies with the monograph on Human Plasma for Fractionation.

The potency of the preparation, reconstituted as stated on the label, is not less than 15 IU of factor VII per ml.

Production

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 2 IU of factor VII per mg of total protein, before the addition of any protein stabiliser.

The factor VII fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

Consistency of the method

The consistency of the method of production with respect to the activities of factors II, IX and X of the preparation, expressed in International Units relative to the activity of factor VII, shall be demonstrated.

The consistency of the method of production with respect to the activity of factor VIIa of the preparation shall be demonstrated. The activity of factor VIIa may be determined, for example, using a recombinant soluble tissue factor that does not activate factor VII but possesses a cofactor function specific for factor VIIa; after incubation of a mixture of the recombinant soluble tissue factor with phospholipids reagent and the dilution of the test sample in factor VII-deficient plasma, *calcium chloride* is added and the clotting time determined; the clotting time is inversely related to the factor VIIa activity of the test sample.

Description. A hygroscopic powder or friable solid that may be white, pale yellow, green or blue.

Reconstitute the preparation under examination as stated on the label immediately before carrying out the Identification, Tests (except those for solubility and water) and Assay.

Identification

It complies with the limits of the Assay.

Tests

pH (2.4.24). 6.5 to 7.5.

Osmolality (2.4.23). Minimum 240 mosmol per kg.

Total protein. If necessary, dilute an accurately measured volume of the reconstituted preparation with a 0.9 per cent solution w/v of *sodium chloride* to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 minutes, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (2.3.30) and calculate the amount of protein by multiplying the result by 6.25.

Activated coagulation factors (2.8.4). For each of the dilutions, the coagulation time is not less than 150 seconds.

Heparin. If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (2.8.10). The preparation under examination contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor VII.

Thrombin. If the preparation under examination contains heparin, determine the amount present as described in the test for heparin and neutralise the heparin by addition of *protamine sulphate* (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test-tubes, mix equal volumes of the reconstituted preparation and a 0.3 per cent solution of *fibrinogen*. Keep one of the tubes at 37° for 6 hours and the other at room temperature for 24 hours. In a third tube, mix a volume of the *fibrinogen solution* with an equal volume of a solution of *human thrombin* (1 IU per ml) and place the tube in a water-bath at 37°. No coagulation occurs in the tubes containing the preparation under examination. Coagulation occurs within 30 seconds in the tube containing thrombin.

Factor II

Determine the assay of human coagulation factor II (2.8.5).

The estimated content is not more than 125.0 per cent of the stated content. The confidence limits (P = 0.95) are not less than 90.0 per cent and not more than 111.0 per cent of the estimated potency.

Factor IX

Determine the assay of human coagulation factor IX (2.8.8).

The estimated content is not more than 125.0 per cent of the stated content. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 125.0 per cent of the estimated potency.

Factor X

Determine the assay of human coagulation factor X (2.8.9).

The estimated content is not more than 125.0 per cent of the stated content. The confidence limits (P = 0.95) are not less than 90.0 per cent and not more than 111.0 per cent of the estimated potency.

Water. Determine by semi-micro determination of water (2.3.43), loss on drying (2.4.19) or near-infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a volume equivalent to not less than 30 IU of factor VII.

Assay

Determine the assay of human coagulation factor VII (2.8.6).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 125.0 per cent of the estimated potency.

Storage. Store protected from light.

Labelling. The label states (1) the number of International Units of factor VII per container; (2) the maximum content of International Units of factor II, factor IX and factor X per container; (3) the amount of protein per container; (4) the name and quantity of any added substances, including where applicable, heparin; (5) the name and volume of the liquid to be used for reconstitution; (6) that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Human Coagulation Factor VIII (rDNA)

Human Coagulation Factor VIII (rDNA) is a freeze-dried preparation of glycoproteins having the same activity as coagulation factor VIII in human plasma. It acts as a cofactor of the activation of factor X in the presence of factor IXa, phospholipids and calcium ions. It circulates in plasma mainly as a two-chain glycosylated protein with 1 heavy

(relative molecular mass of about 2,00,000) and 1 light (relative molecular mass 80,000) chain held together by divalent metal ions. Human coagulation factor VIII (rDNA) is prepared as full-length factor VIII (octocog alfa), or as a shortened two-chain structure (relative molecular mass 90,000 and 80,000), in which the B-domain has been deleted from the heavy chain (moroctocog alfa).

Full-length human rDNA coagulation factor VIII contains 25 potential *N*-glycosylation sites, 19 in the B domain of the heavy chain, 3 in the remaining part of the heavy chain (relative molecular mass 90,000) and 3 in the light chain (relative molecular mass 80,000). The different products are characterised by their molecular size and post-translational modification and/or other modifications.

Production

Human coagulation factor VIII (rDNA) is produced by recombinant DNA technology in mammalian cell culture. It is produced under conditions designed to minimise microbial contamination.

Purified bulk factor VIII (rDNA) may contain added human albumin and/or other stabilising agents, as well as other auxiliary substances to provide, for example, correct pH and osmolality.

The specific activity is not less than 2,000 IU of factor VIII:C per mg of total protein before the addition of any protein stabiliser, and varies depending on purity and the type of modification of molecular structure of factor VIII.

The quality of the bulk preparation is controlled using reference preparations.

Reference preparations

During development, reference preparations are established for subsequent verification of batch consistency during production, and for control of bulk and final preparation. They are derived from representative batches of purified bulk factor VIII (rDNA) that are extensively characterised by tests including those described below and whose procoagulant and other relevant functional properties have been ascertained and compared, wherever possible, with the International Standard for factor VIII concentrate. The reference preparations are suitably characterised for their intended purpose and are stored in suitably sized aliquots under conditions ensuring their stability.

Purified bulk factor VIII (rDNA)

The purified bulk complies with a suitable combination of the following tests for characterisation of integrity of the factor VIII (rDNA). Where any substance added during preparation of the purified bulk interferes with a test, the test is carried out before addition of that substance. Where applicable, the characterisation tests may alternatively be carried out on the finished product.

Specific biological activity or ratio of factor VIII activity to factor VIII antigen

Determine the assay of human coagulation factor VIII (2.8.7). The protein content, or where a protein stabiliser is present, the factor VIII antigen content, is determined by a suitable method and the specific biological activity or the ratio of factor VIII activity to factor VIII antigen is calculated.

Protein composition

The protein composition is determined by a selection of appropriate characterisation techniques which may include peptide mapping, Western blots, HPLC, gel electrophoresis, capillary electrophoresis, mass spectrometry or other techniques to monitor integrity and purity. The protein composition is comparable to that of the reference preparation.

Molecular size. Using size-exclusion chromatography (2.4.16), the molecular size distribution is comparable to that of the reference preparation.

Peptide mapping (2.3.47). There is no significant difference between the test protein and the reference preparation.

Carbohydrates/sialic acid. To monitor batch-to-batch consistency, the monosaccharide content and the degree of sialylation or the oligosaccharide profile are monitored and correspond to those of the reference preparation.

Final lot

It complies with the tests under Identification, Tests and Assay.

Excipients. 80.0 per cent to 120.0 per cent of the stated content, determined by a suitable method, where applicable.

Description. A white or slightly yellow powder or friable mass.

Identification

A. It complies with the limits of the Assay.

B. The distribution of characteristic peptide bands corresponds with that of the reference preparation (SDS-PAGE or Western blot).

Tests

Reconstitute the preparation as stated on the label immediately before carrying out the Tests (except those for solubility and water) and Assay.

pH (2.4.24). 6.5 to 7.5.

Osmolality (2.4.23). Minimum 240 mosmol per kg.

Water. Determine by semi-micro determination of water (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Bacterial endotoxins (2.2.3). Less than 3 IU in the volume that contains 100 IU of factor VIII activity.

Assay

Determine the assay of human coagulation factor VIII (2.8.7).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 80.0 per cent and not more than 120.0 per cent of the estimated potency.

Storage. Store protected from light.

Labelling. The label states (1) the factor VIII content in International Units; (2) the name and amount of any excipient; (3) the composition and volume of the liquid to be used for reconstitution.

Human Normal Immunoglobulin

Normal Immunoglobulin; Immune Human Serum Globulin; Human Gamma Globulin

Human Normal Immunoglobulin is a sterile solution or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG), together with smaller amounts of other plasma proteins.

Production

It is obtained from source materials such as the blood, plasma, serum or placentae frozen immediately after collection from healthy donors who must as far as can be ascertained after clinical examination, laboratory tests on their blood and a study of their medical history, be free from disease transmissible by transfusion of blood or blood products. The examinations and tests to be carried out are decided by the National Regulatory Authority; in particular, tests for hepatitis B surface antigen, HCV antibodies and for HIV antibodies must be carried out by suitable sensitive methods and must show negative results in both cases. Plasma, serum or placentae obtained from donors who do not meet all the requirements stated above may be used as source material provided that it has been demonstrated to the national authority that process of fractionation and production removes any known agent capable of adversely affecting the health of subjects treated with the preparation. No antibiotic is added to the source materials used for the preparation of immunoglobulin.

It is prepared from pooled material of a minimum volume of 25 litres by a method which has been shown (a) to be capable of concentrating tenfold from source material at least two different antibodies, one viral and one bacterial, for which an International Standard or Reference Preparation is available; (b) not to affect the integrity of the globulins; (c) to consistently yield a product which is safe for intramuscular injection and; (d) to yield a product that does not transmit viral hepatitis or any other infection.

The liquid preparation is prepared as a stabilised solution in *saline solution* or a 2.25 per cent w/v solution of *glycine* or other suitable agent and is sterilised by filtration and distributed into previously sterilised containers which are then sealed so as to exclude micro-organisms. An antimicrobial preservative may be added except when the preparation is to be freeze-dried. Any antimicrobial preservative or stabilising agent added must be such that neither deleterious effect on the final product in the amounts present nor capability to cause untoward reactions in human beings is demonstrated.

An accelerated degradation test is carried out on the final liquid or freeze-dried preparation by heating at 37° for 4 weeks. The difference between the percentages of protein eluted in the fractions following the main peak as determined by size-exclusion chromatography (2.4.16), before and after exposure at 37° does not exceed 5.0 per cent.

Human Normal Immunoglobulin contains not less than 90.0 per cent and not more than 110.0 per cent of the quantity of protein stated on the label and in any case, not less than 10.0 per cent w/v and not more than 18.0 per cent w/v of protein.

Description. The liquid preparation is clear pale yellow or brownish in colour; on storage it may show turbidity or a small amount of particulate matter. The freeze-dried preparation is a white to slightly yellow powder or solid, friable mass.

Identification

A. Precipitation tests with a suitable range of species-specific antisera which give positive results for the presence of proteins of human origin and negative results with antisera specific to plasma proteins of the other species.

B. Examine by electrophoresis (2.4.12), using the moving boundary technique and a 1.0 per cent w/v solution in *barbitone buffer solution pH 8.6* of ionic strength 0.1. At least 90.0 per cent w/v of the protein has a mobility not greater than -2.8×10^{-5} $cm^2V^{-1}S^{-1}$.

Tests

pH (2.4.24). 6.4 to 7.2, determined in a solution prepared by dilution of a quantity with saline solution so as to contain 1.0 per cent w/v of protein.

Protein composition. Determine by cellulose acetate electrophoresis (2.4.12), but applying an electric field such that the albumin band of normal human serum applied in a control strip migrates at least 30 mm and using one strip of cellulose acetate for each of the following solutions:

Test solution. Dilute the preparation under examination with *saline solution* to produce a solution containing 5 per cent w/v of protein.

Reference solution. Reconstitute *human immunoglobulin for electrophoresis RS* with *saline solution* to produce a solution containing 5 per cent w/v of protein.

Calculate the result as the mean of three measurements of the absorbance of each strip. In the electrophoretogram obtained with test solution not more than 10.0 per cent of the protein is contained in bands other than the principal band. The test is not valid unless the proportion of protein in the principal band in the electrophoretogram obtained with reference solution is within the limits stated in the leaflet supplied with *human immunoglobulin for electrophoresis RS.*

Molecular size. Determine by size-exclusion chromatography (2.4.16), applying 2 ml of the preparation under examination diluted with *mixed phosphate buffer pH 7.0* with *azide* to produce a solution containing 4.0 to 5.0 per cent w/v of protein.

Chromatographic system

- a column 1 m x 25 mm packed with agarose trapped within a cross-linked polyacrylamide network and having a linear fraction range suitable for fractionation of globular proteins in the range of molecular weights from 20,000 to 350,000,
- mobile phase: *mixed phosphate buffer pH 7.0 with azide*,
- flow rate. 20 ml per hour (4 ml per cm^2 of column cross-sectional area),
- spectrophotometer set at 280 nm.

Collect the eluate in fractions of about 4 ml. Examine the chromatogram in comparison with that in Fig.1. The sum of the areas of the peaks containing IgG monomer, dimer, albumin and other proteins of similar molecular size (area B) is not less than 85.0 per cent of the total area of the chromatogram. Not more than 10.0 per cent of the total area of the chromatogram represents proteins eluted ahead of IgG dimer (area A); if area A can be subdivided into two distinct areas, the area corresponding to larger proteins does not exceed 5.0 per cent of the total area of the chromatogram. Not more than 5.0 per cent of the total area of the chromatogram represents proteins eluted after IgG monomer and albumin (area C).

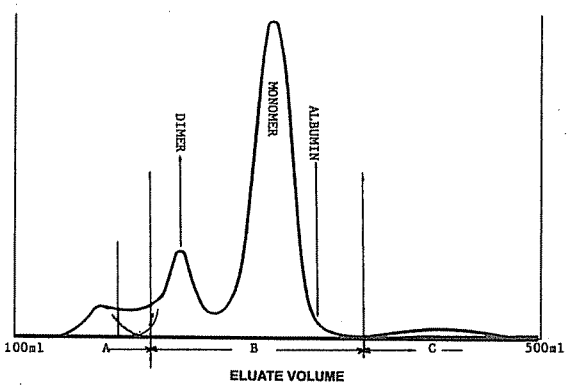


Fig.1 Typical Chromatogram for Human Normal Immunoglobulin

Stability. Heat approximately 2 ml at 57° for 4 hours in a stoppered glass tube (75 mm x 12 mm); no gelation or flocculation occurs.

Pyrogens (2.2.8). Complies with the test for pyrogens, using 1 ml of the preparation under examination per kg of the rabbit's weight.

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity, Method A, injecting 0.5 ml into each mouse and 5 ml into each guinea-pig.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute a suitable volume with *water* to produce a solution containing 1.0 per cent w/v of protein. Take 1.5 ml of the dilution in a round-bottomed centrifuge tube. Add 5 ml of *water,* mix, add 0.2 ml of 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture consisting of 1 volume *nitrogen free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for five minutes, decant the supernatant liquid and allow the inverted tube to drain on filter paper. To the residue in the tube add three drops of a 30 per cent w/v solution of *copper sulphate* and 1 ml of *nitrogen-free sulphuric acid* and boil gently for 10 minutes; cool, add 1 g of *anhydrous sodium sulphate* and 10 mg of *selenium*, boil gently for 1 hour and cool. Transfer to an ammonia distillation apparatus, add 6 ml of a *saturated solution of sodium hydroxide* and pass steam through the flask; distil for seven minutes, collecting the distillate in a mixture of 5 ml of a *saturated solution of boric acid*, 5 ml of *water*, and 1 drop *saturated solution of methyl red* in *alcohol* containing 0.1 per cent of *methylene blue*, and titrate with *0.02 M hydrochloric acid.*

1 ml of *0.02 M hydrochloric acid* is equivalent to 0.00175 g of protein.

Freeze-dried Human Normal Immunoglobulin complies with the following additional requirements.

Solubility rate. Add the volume of the liquid stated on the label and allow it to stand for 15 minutes at a temperature of 20° to 25°; it dissolves completely.

Loss on drying (2.4.19). Not more than 2.0 per cent, determined on 0.5 g, by drying over *phosphorus pentoxide* at a pressure not exceeding 3 Pa for 24 hours.

Human Normal Immunoglobulin intended for use in the prevention of infective hepatitis (hepatitis A) complies with the following additional requirement.

Anti-hepatitis A activity. Determine the anti-hepatitis A activity by comparison with the activity of the Standard preparation, using an immunoassay of suitable sensitivity and specificity. The stated potency is not less than 100 Units per ml. The estimated potency is not less than the stated potency. The fiducial limits of error are not less than 80.0 per cent and not more than 125.0 per cent.

Standard Preparation. The Standard Preparation is the 1st International Reference Preparation for Hepatitis A immunoglobulin, established in 1981, consisting of freeze-dried material derived from fractionated plasma (supplied in ampoules containing 100 Units), or another suitable preparation the antigen binding of which has been determined in relation to the International Reference Preparation.

Storage. Store protected from light, the liquid preparation in sealed, colourless, glass containers, at a temperature between 2° and 8°. Store the freeze-dried preparation under vacuum or under an inert gas.

Labelling. The label states (1) the volume and the protein concentration expressed in g per litre or, for freeze-dried preparations, the total amount of protein in the container; (2) the type of source material; (3) the name and quantity of any added preservative or stabilising agent; (4) the recommended human dose; (5) that it is meant for intramuscular injection only; (6) the storage conditions.

Human Plasma Protein Fraction

Plasma Protein Solution; Human Albumin Fraction (Saline); Plasma Protein Fraction; PPF

Human Plasma Protein Fraction is a sterile isotonic aqueous solution of proteins of plasma or serum containing albumin and globulins. It is prepared as an isotonic solution containing 4.0 to 5.0 per cent w/v of total protein. It contains no fibrinogen or antibodies.

Production

The plasma or serum is obtained from healthy human donors who must, after clinical examination, laboratory tests on their blood and a study of their medical history, be free from detectable agents of infection transmissible by transfusion of blood or blood derivatives. The examinations and tests to be carried out are decided by the National Regulatory Authority; in particular tests for hepatitis B surface antigen and for HIV antibodies are carried out by suitable sensitive methods and must give negative results in both cases. Other disease-causative agents that are not destroyed or removed by the processing method must not be present. Plasma or serum obtained from donors who do not meet all the stated requirements may be used as source material provided that it has been demonstrated to the national authority that the process of fractionation will remove any known agent capable of adversely affecting the health of recipients of the Human Plasma Protein Fraction.

The separation of the protein may be done by precipitation with suitable organic solvents under controlled conditions, particularly of pH, ionic strength and temperature, so that in the final product not less than 85.0 per cent of the total protein is albumin. Residual solvent, if present, may be removed by freeze-drying or other suitable treatment. Alternative methods of preparation which shall not affect the integrity of the product and shall have been shown to yield consistently a product which is safe for intravenous injection may be adopted.

The product is dissolved in *water* and sufficient quantities of a suitable stabiliser against the effect of heat, like *sodium caprylate* in a suitable concentration, and sufficient *sodium chloride* to adjust the sodium ions to between 130 and 160 millimoles per litre may be added but no antibiotic or antimicrobial preservative is added at any stage during preparation. The solution is sterilised by filtration through a bacteria-retentive filter and distributed aseptically into sterile containers which are then closed so as to prevent microbial contamination. The solution in its final container is heated at 60° ± 0.5° and maintained at this temperature for 10 hours. The containers are then incubated at 30° to 32° for not less than 14 days or at 20° to 25° for not less than 4 weeks and examined visually for evidence of microbial contamination. Those showing abnormalities such as abnormal colour, turbidity, presence of atypical particles or microbial contamination are discarded.

Human Plasma Protein Fraction contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of total protein.

Description. A clear, almost colourless or pale yellow liquid; almost odourless. On storage a dust-like precipitate may develop but it disappears on shaking.

Identification

A. Precipitation tests with specific antisera show that the preparation consists only of plasma proteins of human origin only and gives negative results with antisera specific to plasma proteins of other species.

B. Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal

human serum and the preparation under examination, both diluted to contain 1.0 per cent w/v of protein. The main component of the preparation under examination corresponds to the main component of the normal human serum. The solution may show the presence of small quantities of other plasma proteins.

Tests

pH (2.4.24). 6.7 to 7.3, determined in a solution prepared by diluting with sufficient of *saline solution* to produce a solution containing 1.0 per cent w/v of protein.

Polymers and aggregates. Determine by size-exclusion chromatography (2.4.16), applying 2 ml of the preparation under examination.

Chromatographic system
- a column 1 m x 25 mm packed with a cross-linked dextran suitable for fractionation of globular proteins in the range of molecular weights from 5,000 to 3,50,000 (such as Sephadex G-150),
- mobile phase: *mixed phosphate buffer pH 7.0 with azide*,
- flow rate. 20 ml per hour (4 ml per square centimeter of column cross-sectional area),
- spectrophotometer set at 280 nm.

Collect the eluate in fractions of about 4 ml and combine the fractions corresponding to each peak. For each combined fraction, determine by Method E for determination of nitrogen (2.3.30).

1 ml of *0.02M hydrochloric acid* is equivalent to 0.00028 g of nitrogen. Not more than 10.0 per cent of the total nitrogen is present in the combined fraction associated with non-retained proteins.

Protein composition. Carry out by cellulose acetate electrophoresis (2.4.12), Method II using one strip for each solution.

Test solution. Dilute the preparation under examination with *saline solution* to obtain a solution containing 2.0 per cent w/v of protein.

Reference solution. Dilute *human plasma protein fraction for electrophoresis RS* with *saline solution* to obtain a solution containing 2.0 per cent w/v of protein.

Calculate the result as the mean of three measurements of the absorbance of each of the 10 strips. In the electrophoretogram obtained with test solution not more than 15.0 per cent of the protein is contained in bands other than the principal band. The test is not valid unless the proportion of protein in the principal band in the electrophoretogram obtained with reference solution is within the limits stated in the leaflet supplied with *human plasma protein fraction for electrophoresis RS*.

Sodium. Not less than 95.0 per cent and not more than 105.0 per cent of the stated amount and, in any case not more than 160 millimoles of Na per litre, determined by Method A for atomic absorption spectrophotometry (2.4.2), measuring at 589 nm and using *sodium solution AAS* suitably diluted with *water* to prepare the standard solutions.

Potassium. Not more than 50 µmol of K per g of protein, determined by atomic absorption spectrophotometry (2.4.2), measuring at 766 nm and using *potassium solution AAS*, suitably diluted with *water* to prepare the standard solutions.

Alkaline phosphatase. Not more than 0.1 Unit per g of protein, determined by the following method. Transfer a mixture of 0.5 ml of the substance under examination and 0.5 ml of *diethanolamine buffer pH 10.0* to a spectrophotometer cell maintained at a temperature of 37° ± 0.2° and add 0.1 ml of *nitrophenyl phosphate solution*. Record the absorbance of the solution at about 405 nm (2.4.7), over a period of at least 30 seconds from the time of addition of the *nitrophenyl phosphate solution*. Calculate the alkaline phosphatase activity at 37° in Units per g of protein from the expression 118.3x/P, where x is the rate of increase of absorbance per minute and P is the content of total protein in g per litre, as determined in the Assay.

Haem content. Dilute with sufficient *saline solution* to produce a solution containing 1.0 per cent w/v of protein; absorbance of the resulting solution at about 403 nm, not more than 0.15 (2.4.7).

Sterility (2.2.11). Complies with the tests for sterility.

Abnormal toxicity (2.2.1). Complies with the test for abnormal toxicity, using Method B and 0.5 ml of the solution for each mouse and 5 ml for each guinea-pig irrespective of the protein content.

Pyrogens (2.2.8). Complies with the test for pyrogens, using 3 ml per kg of the rabbit's weight in rabbits that have not previously received blood products.

Assay

For protein. Dilute to about 0.75 per cent w/v of total protein with *saline solution*. Take 2 ml of this solution in a round-bottomed centrifuge tube, add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture of 30 volumes of *water* and 1 volume of *nitrogen-free sulphuric* acid. Shake, centrifuge for 5 minutes, decant the supernatant liquid and let the inverted tube stand on a filter paper to drain the fluid. Carry out Method E for determination of nitrogen (2.3.30), on the residue thus obtained and multiply the result by 6.25 to obtain the protein content.

Storage. Store protected from light at a temperature between 2° and 25°.

Labelling. The label states (1) the volume in the container; (2) the total amount of protein in the container expressed as a percentage or in grams per litre; (3) the concentration of sodium ions in millimoles per litre; (4) the names and concentrations of stabilising agents and of any other added substances present in the final solution; (5) that the solution should not be used if the solution is cloudy or shows a deposit which does not disappear on shaking; (6) that, once the container has been penetrated, the contents must be used within 4 hours and any unused solution discarded; (7) the storage conditions.

Human Prothrombin Complex

Human Prothrombin Complex is a plasma protein fraction containing blood coagulation factor IX together with variable amounts of coagulation factors II, VII and X; the presence and proportion of these additional factors depends on the method of fractionation. It is obtained from human plasma that complies with the monograph on Human Plasma for Fractionation.

The potency of the preparation, reconstituted as stated on the label, is not less than 20 IU of factor IX per ml.

Production

The method of preparation is designed to minimise activation of any coagulation factor (to minimise potential thrombogenicity) and includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and that any residues are such as not to compromise the safety of the preparation for patients.

The specific activity is not less than 0.6 IU of factor IX per mg of total protein, before the addition of any protein stabiliser.

The prothrombin complex fraction is dissolved in a suitable liquid. Heparin, antithrombin and other auxiliary substances such as a stabiliser may be added. No antimicrobial preservative is added. The solution is passed through a bacteria-retentive filter, distributed aseptically into the final containers and immediately frozen. It is subsequently freeze-dried and the containers are closed under vacuum or under an inert gas.

Description. A white or slightly coloured powder or friable solid, very hygroscopic.

Reconstitute the preparation under examination as stated on the label immediately before carrying out the Identification, Tests (except those for solubility and water) and Assay.

Identification

It complies with the limits of the assay for coagulation factor IX activity and, where applicable, those for factors II, VII and X.

Tests

pH (2.4.24). 6.5 to 7.5.

Osmolality (2.4.23). Minimum 240 mosmol per kg.

Total protein. If necessary, dilute an accurately measured volume of the reconstituted preparation with a 0.9 per cent w/v solution of *sodium chloride* to obtain a solution expected to contain about 15 mg of protein in 2 ml. To 2.0 ml of the solution in a round-bottomed centrifuge tube add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 minutes, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (2.3.30) and calculate the amount of protein by multiplying the result by 6.25.

Activated coagulation factors (2.8.4). If necessary, dilute the preparation under examination to contain 20 IU of factor IX per ml. For each of the dilutions, the coagulation time is not less than 150 seconds.

Heparin. If heparin has been added during preparation, determine the amount present by the assay of heparin in coagulation factor concentrates (2.8.10). The preparation under examination contains not more than the amount of heparin stated on the label and in any case not more than 0.5 IU of heparin per International Unit of factor IX.

Thrombin. If the preparation under examination contains heparin, determine the amount present as described in the test for heparin and neutralise it by addition of *protamine sulphate* (10 µg of protamine sulphate neutralises 1 IU of heparin). In each of 2 test-tubes, mix equal volumes of the reconstituted preparation and a 0.3 per cent w/v solution of *fibrinogen*. Keep one of the tubes at 37° for 6 hours and the other at room temperature for 24 hours. In a third tube, mix a volume of the fibrinogen solution with an equal volume of a solution of *human thrombin* (1 IU per ml) and place the tube in a water-bath at 37°. No coagulation occurs in the tubes containing the preparation under examination. Coagulation occurs within 30 seconds in the tube containing thrombin.

Water. Determine by semi-micro determination of water (2.3.43), loss on drying (2.4.19) or near-infrared spectrometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a volume of the reconstituted preparation equivalent to not less than 30 IU of factor IX.

Assay

Factor IX

Determine the assay of human coagulation factor IX (2.8.8).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 80.0 per cent to 125.0 per cent.

Factor II

Determine the assay of human coagulation factor II (2.8.5).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 90.0 per cent to 111.0 per cent.

Factor VII

If the label states that the preparation contains factor VII, Determine the assay of human coagulation factor VII (2.8.6).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 80.0 per cent to 125.0 per cent.

Factor X

Determine the assay of human coagulation factor X (2.8.9).

The estimated potency is not less than 80.0 per cent and not more than 125.0 per cent of the stated potency. The confidence interval (P = 0.95) of the estimated potency is not greater than 90.0 per cent to 111.0 per cent.

Storage. Store protected from light.

Labelling. The label states (1) the number of International Units of factor IX, factor II and factor X per container; (2) where applicable, the number of International Units of factor VII per container; (3) where applicable, that the preparation contains protein C and/or protein S; (4) the amount of protein per container; (5) the name and quantity of any added substances, including where applicable, heparin; (6) the name and quantity of the liquid to be used for reconstitution; (7) that the transmission of infectious agents cannot be totally excluded when medicinal products prepared from human blood or plasma are administered.

Human Normal Immunoglobulin for Intravenous Use

Human Normal Immunoglobulin for Intravenous Administration

Human Normal Immunoglobulin for Intravenous Administration is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G (IgG). Other proteins may be present. Human normal immunoglobulin for intravenous administration contains the IgG antibodies of normal subjects. This monograph does not apply to products intentionally prepared to contain fragments or chemically modified IgG.

Human normal immunoglobulin for intravenous administration is obtained from plasma that complies with the requirements of the monograph on Human plasma for fractionation. No antibiotic is added to the plasma used.

Production

The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin.

The product shall have been shown, by suitable tests in animals and evaluation during clinical trials, to be well tolerated when administered intravenously.

Human normal immunoglobulin for intravenous administration is prepared from pooled material from not fewer than 1,000 donors by a method that has been shown to yield a product that (a) does not transmit infection; (b) at an immunoglobulin concentration of 50 g per litre, contains antibodies for at least 2 of which (one viral and one bacterial) an International Standard or Reference Preparation is available, the concentration of such antibodies being at least 3 times that in the initial pooled material; (c) has a defined distribution of immunoglobulin G subclasses; (d) complies with the test for Fc function of immunoglobulin.

Test for Fc function of immunoglobulin

Stabilised human blood. Collect group O human red blood into ACD anticoagulant solution. Store the stabilised blood at 4° for not more than 3 weeks.

Phosphate buffered saline pH 7.2. Dissolve 1.022 g of *anhydrous disodium hydrogen phosphate*, 0.336 g of *anhydrous sodium dihydrogen phosphate* and 8.766 g of *sodium chloride* in 800 ml of *water* and dilute to 1,000 ml with the same solvent.

Magnesium and calcium stock solution. Dissolve 1.103 g of *calcium chloride* and 5.083 g of *magnesium chloride* in *water* and dilute to 25 ml with the same solvent.

Barbital buffer stock solution. Dissolve 207.5 g of *sodium chloride* and 25.48 g of *barbital sodium* in 4,000 ml of *water* and adjust to pH 7.3 using *1 M hydrochloric acid.* Add 12.5 ml of *magnesium and calcium stock solution* and dilute to 5,000 ml with *water.* Filter through a membrane filter (pore size 0.22 µm). Store at 4° in glass containers.

Albumin barbital buffer solution. Dissolve 0.150 g of *bovine albumin* in 20 ml of *barbital buffer stock solution* and dilute to 100 ml with *water.*

Tannic acid solution. Dissolve 10 mg of *tannic acid* in 100 ml of *phosphate-buffered saline pH 7.2.* Prepare immediately before use.

Guinea-pig complement. Prepare a pool of serum from the blood of not fewer than 10 guinea-pigs. Separate the serum from the clotted blood by centrifugation at about 4°. Store the serum in small amounts below -70°. Immediately before starting complement-initiated haemolysis, dilute to 125 to 200 CH_{50} per ml with albumin barbital buffer solution and store in an ice-bath during the test.

Rubella antigen. Suitable rubella antigen for haemagglutination-inhibition titre (HIT). Titre > 256 HA units.

Preparation of tanned human red blood cells. Separate human red blood cells by centrifuging an appropriate volume of stabilised human blood and wash the cells at least 3 times with *phosphate-buffered saline pH 7.2* and suspend at 2 per cent v/v in *phosphate-buffered saline pH 7.2.* Dilute 0.1 ml of *tannic acid solution* to 7.5 ml with *phosphate-buffered saline pH 7.2* (final concentration 1.3 mg per litre). Mix 1 volume of the freshly prepared dilution with 1 volume of human red blood cell suspension and incubate at 37° for 10 minutes. Collect the cells by centrifugation (400 to 800 g for 10 minutes), discard the supernatant and wash the cells once with *phosphate-buffered saline pH 7.2.* Resuspend the tanned cells at 1.0 per cent v/v in *phosphate-buffered saline pH 7.2.*

Antigen coating of tanned human red blood cells. Take a suitable volume (V_s) of tanned cells, add 0.2 ml of *rubella antigen* per 1.0 ml of tanned cells and incubate at 37° for 30 minutes Collect the cells by centrifugation (400 to 800 g for 10 minutes) and discard the supernatant, leaving a volume of 200 µl. Add a volume of *albumin barbital buffer solution* equivalent to the discarded supernatant, resuspend and collect the cells as described and repeat the washing procedure. Make up the remaining 200 µl to three-quarters of V_s, thereby obtaining the initial volume (V_i). Mix 900 µl of *albumin barbital buffer solution* with 100 µl of V_i, which is thereby reduced to the residual volume (V_r), and determine the initial absorbance at 541 nm (A). Dilute V_r by a factor equal to A using *albumin barbital buffer solution*, thereby obtaining the final adjusted volume $V_f = V_r \times A$ of sensitised human red blood cells and adjusting A to 1.0 ± 0.1 for a tenfold dilution.

Antibody binding of antigen-coated tanned human red blood cells. Prepare the following solutions in succession and in duplicate, using for each solution a separate half-micro cuvette (for example, disposable type) or test-tube:

Test solutions. If necessary, adjust the immunoglobulin under examination to pH 7, for example by addition of *1 M sodium hydroxide.* Dilute volumes of the preparation under examination containing 30 mg and 40 mg of immunoglobulin with *albumin barbital buffer solution* and adjust the volume to 900 µl.

Reference solutions. Prepare as for the test solutions using *human immunoglobulin RS.*

Complement control. 900 µl of *albumin barbital buffer solution.*

Add to each cuvette/test-tube 100 µl of sensitised *human red blood cells* and mix well.

Incubate at room temperature for 15 minutes, add 1,000 µl of *albumin barbital buffer solution,* collect the cells by centrifugation (1,000 *g* for 10 min) of the cuvette/test-tube and remove 1,900 µl of the supernatant. Replace the 1,900 µl with *albumin barbital buffer solution* and repeat the whole of the washing procedure, finally leaving a volume of 200 µl. Test samples may be stored in sealed cuvette/test-tubes at 4° for 24 hours.

Complement-initiated haemolysis. To measure haemolysis, add 600 µl of *albumin barbital buffer solution* warmed to 37° to the test sample, re-suspend the cells carefully by repeated pipetting (not fewer than 5 times) and place the cuvette in the thermostatted cuvette holder of a spectrophotometer. After 2 minutes, add 200 µl of *diluted guinea-pig complement* (125 to 200 CH_{50}/ml), mix thoroughly by pipetting twice and start immediately after the second pipetting the time-dependent recording of absorbance at 541 nm, using *albumin barbital buffer solution* as the compensation liquid. Stop the measurement if absorbance as a function of time has clearly passed the inflexion point.

Evaluation. Determine the slope (S) of the haemolysis curve at the approximate inflexion point by segmenting the steepest section in suitable time intervals Ät (for example, Ät = 1 minute) and calculate S between adjacent intersection points, expressed as ÄA per minute. The largest value for S serves as (S_{exp}). In addition, determine the absorbance at the start of measurement (A_s) by extrapolating the curve, which is almost linear and parallel to the time axis within the first few minutes. Correct (S_{exp}) using the expression:

$$S' = \frac{S_{exp}}{A_s}$$

Calculate the arithmetic mean of the values of S' for each preparation. Calculate the index of Fc function (I_{Fc}) from the expression:

$$I_{Fc} = \frac{100 \times \left(\overline{S'} - \overline{S'}_c\right)}{\overline{S'}_s - \overline{S'}_c}$$

$\overline{S'}$ = arithmetic mean of the corrected slope for the preparation under examination,

$\overline{S'}s$ = arithmetic mean of the corrected slope for the reference preparation,

$\overline{S'}c$ = arithmetic mean of the corrected slope for the complement control.

Calculate the index of Fc function for the preparation under examination; the value is not less than that stated in the leaflet accompanying the reference preparation.

Human normal immunoglobulin for intravenous administration is prepared as a stabilised solution or as a freeze-dried preparation. A stabiliser may be added. In both cases the preparation is passed through a bacteria-retentive filter. The preparation may subsequently be freeze-dried and the containers closed under vacuum or under an inert gas. No antimicrobial preservative is added either during fractionation or at the stage of the final bulk solution.

The stability of the preparation is demonstrated by suitable tests carried out during development studies.

Description. The liquid preparation is clear or slightly opalescent and colourless or pale yellow. The freeze-dried preparation is a hygroscopic, white or slightly yellow powder or solid friable mass.

For the freeze-dried preparation, reconstitute as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

Identification

Examine by a suitable immunoelectrophoresis technique. Using antiserum to normal human serum, compare normal human serum and the preparation under examination, both diluted to contain 1.0 per cent w/v of protein. The main component of the preparation under examination corresponds to the IgG component of normal human serum. The preparation under examination may show the presence of small quantities of other plasma proteins; if human albumin has been added as a stabiliser, it may be seen as a major component.

Tests

pH (2.4.24). 4.0 to 7.4.

Dilute the preparation under examination with a 0.9 per cent w/v solution of *sodium chloride* to obtain a solution containing 1.0 per cent of protein.

Osmolality (2.4.23). Minimum 240 mosmol per kg.

Total protein. Minimum 3.0 per cent w/v and between 90.0 to 110.0 per cent of the quantity of protein stated on the label.

Dilute the preparation under examination with a 0.9 per cent solution of *sodium chloride* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 7.5 per cent w/v solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 minutes, decant the supernatant liquid and allow the inverted tube to drain on filter paper. Determine the nitrogen in the centrifugation residue by the method of sulphuric acid digestion (2.3.30) and calculate the content of protein by multiplying the result by 6.25.

Protein composition. Determine by zone electrophoresis (2.4.12).

Use strips of suitable cellulose acetate gel as the supporting medium and *barbital buffer solution pH 8.6* as the electrolyte solution.

Test solution. Dilute the preparation under examination with a 0.9 per cent w/v solution of *sodium chloride* to an immunoglobulin concentration of 3.0 per cent w/v.

Reference solution. Reconstitute *human immunoglobulin for electrophoresis reference preparation* and dilute with a 0.9 per cent w/v solution of *sodium chloride* to a protein concentration of 3.0 per cent w/v.

To a strip apply 4 µl of the test solution as a 10 mm band or apply 0.4 µl per mm if a narrower strip is used. To another strip apply in the same manner the same volume of the reference solution. Apply a suitable electric field such that the albumin band of normal human serum applied on a control strip migrates at least 30 mm. Stain the strips with *amido black 10B solution* for 5 minutes. Decolourise with a mixture of 10 volumes of *glacial acetic acid* and 90 volumes of *methanol* so that the background is just free of colour. Develop the transparency of the strips with a mixture of 19 volumes of *glacial acetic acid* and 81 volumes of *methanol*. Measure the absorbance of the bands at 600 nm in an instrument having a linear response over the range of measurement. Calculate the result as the mean of 3 measurements of each strip.

System suitability. In the electropherogram obtained with the reference preparation, the proportion of protein in the principal band is within the limits stated in the leaflet accompanying the reference preparation.

Results. In the electropherogram obtained with the test solution, not more than 5.0 per cent of protein has a mobility different from that of the principal band. This limit is not applicable if albumin has been added to the preparation as a stabiliser; for such preparations, a test for protein composition is carried out during manufacture before addition of the stabiliser.

Molecular size. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the preparation under examination with a 0.9 per cent w/v solution of *sodium chloride* to obtain a concentration in the range of 0.4 to1.2 per cent w/v and injection of 50 to 600 µg of protein are usually suitable.

Reference solution. Dilute *human immunoglobulin RS* with a 0.9 per cent w/v solution of *sodium chloride* to the same protein concentration as the test solution.

Chromatographic system

- a stainless steel column 60 cm x 7.5 mm or 30 cm x 7.8 mm packed with hydrophilic silica,
- mobile phase. dissolve 4.873 g of *disodium hydrogen phosphate dihydrate*, 1.741 g of *sodium dihydrogen phosphate monohydrate*, 11.688 g of *sodium chloride* and 50 mg of *sodium azide* in 1000 ml of *water*.
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 280 nm.

In the chromatogram obtained with the reference solution, the principal peak corresponds to IgG monomer and there is a peak corresponding to dimer with a relative retention to the principal peak of about 0.85. Identify the peaks in the chromatogram obtained with the test solution by comparison with the chromatogram obtained with the reference solution; any peak with a retention time shorter than that of dimer corresponds to polymers and aggregates.

Results. In the chromatogram obtained with the test solution:

a. *relative retention:* for monomer and dimer, the relative retention to the corresponding peak in the chromatogram obtained with the reference solution is 1 ± 02;

b. *peak area:* the sum of the peak areas of monomer and dimer represent not less than 90.0 per cent of the total area of the chromatogram and the sum of the peak area of polymers and aggregates represents not more than 3.0 per cent of the total area of the chromatogram. This requirement does not apply to products where albumin has been added as a stabiliser; for products stabilised with albumin, a test for distribution of molecular size is carried out during manufacture before addition of the stabiliser.

Anticomplementary activity. The consumption of complement is not greater than 50.0 per cent (1 CH_{50}/mg of immunoglobulin).

Test for anticomplementary activity of immunoglobulin

For the measurement of anticomplementary activity (ACA) of immunoglobulin, a defined amount of test material (10 mg of immunoglobulin) is incubated with a defined amount of guinea-pig complement (20 CH_{50}) and the remaining complement is titrated; the anticomplementary activity is expressed as the percentage consumption of complement relative to the complement control considered as 100.0 per cent.

The haemolytic unit of complement activity (CH_{50}) is the amount of complement that, in the given reaction conditions, will produce the lysis of 2.5×10^8 out of a total of 5×10^8 optimally sensitised red blood cells.

Magnesium and calcium stock solution. Prepare as stated earlier.

Barbital buffer stock solution. Prepare as stated earlier.

Gelatin solution. Dissolve 12.5 g of *gelatin* in about 800 ml of *water* and heat to boiling in a water-bath. Cool to 20° and dilute to 10 litres with *water*. Filter through a membrane filter (pore size: 0.22 µm). Store at 4°. Use clear solutions only.

Citrate solution. Dissolve 8.0 g of *sodium citrate*, 4.2 g of *sodium chloride* and 20.5 g of *glucose* in 750 ml of *water*. Adjust to pH 6.1 using a 10.0 per cent w/v solution of *citric acid* and dilute to 1,000 ml with *water*.

Gelatin barbital buffer solution. Add 4 volumes of *gelatin solution* to 1 volume of *barbital buffer stock solution* and mix. Adjust to pH 7.3, if necessary, using *1 M sodium hydroxide* or *1 M hydrochloric acid*. Maintain at 4°. Prepare fresh solutions daily.

Stabilised sheep blood. Collect one volume of sheep blood into one volume of citrate solution and mix. Store at 4° for not less than 7 days and not more than 28 days. (Stabilised sheep blood and sheep red blood cells are available from a number of commercial sources.)

Haemolysin. Antiserum against sheep red blood cells prepared in rabbits.

Guinea-pig complement. Prepare a pool of serum from the blood of not fewer than ten guinea-pigs. Separate the serum from the clotted blood by centrifugation at about 4°. Store the serum in small amounts below -70°.

Method

Preparation of standardised 5 per cent sheep red blood cell suspension. Separate sheep red blood cells by centrifuging an appropriate volume of stabilised sheep blood and wash the cells at least three times with *gelatin barbital buffer solution* and prepare as a 5.0 per cent v/v suspension in the same solution. Measure the cell density of the suspension as follows: add 0.2 to 2.8 ml of *water* and centrifuge the lysed solution for 5 minutes at 1,000 g; the cell density is suitable if the absorbance (2.4.7) of the supernatant liquid at 541 nm is

0.62 ± 0.01. Correct the cell density by adding *gelatin barbital buffer solution* according to the formula:

$$V_f = \frac{V_i \times A}{0.62}$$

V_f = final adjusted volume,

V_i = initial volume,

A = absorbance of the original suspension at 541 nm.

The adjusted suspension contains about 1×10^9 cells per ml.

Haemolysin titration

Prepare haemolysin dilutions as shown in Table 1.

Add 1.0 ml of 5.0 per cent *sheep red blood cell suspension* to each tube of the haemolysin dilution series, starting at the 1:75 dilution, and mix. Incubate at 37° for 30 minutes.

Transfer 0.2 ml of each of these incubated mixtures to new tubes and add 1.10 ml of *gelatin barbital buffer solution* and 0.2 ml of diluted *guinea-pig complement* (for example, 1:150). Perform this in duplicate.

As the unhaemolysed cell control, prepare three tubes with 1.4 ml of *gelatin barbital buffer solution* and 0.1 ml of *5.0 per cent sheep red blood cell suspension.*

As the fully haemolysed control, prepare three tubes with 1.4 ml of *water* and 0.1 ml of *5.0 per cent sheep red cell suspension.*

Incubate all tubes at 37° for 60 minutes and centrifuge at 1,000 g for 5 minutes. Measure the absorbance (2.4.7) of the supernatants at 541 nm and calculate the percentage degree of haemolysis in each tube using the expression:

$$\frac{A_a - A_1}{A_b - A_1} \times 100$$

A_a = absorbance of tubes with haemolysin dilution,

A_b = mean absorbance of the three tubes with full haemolysis,

A_1 = mean absorbance of the three tubes with no haemolysis.

Plot the percentage degree of haemolysis as the ordinate against the corresponding reciprocal value of the haemolysin dilution as the abscissa on linear graph paper. Determine the optimal dilution of the haemolysin from the graph by inspection. Select a dilution such that further increase in the amount of haemolysin does not cause appreciable change in the degree of haemolysis. This dilution is defined as one minimal haemolytic unit (1 MHU) in 1.0 ml. The optimal haemolytic haemolysin dilution for preparation of sensitised sheep red blood cells contains 2 MHU per ml.

The haemolysin titration is not valid unless the maximum degree of haemolysis is 50.0 per cent to 70.0 per cent. If the maximum degree of haemolysis is not in this range, repeat the titration with more or less diluted complement solution.

Table - 1

Required dilution of haemolysin	Prepared using		
	Gelatin barbital buffer solution		Haemolysin
	Volume ml	Dilution (1 : ...)	Volume ml
7.5	0.65	undiluted	0.1
10	0.90	undiluted	0.1
75	1.80	7.5	0.2
100	1.80	10	0.2
150	1.00	75	1.0
200	1.00	100	1.0
300	1.00	150	1.0
400	1.00	200	1.0
600	1.00	300	1.0
800	1.00	400	1.0
1200	1.00	600	1.0
1600	1.00	800	1.0
2400	1.00	1200	1.0
3200*	1.00	1600	1.0
4800*	1.00	2400	1.0

* discard 1.0 ml of the mixture

Preparation of optimised sensitised sheep red blood cells (haemolytic system)

Prepare an appropriate volume of diluted haemolysin containing 2 MHU/ml and an equal volume of *standardised 5.0 per cent sheep red blood cell suspension.* Add the haemolysin dilution to the standardised cell suspension and mix. Incubate at 37° for 15 minutes, store at 2° to 8° and use within 6 hours.

Titration of complement. Prepare an appropriate dilution of complement (for example, 1:250) with *gelatin barbital buffer solution* and perform the titration in duplicate as shown in Table 2.

Add 0.2 ml of sensitised sheep red blood cells to each tube, mix well and incubate at 37° for 60 minutes. Cool the tubes in an ice-bath and centrifuge at 1,000 g for 5 minutes. Measure the absorbance of the supernatant liquid at 541 nm and calculate the degree of haemolysis (Y) using the expression:

$$\frac{A_c - A_1}{A_b - A_1}$$

A_c = absorbance of tubes 1 to 12,
A_b = mean absorbance of tubes with 100 per cent haemolysis,
A_1 = mean absorbance of cell controls with 0 per cent haemolysis.

Plot $Y/(1 - Y)$ as the abscissa against the amount of diluted complement in ml as the ordinate on log–log graph paper. Fit the best line to the points and determine the ordinate for the 50.0 per cent haemolytic complement dose where $Y/(1 - Y) = 1.0$. Calculate the activity in haemolytic units (CH_{50}/ml) from the expression:

$$\frac{C_d}{C_a \times 5}$$

C_d = reciprocal value of the complement dilution,
C_a = volume of diluted complement in ml resulting in 50.0 per cent haemolysis,
5 = scaling factor to take account of the number of red blood cells.

The test is not valid unless the plot is a straight line between 15.0 per cent and 85.0 per cent haemolysis and the slope is 0.15 to 0.40, and preferably 0.18 to 0.30.

Table - 2

Tube Number	Volume of diluted complement in ml (for example 1:250)	Volume of gelatin barbital buffer solution in ml
1	0.1	1.2
2	0.2	1.1
3	0.3	1.0
4	0.4	0.9
5	0.5	0.8
6	0.6	0.7
7	0.7	0.6
8	0.8	0.5
9	0.9	0.4
10	1.0	0.3
11	1.1	0.2
12	1.2	0.1
Three tube as cell control at 0 per cent haemolysis	-	1.3
Three tube at 100 per cent haemolysis	-	1.3 ml of water

Test for anticomplementary activity

Prepare a complement dilution having 100 CH_{50} per ml by diluting titrated *guinea-pig complement* with *gelatin barbital buffer solution*. If necessary, adjust the immunoglobulin under examination to pH 7. Prepare incubation mixtures as follows for an immunoglobulin containing 50 mg per ml:

Table - 3

Immunoglobulin under examination	Complement control (in duplicate)	
Immunoglobulin (50 mg per ml)	0.2 ml	-
Gelatin barbital buffer	0.6 ml	0.8 ml
Complement	0.2 ml	0..2 ml

Carry out the test on the immunoglobulin under examination and prepare ACA negative and positive controls using *human immunoglobulin RS*, as indicated in the leaflet accompanying the reference preparation. Higher or lower volumes of sample and of *gelatin barbital buffer solution* are added if the immunoglobulin concentration varies from 50 mg per ml; for example, 0.47 ml of *gelatin barbital buffer solution* is added to 0.33 ml of immunoglobulin containing 30 mg per ml to give 0.8 ml. Close the tubes and incubate at 37° for 60 minutes. Add 0.2 ml of each incubation mixture to 9.8 ml of *gelatin barbital buffer solution* to dilute the complement. Perform complement titrations as described above on each tube to determine the remaining complement activity (Table 2). Calculate the anticomplementary activity of the preparation under examination relative to the complement control considered as 100.0 per cent, from the expression:

$$\frac{a - b}{a} \times 100$$

a = mean complement activity (CH_{50}/ml) of complement control,
b = complement activity (CH_{50}/ml) of tested sample.

The test is not valid unless:

a. the anticomplementary activities found for ACA negative control and ACA positive control are within the limits stated in the leaflet accompanying the reference preparation,

b. the complement activity of the complement control (a) is in the range 80 to 120 CH_{50} per ml.

Prekallikrein activator. Maximum 35 IU per ml, calculated with reference to a dilution of the preparation under examination containing 3.0 per cent w/v of immunoglobulin.

Test for prekallikrein activator

Prekallikrein activator (PKA) activates prekallikrein to kallikrein and may be assayed by its ability to cleave a chromophore

from a synthetic peptide substrate so that the rate of cleavage can be measured spectrophotometrically and the concentration of PKA calculated by comparison with a reference preparation calibrated in International Units.

The International Unit is the activity of a stated amount of the International Standard which consists of freeze-dried prekallikrein activator. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Reagents. *Prekallikrein activator in albumin RS* is calibrated in International Units by comparison with the International Standard.

Buffer A. Dissolve 6.055 g of *tris(hydroxymethyl)-aminomethane*, 1.17 g of *sodium chloride*, 50 mg of *hexadimethrine bromide* and 0.100 g of *sodium azide* in *water*. Adjust to pH 8.0 with *2 M hydrochloric acid* and dilute to 1000 ml with *water*.

Buffer B. Dissolve 6.055 g of *tris(hydroxymethyl)-aminomethane* and 8.77 g of *sodium chloride* in *water*. Adjust to pH 8.0 with *2 M hydrochloric acid* and dilute to 1,000 ml with *water*.

Preparation of prekallikrein substrate

To avoid coagulation activation, blood or plasma used for the preparation of prekallikrein must come into contact only with plastics or silicone-treated glass surfaces.

Draw 9 volumes of human blood into 1 volume of anticoagulant solution (ACD, CPD or 3.8 per cent w/v solution of *sodium citrate*) to which 1 mg per ml of *hexadimethrine bromide* has been added. Centrifuge the mixture at 3,600 g for 5 minutes. Separate the plasma and centrifuge again at 6,000 g for 20 minutes to sediment platelets. Separate the platelet-poor plasma and dialyse against 10 volumes of buffer A for 20 hours. Apply the dialysed plasma to a chromatography column containing agarose-DEAE for ion exchange chromatography which has been equilibrated in buffer A and is equal to twice the volume of the plasma. Elute from the column with buffer A at 20 ml per cm^2 per hours. Collect the eluate in fractions and record the absorbance (2.4.7) at 280 nm. Pool the fractions containing the first protein peak so that the volume of the pool is about 1.2 times the volume of the platelet-poor plasma.

Test the substrate pool for absence of kallikrein activity by mixing 1 part with 20 parts of the pre-warmed *chromogenic substrate solution* to be used in the assay and incubate at 37° for 2 minutes. The substrate is suitable if the increase in absorbance is less than 0.001 per minute. Add to the pooled solution 0.7 per cent w/v of *sodium chloride* and filter using a membrane filter (porosity 0.45 µm). Freeze the filtrate in portions and store at -25°; the substrate may be freeze-dried before storage.

Carry out all procedures from the beginning of the chromatography to freezing in portions during a single working day.

Method. The assay may be carried out using an automated enzyme analyser or a suitable microtitre plate system allowing kinetic measurements, with appropriate software for calculation of results. Standards, samples and prekallikrein substrate may be diluted as necessary using buffer B.

Incubate diluted standards or samples with prekallikrein substrate for 10 minutes such that the volume of the undiluted sample does not exceed 1/10 of the total volume of the incubation mixture to avoid errors caused by variation in ionic strength and pH in the incubation mixture. Incubate the mixture or a part thereof with at least an equal volume of a solution of a suitable synthetic chromogenic substrate, known to be specific for kallikrein (for example, *N-benzoyl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine 4-nitroanilide acetate* or D-*prolyl*-L-*phenylalanyl*-L-*arginine-4-nitroanilide-dihydrochloride),* dissolved in buffer B. Record the rate of change in absorbance per minute for 2 to 10 minutes at the wavelength specific for the substrate used. Prepare a blank for each mixture of sample or standard using buffer B instead of prekallikrein substrate.

Depending on the method used, ΔA per minutes has to be corrected by subtracting the value obtained for the corresponding blank without the prekallikrein substrate. The results may be calculated using a standard curve, a parallel-line or a slope ratio assay or any other suitable statistical method. Plot a calibration curve using the values thus obtained for the reference preparation and the respective concentrations; use the curve to determine the PKA activity of the preparation under examiantion.

Anti-A and anti-B haemagglutinins. Carry out the tests for anti-A and anti-B haemagglutinins as stated under Dried human haemophilic fraction. If the preparation under examination contains more than 3.0 per cent of immunoglobulin, dilute to this concentration before preparing the dilutions to be used in the test. The 1 to 64 dilutions do not show agglutination.

Anti-D antibodies. It complies with the test for anti-D antibodies in human immunoglobulin for intravenous administration.

Test for anti-D antibodies in human immunoglobulin for intravenous administration

Materials

Phosphate-buffered saline (PBS). Dissolve 8.0 g of *sodium chloride*, 0.76 g of *anhydrous disodium hydrogen phosphate*, 0.2 g of *potassium chloride*, 0.2 g of *potassium dihydrogen*

phosphate and 0.2 g of *sodium azide* in *water* and dilute to 1,000 ml with the same solvent.

Papain solution. Use serological grade papain from a commercial source, the activity of which has been validated.

Red blood cells. Use pooled red blood cells from not fewer than 3 donors of group OR_2R_2 and 3 donors of group Orr respectively. Wash the cells 4 times with PBS or until the supernatant is clear. Centrifuge the cells at 1, 800 g for 5 minutes to pack. Treat the packed red cells with papain solution according to the manufacturer's instructions. Store in Alsever's solution for not more than 1 week.

Microtitre plates. Use V-bottomed rigid micro-titre plates.

Reference standards. Immunoglobulin (anti-D antibodies test) reference preparation and *Immunoglobulin (anti-D antibodies test negative control) reference preparation* are suitable for use as the reference preparation and negative control, respectively.

Method

Reference preparation and negative control solutions. Reconstitute the reference preparation and the negative control according to instructions. Dilute the reconstituted preparations with an equal volume of *PBS* containing 0.2 per cent w/v of *bovine albumin* and then prepare a further 7 serial two-fold dilutions using *PBS* containing 0.2 per cent w/v of *bovine albumin* to give a total dilution range from 1/2 to 1/256. Make 2 independent sets of dilutions for each preparation. Add 20 µl of each dilution to the microtitre plate.

Test solutions. Initially dilute the test samples to give a starting immunoglobulin G (IgG) concentration of 2.5 per cent w/v using *PBS* containing 0.2 per cent w/v of *bovine albumin* and then prepare a further 7 serial two-fold dilutions using *PBS* containing 0.2 per cent w/v of *bovine albumin* to give a total dilution range from 1/2 to 1/256. Make 2 independent sets of dilutions for each test sample. Add 20 µl of each dilution to the microtitre plate.

Prepare 3.0 per cent v/v suspensions of papain-treated D-positive (OR_2R_2) and D-negative (Orr) red cells in *PBS* containing 0.2 per cent w/v of *bovine albumin*. Add 20 µl of D-positive cells to one dilution series of each of the test sample, the reference preparation and the negative control, and 20 µl of *D-negative cells* to the other dilution series of each of the test samples, the reference preparation and the negative control. Mix by shaking the plate on a shaker for 10 seconds.

Centrifuge the plate at 80 g for 1 minutes to pellet the cells.

Place the plate at an angle of approximately 70°. Read after 4 to 5 minutes (or until the cells have streamed in the wells containing the negative control and the wells where the D-negative cells have been added). A cell button at the bottom of the well indicates a positive result. A stream of cells represents a negative result.

Record the endpoint titre as the reciprocal of the highest dilution that gives rise to a positive result.

The titre of the preparation under examination is not greater than the titre of the reference preparation.

Water. Determine by semi-micro determination of water (2.3.43), loss on drying (2.4.19) or near infrared spectrophotometry (2.4.6), the water content is within the limits approved by the competent authority.

Sterility (2.2.11). Complies with the test for sterility.

Pyrogens (2.2.8). Complies with the test for pyrogens. Inject per kg of the rabbit's mass a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml per kg of body mass.

Antibody to hepatitis B surface antigen

Minimum 0.5 IU per g of immunoglobulin, determined by a suitable immunochemical method.

Storage. For the liquid preparation, store in a colourless glass container, protected from light, at the temperature stated on the label. For the freeze-dried preparation, store in an airtight colourless glass container, protected from light, at a temperature not exceeding 25°.

Labelling. The label states (1) for liquid preparations, the volume of the preparation in the container and the protein content expressed in grams per litre; (2) for freeze-dried preparations, the quantity of protein in the container; (3) the amount of immunoglobulin in the container; (4) the route of administration; (5) for freeze-dried preparations, the name or composition and the volume of the reconstituting liquid to be added; (6) the distribution of subclasses of immunoglobulin G present in the preparation; (7) where applicable, the amount of albumin added as a stabilizer; (8) the maximum content of immunoglobulin A.

Plasma for Fractionation

Human Plasma for Fractionation

Human Plasma for Fractionation is the liquid part of human blood remaining after separation of the cellular elements from blood collected in a receptacle containing an anticoagulant, or separated by continuous filtration or centrifugation of anticoagulated blood in an apheresis procedure; it is intended for the manufacture of plasma-derived products.

Production

Donors

Only a carefully selected, healthy donor who, as far as can be ascertained after medical examination, laboratory blood tests

and a study of the donor's medical history, is free from detectable agents of infection transmissible by plasma-derived products may be used.

Immunisation of donors

Immunisation of donors to obtain immunoglobulins with specific activities may be carried out when sufficient supplies of material of suitable quality can not be obtained from naturally immunised donors. Recommendations for such immunisations are formulated by the World Health Organisation (*Requirements for the collection, processing and quality control of blood, blood components and plasma derivatives,* WHO Technical Report Series, No. 840, 1994 or subsequent revision).

Records

Records of donors and donations made are kept in such a way that, while maintaining the required degree of confidentiality concerning the donor's identity, the origin of each donation in a plasma pool and the results of the corresponding acceptance procedures and laboratory tests can be traced.

Laboratory tests

Laboratory tests are carried out for each donation to detect the following viral markers:

1. Antibodies against human immunodeficiency virus 1 (anti-HIV-1),
2. Antibodies against human immunodeficiency virus 2 (anti-HIV-2),
3. Antibodies against hepatitis C virus (anti-HCV),
4. Hepatitis B surface antigen (HBsAg).

Pending complete harmonisation of the laboratory tests to be carried out, the competent authority may require that a test for alanine aminotransferase (ALT) also be carried out.

The test methods used are of suitable sensitivity and specificity and comply with the regulations in force. If a repeat-reactive result is found in any of these tests, the donation is not accepted.

Individual plasma units

The plasma is prepared by a method that removes cells and cell debris as completely as possible. Whether prepared from whole blood or by plasmapheresis, the plasma is separated from the cells by a method designed to prevent the introduction of micro-organisms. No antibacterial or antifungal agent is added to the plasma. The containers comply with the requirements for plastic containers for blood and blood components (6.2). The containers are closed so as to prevent any possibility of contamination.

If 2 or more units are pooled prior to freezing, the operations are carried out using sterile connecting devices or under aseptic conditions and using containers that have not previously been used.

When obtained by plasmapheresis, plasma intended for the recovery of proteins that are labile in plasma is frozen by cooling rapidly in a chamber at -30° or below as soon as possible and at the latest within 24 hours of collection.

When obtained from whole blood, plasma intended for the recovery of proteins that are labile in plasma is separated from cellular elements and is frozen by cooling rapidly in a chamber at -30° or below as soon as possible and at the latest within 24 hours of collection.

When obtained from whole blood, plasma intended solely for the recovery of proteins that are not labile in plasma is separated from cellular elements and frozen in a chamber at -20° or below as soon as possible and at the latest within 72 hours of collection.

It is not intended that the determination of total protein and factor VIII shown below be carried out on each unit of plasma. They are rather given as guidelines for good manufacturing practice, the test for factor VIII being relevant for plasma intended for use in the preparation of concentrates of labile proteins.

The total protein content of a unit of plasma depends on the serum protein content of the donor and the degree of dilution inherent in the donation procedure. When plasma is obtained from a suitable donor and using the intended proportion of anticoagulant solution, a total protein content complying with the limit of 5 per cent is obtained. If a volume of blood or plasma smaller than intended is collected into the anticoagulant solution, the resulting plasma is not necessarily unsuitable for pooling for fractionation. The aim of good manufacturing practice must be to achieve the prescribed limit for all normal donations.

Preservation of factor VIII in the donation depends on the collection procedure and the subsequent handling of the blood and plasma. With good practice, 0.7 IU/ml can usually be achieved, but units of plasma with a lower activity may still be suitable for use in the production of coagulation factor concentrates. The aim of good manufacturing practice is to conserve labile proteins as much as possible.

Total protein. Carry out the test using a pool of not less than 10 units. Dilute the pool with a 0.9 per cent solution of *sodium chloride* to obtain a solution containing about 15 mg of protein in 2 ml. To 2.0 ml of this solution in a round-bottomed centrifuge tube add 2 ml of a 7.5 per cent solution of *sodium molybdate* and 2 ml of a mixture of 1 volume of *nitrogen-free sulphuric acid* and 30 volumes of *water*. Shake, centrifuge for 5 minutes, decant the supernatant liquid and allow the inverted tube to

drain on filter paper. Determine the nitrogen in the residue by the method of sulphuric acid digestion (2.3.30) and calculate the protein content by multiplying the quantity of nitrogen by 6.25. The total protein content is not less than 5.0 per cent.

Factor VIII

Carry out the test using a pool of not less than 10 units. Thaw the samples under examination, if necessary, at 37°. Determine the assay of factor VIII (2.8.7), using a reference plasma calibrated against the International Standard for human coagulation factor VIII in plasma. The activity is not less than 0.7 IU per ml.

Pooled plasma

During the manufacture of plasma products, the first homogeneous pool of plasma (for example, after removal of cryoprecipitate) is tested for HBsAg, HCV antibodies and for HIV antibodies using test methods of suitable sensitivity and specificity; the pool must give negative results in these tests.

The plasma pool is also tested for hepatitis C virus RNA using a validated nucleic acid amplification technique (2.8.1). A positive control with 100 IU per ml of hepatitis C virus RNA and, to test for inhibitors, an internal control prepared by addition of a suitable marker to a sample of the plasma pool are included in the test. The test is invalid if the positive control is non-reactive or if the result obtained with the internal control indicates the presence of inhibitors. The plasma pool complies with the test if it is found non-reactive for hepatitis C virus RNA.

Description. Before freezing, a clear to slightly turbid liquid without visible signs of haemolysis; it may vary in colour from light yellow to green.

Storage. Frozen plasma is stored in conditions designed to maintain the temperature at or below -20°; for accidental reasons, the storage temperature may rise above -20° on one or more occasions during storage but the plasma is nevertheless considered suitable for fractionation if all the following conditions are fulfilled (1) the total period of time during which the temperature exceeds -20° does not exceed 72 hours; (2) the temperature does not exceed -15° on more than one occasion; (3) the temperature at no time exceeds -5°.

Labelling. The label enables each individual unit to be traced to a specific donor.

Platelet Concentrate

Platelets separated from whole blood within 4 to 6 hours of collection and suspended in 40 to 50 ml of plasma are designated as platelet concentrate.

Production

Platelet Concentrate is prepared from units of whole blood that have not been allowed to cool below 20°. Platelet rich plasma (PRP) is separated within 4-6 hours after completion of the phlebotomy. The final component should contain resuspended platelets in an amount of plasma adequate to maintain an acceptable pH - generally 40 to 70 ml is used.

Tests

Four PC per month should be assayed for pH and for platelet, RBC and leucocyte counts.

PRP

Counts	3.0 - 4.5 x 10^5 per µl (approximately)
Volume	170 - 250 ml (approximately)
Total Count	9 x 10^{10} per bag (approximately)

PRC

Counts	8 - 10 x 10^5 per µl 350 ml bag (approximately)
	10 - 12 x 10^5 per µl 450 ml bag (approximately)
Volume	40 - 50 ml per bag

Platelet counts > 5.5 x 10^{10} per bag in 75 per cent of bags (if prepared from 450 ml bags)

> 4.2 x 10^{10} per bag in 75 per cent of bags (if prepared from 350 ml bags)

Leucocyte count < 0.12 x 10^9 per bag.

RBC counts < 1.2 x 10^9 per bag.

pH < 6.3 at the end of 5 days storage.

Expiration time. The expiration time is not more than 72 hours from the time of collection of the source material.

Storage. Store at 20° to 22° in polyvinylchloride plastic bags. Preserve at the temperature relevant to the volume of resuspension plasma, either between 20° and 22° or between 1° and 6°, the latter except during shipment, when the temperature may be between 1° and 10°.

Labelling. In addition to the labelling requirements of Whole Blood applicable to this product, label it to state the volume of original plasma present, the kind and volume of anticoagulant solution present in the original plasma, the blood group designation of the source blood, and the hour of expiration on the stated expiration date. Where labelled for storage at 20° to 22°, label it also to state that a continuous gentle agitation shall be maintained, or where labelled for storage at 1° to 6°, to state that such agitation is optional. Label it also with the type and result of a serologic test for syphilis, or to indicate that it

was nonreactive in such test; with the type and result of a test for hepatitis B surface antigen, or to indicate that it was nonreactive in such test; with a warning that it is to be used as soon as possible but not more than 6 hours after entering the container; to state that a filter is to be used in the administration equipment; and to state that the instruction circular provided is to be consulted for directions for use.

Whole Human Blood

Whole Blood (Human)

Whole Human Blood is blood drawn aseptically from selected human donors and mixed with a suitable anticoagulant.

Whole Human Blood is the final mixture of blood and anticoagulant solution contains not less than 9.7 per cent w/v of haemoglobin, calculated from the haemoglobin content of the donor's blood and the dilution due to the anticoagulant solution. It is obtained from healthy donors who must:

(a) be in the age range of 18 to 60 years and be in good health as indicated in part by normal temperature and blood pressure within normal limits;

(b) not be pregnant, if females;

(c) not have undergone major surgery within 6 months of donation;

(d) as far as can be ascertained after clinical and laboratory examination and the study of medical history of the donor be free from disease transmissible by blood transfusion;

(e) have blood containing not less than 12.5 per cent w/v of haemoglobin;

(f) be free from acute respiratory diseases;

(g) be free from any infectious skin disease at the site of phlebotomy;

(h) have no history of malarial fever within 12 months of donation;

(i) have no history of viral hepatitis or of close contact with an individual having viral hepatitis within 12 months of donation and have blood that has given negative results in tests for the presence of hepatitis-B antigen;

(j) have blood that has been tested with negative results for evidence of syphilitic infection, HCV antibodies, HIV antibodies and malarial parasites.

The examinations and tests to be carried out are decided by the National Regulatory Authority.

The frequency of donations of whole blood shall not exceed once every 3 months with a maximum volume of 1.5 litres in any consecutive 12 month period.

The blood is drawn aseptically through a closed system into a suitable sterile container containing a specific amount of Anticoagulant Citrate Dextrose Solution (ACD Solution) or Anticoagulant Citrate Phosphate Dextrose Solution (CPD Solution) which is placed before the container is sterilised. The quantity of anticoagulant solution should not exceed 22.0 per cent v/v of the final volume of the mixture. No antimicrobial preservative is added.

During the withdrawal of blood the container is gently agitated to ensure thorough mixing. When withdrawal is complete the container is immediately sealed and cooled to 2° to 8°. It is not opened until immediately before transfusion. With every container of blood, a separate sample mixed with the appropriate quantity of anticoagulant solution, is collected for compatibility and other tests; this small container is firmly attached to the main container.

Whole Human Blood in containers from which samples have been removed for tests should not be used for transfusion. Consequently, it is not intended that the Tests and the Assay should be carried out on the contents of the container. The Blood Bank or the service collecting the blood is responsible for ensuring that the conditions in which the blood is collected and stored are such that, if and when tested, the blood will comply with the requirements of the monograph.

Blood group. Determine the blood group (in the sample accompanying each donation) under the ABO system (2.8.11), by examination of both corpuscles and serum, and under the Rh system (2.8.11), by examination of the corpuscles.

Description. Deep red fluid which, on standing separates into a lower layer of sedimented-red cells and a yellowish, almost clear upper layer of plasma, free from visible signs of haemolysis, with a greyish layer between the two consisting of leucocytes and thrombocytes. A layer containing emulsified fat may form on the surface.

Tests

Sterility (2.2.11). Complies with the tests for sterility, determined by Method B.

Assay. Determine the haemoglobin content by photometric haemoglobinometry (2.8.12).

Storage. Store in colourless, transparent and sterile containers into which it was originally drawn. The containers should be provided with a hermetic, contamination-proof closure. Store at a temperature between 2° to 8°.

Labelling. The label states (1) the distinctive code number by reference to which the details of the donor are available; (2) the ABO group with the approved colour scheme for different groups as specified by the National Regulatory Authority; (3) the Rh group; (4) the total volume of fluid, the proportion of

blood, and the nature and volume of anticoagulant solution; (5) the date on which the blood was withdrawn; (6) the expiry date which should not exceed 21 days from the date of withdrawal of blood; (7) the storage conditions; (8) that the blood must not be used for transfusion if there is any visible evidence of haemolysis or other deterioration; (9) for blood of group O whether haemolysins are present or not and if they are, that the blood must be administered only to recipients of blood group O; (10) that the blood has given negative results in the tests for the presence of malarial parasites, hepatitis-B antigen, syphilis and HIV antibodies and any other tests prescribed by the National Regulatory Authority.

BIOTECHNOLOGY PRODUCTS

General Monograph

Monographs

Biotechnology Derived Therapeutic Products

Biotechnology has brought about the discovery and development of a new class of human therapeutics. Advances in genetics, cellular and molecular biology have allowed scientists to identify and develop a number of new products. These products provide significant clinical benefits, and in many cases, provide therapies where no effective treatment existed.

Proteins are the workhorse molecules of the cell. Due to their biological significance, proteins can make excellent drug candidates. In the living cell, genes, in the form of DNA, provide the basic instructions in creating biologically functional proteins. Biotechnology is the process that produces protein molecules in sufficient quantities for therapeutic use, which have very specific physiological roles that already exist in the human body *albeit* in limited quantities. Techniques of genetic engineering using bacteria, yeast and eukaryotic cells in culture or cultured animal cells are employed to incorporate in these cells the information needed to produce a human protein with therapeutic potential. Once engineered, these cells can be grown in large quantities by fermentation or large-scale cultivation of animal or human cells. The addition of genetic material to cells gives the engineered proteins their name, recombinant proteins

Gene Cloning and Protein Expression

The basic principle involved in gene cloning includes the insertion of a select fragment of DNA representing the gene for a peptide/protein into an episomal circular DNA, called the vector, which transports the gene into a host cell. The vehicle containing the inserted gene then replicates along with the host cell. The cellular machinery of the host cell transcribes and translates the genes including the inserted gene into proteins. The vector with an inserted foreign gene is called a *recombinant DNA molecule* and the protein produced as a consequence of the inserted gene by the host cell is called *recombinant Protein.* The commonly used vectors for bacterial transformations are *Plasmids,* which are small circular DNAs found in bacteria and other microorganisms. Other vectors include viruses such as bacteriophages (for bacterial hosts) or animal viruses for eukaryotic host cells (e.g., Epstein-Barr virus, Simian Virus etc). Major advances have been made in the past decade for efficient and stable expression of foreign proteins in mammalian cells in culture.

In addition, transgenic animals and plants have been produced which express foreign genes inserted into their genome for tissue/organ specific expression. Although large-scale production of therapeutic proteins has been accomplished, the current edition of the Indian Pharmacopoeia does not contain products produced by this process and hence no further discussion on this aspect is included in this chapter

Monoclonal Antibodies

Antibodies are proteins produced by differentiated b-lymphocytes. Kohler and Milstein first fused a normally short-lived antibody producing b-cell obtained from the spleen of mice immunized with an antigen to a rapidly dividing cancer cell (mouse myeloma) to produce a cell line (hybridoma), which was both immortal and a producer of antibodies that recognized a single epitope on an antigen, hence, the name monoclonal antibodies (mAbs). Monoclonal antibodies can be produced in cell culture or in live animals. When the hybridoma cells are injected into the peritoneal cavity of mice, they produce tumors containing an antibody-rich fluid called ascites fluid. Production in cell culture is usually preferred since cell culture in fermentation chambers can be used to produce antibodies on a larger scale.

Humanized monoclonal antibodies

The standard procedure of producing monoclonal antibodies yields mouse antibodies. For therapeutic applications in humans these proteins are unsuitable since the human immune system recognizes mouse antibodies as foreign, causing systemic inflammatory effects and are removed rapidly from circulation. Application of recombinant DNA technology has overcome this problem. The DNA that encodes the binding portion of monoclonal mouse antibodies is ligated to the human antibody producing DNA. Such a construct is then cloned suitably in a vector and introduced into a mammalian expression system to produce these half-mouse and half-human antibodies. Depending on how big a part of the mouse antibody is used, such proteins are either referred to as Chimeric or humanized monoclonal antibodies. Monoclonal antibodies have been generated and approved to treat cancer, cardiovascular disease, inflamatory diseases, muscular degeneration, transplant rejection, viral infection and others.

Therapeutic recombinant monoclonal antibodies

Recombinant monoclonal antibodies for therapeutic applications belong to the IgG class of immunoglobulins. Immunoglobulins are made up of four polypeptide chains, two light (λ and κ) and two heavy chains (α, β, γ, ε, μ). The type of heavy chain determines the immunoglobulin isotype (IgA, IgD, IgG, IgE and IgM respectively). The four-polypeptide chains are cross-linked by disulfide bridges (-S-S- bonds). Light chains comprise 220 amino acid residues whereas heavy chains are made of 440-550 amino acids. Each chain has two distinct regions namely, "constant" and "variable", the former being proximal to the C-terminal region (111-220 or 440-550 residues) whereas the latter is towards the N- terminal sequence of the polypeptide (1-110). The amino

acids of the constant region of immunoglobulin are uniform from one antibody to another within the same isotype (e.g., IgG), while the variable region found in both the light and heavy chains consist of different amino acids. These regions are referred to as hypervariable region or "Complementary Determining Regions (CDRs)"representing antibody diversity, each specific for the recognition of the antigen. An antibody molecule is generally represented as a "Y" shaped structure. The arms of the Y structure are connected to the stem region by a flexible hinge. IgG can be proteolytically cleaved by partial digestion with papain, which results in three distinct ~50 KD fragments: two identical fragments designated Fab fragments and one Fc fragment. The Fab fragments are the arms of the antibody containing the entire light (L) chain and the N-terminal half of the Heavy (H) chain. These fragments contain the IgG's antigen binding domains. The Fc fragment derives from the stem portion of the Y structure and contains the c-terminal halves of the two H chains. The Fc region is responsible for the activation of phagocytosis, complement dependent cellular toxicity and antibody dependent cellular cytotoxicity (ADCC).

The IgG-Fc region is glycosylated at Asn 297, which is essential for the conformation of the antibody molecule that allows interaction with effector ligands. The IgG-Fc glycoform pattern of monoclonal chimeric and "humanized" recombinant therapeutic antibodies produced in CHO or NSO cells show wide variation from clone to clone, which is dependent on the process and cell culture conditions. Under certain conditions a number of abnormally glycosylated products that lack potency and potentially immunogenic products are produced (e.g., galactose-α (1,3)-galactose and N-glycolylneuraminic acid) which are not therapeutically acceptable. However, successive rounds of selection of cell lines overcome these problems. Another important variable in efficacy due to glycosylation is the ability of the IgG molecule to mediate ADCC. This property is particularly important in antibody therapeutics for oncology indications. The ability to elicit ADCC greatly varies with different glycoforms. Therefore, the cell lines have been engineered by the introduction of a glycosyl transferase (GnTIII) and/or inactivation/deletion of fucosyl transferase in the CHO cells, which has resulted in products with 20 to 100-fold increase in ADCC.

Processes for the production of recombinant therapeutic proteins including mAbs

Prokaryotes

The qualitative and quantitative demand for recombinant proteins is steadily increasing. Molecular biologists are constantly challenged by the need to improve and optimize the existing expression systems to meet the steadily increasing qualitative and quantitative demand for recombinant proteins. The continuous evolution of novel expression systems is paralleled by growing concerns about the safety of these novel pharmaceuticals, which has resulted in the regulatory authorities setting high standards for certification. One of the strategies used by researchers in this field involves sourcing new genetic elements for incorporation into expression systems by systematically analyzing the rich natural diversity of microorganisms. There are, in addition, numerous tools for modifying microorganisms and for re-engineering existing biological pathways or processes to meet the needs of the pharmaceutical industry. Although many prokaryotic hosts are described in literature for the production of recombinant proteins, *E.coli* is the most preferred host because its genetics are well understood. The popular cloning host has been *E.coli* BL21 and its derivative BL 21λDE3. However there are some disadvantages such as the recombinant protein produced may not have a proper secondary structure due to the non-formation of intra-molecular disulfide bonds. Many recombinant proteins tend to aggregate when over expressed in *E.coli*. *E.coli* proteins begin their sequence with an N-formyl methionine, which may not be removed by its enzyme systems. Proteolytic enzymes of *E.coli* may partially degrade the recombinant protein.

The principal element in recombinant protein expression technology is the recombinant plasmid, which contains the gene that codes for the protein of interest. The gene expression regulatory elements such as the promoter sequence in the plasmid play a key role in the production of mRNA of the recombinant gene. The other important regulatory process employed in gene expression is the application of gene induction. Induction is the phenomenon wherein the transcription of the gene is turned on by the addition of a chemical to the growth medium. The most popular system used in academic research is the *lac* promoter, which is induced by Isopropyl thiogalactopyranoside (IPTG). However, in the industrial production of recombinant therapeutic proteins, addition of chemical inducers is not desired, hence auto-inducible promoters which are up-regulated upon changes in the culture medium or the growth phase of the bacteria or the regulation of cell cycle, are employed for improvement in production efficiency. Overproduction of proteins most often results in misfolded structures, which are insoluble and accumulate in the cytoplasm as "inclusion bodies". Cloning and expressing a group of proteins collectively called "Chaperones" overcome this problem. Some of the chaperones whose mode of action is partially understood include, Gro EL/ Gro ES, DnaK, DnaJ and GrpE. Fusion of the gene of interest with a partner gene results in a recombinant protein, which produces both gene products linked in tandem. Such fusions also increase expression levels as well as provide a means for a convenient process of purification by affinity chromatography. A number of fusion partner genes are reported in literature but the most common partner proteins are maltose binding protein, glutathione-S-transferase and thioredoxin.

For ease of recombinant protein purification, short sequence tags at N- or C-terminal are genetically attached which result in recombinant protein products with additional amino acid sequences. These additional sequences have to be cleaved from the final therapeutic product. Several methods for precise excision of tag sequences have been described which include enzymatic and chemical processes. Another strategy employed for enhancing protein expression is the use of "bicistronic" vectors. In this strategy, a short sequence of a gene efficiently expressed is linked as a non-translatable transcriptional fusion to the gene of interest. Secretion of recombinant proteins or their translocation to periplasmic space of bacteria is a procedure employed in the expression of foreign proteins in prokaryotes. This procedure requires the addition of signal sequences from secretory pathways or leader sequences for transport to periplamic space. *OmpA* and *PelB* signal peptides have been shown to efficiently transport recombinant proteins to bacterial periplasm. A good example is the production of correctly folded full-length antibodies within the periplasm of *E. coli.*

Eukaryotes

Yeasts

Similar to prokaryotes in growth kinetics achieving high density, yeasts secrete large amounts of recombinant proteins. *Saccharomyces sp, Pichia pastoris, Hansenula polymorpha,* and *Kluyveromyces lactis* are some of the yeasts, which are currently in use for the production of therapeutic recombinant proteins. Yeasts are the preferred eukaryotes for the production of recombinant therapeutic proteins that cannot be produced from prokaryotes because of incorrect folding or the requirement for glycosylation. *Sacharomyces cerevisiae* is genetically well characterized, its growth physiology well understood and hence, it is the prevalent yeast species in pharmaceutical production process. However, *Pichia pastoris* is also gaining ground as a preferred host for heterologous protein expression because of its superior secretion efficiency and high yields. *Pichia pastoris* and *Hansenula polymorpha* are methanol metabolizing yeasts (methylotrophic yeasts), which contain strong promoters for the genes of methanol assimilating enzymes such as methanol oxidase and alcohol oxidase. As the enzymes of these pathways account for more than 30% of the total protein content of the organism, the metabolic efficiency with respect to secreted protein is significantly higher. Although attempts are being made to humanize N-linked glycosylation in the yeast *Pichia pastoris,* the employability of yeasts for the expression of human therapeutic proteins might reach a limit if the product has a highly specific post-translational modification for its pharmacological activity/properties. In such cases, mammalian cell expression systems are employed for the production of therapeutic proteins.

Mammalian Cell Lines

Animal Cell expression systems produce proteins, which have high degree of similarity to human proteins with respect to the pattern and capacity of post translational modifications. The most commonly used cell lines for the expression of therapeutic proteins are as follows:

Chinese Hamster Ovary (CHO) Cell Line

Baby Hamster Kidney (BHK) Cell Line

HEK Human Embryo Kidney (HEK) Cell Line

Human Retinal Embryonic Cell Line (PER C.6)

Mouse Myeloma Cell Line (NSO)

Mammalian cell lines produce therapeutic proteins with proper folding, assembly and post-translational modifications, which results in pharmacologically active products in terms of quality and reproducibility. However, there remains a possibility of transmission of viral or pyrogenic substances carried over in the final product. There is a trend now to use cell lines, which are of human origin since they possess all mechanisms to produce products most similar if not identical to naturally occurring proteins in the human body.

Immortalized cell lines transfected with foreign therapeutic protein genes are used for the production of the respective proteins. There are basically two processes of immortalizing mammalian cells; (1) *Viral transformation*: Induction of immortalization by inactivating the tumor suppressor genes of mammalian cells. Viral genes including Epstein - Barr virus (EBV), Simian Virus 40 (SV 40) T antigen, adenovirus and human papillomavirus (HPV) can induce immortalization. Although the method is quite reliable, there are reports of aneuploidy and loss of some properties of the primary cells. (2) *Modification of telomere of somatic cells*. Telomerase reverse transcriptase protein (TERT), which is inactive in most somatic cells, when exogenously expressed, the cells are able to maintain telomere lengths sufficient to avoid replicative senescence thus, rendering the cells immortal. The latter method is preferred for therapeutic protein expression. Analysis of several telomere-immortalized cell lines has verified that the cells maintain a stable genotype and retain critical phenotype markers.

Development of a cell line stably expressing a desired protein gene starts with the construction of expression vectors. Different expression vectors both viral and non-viral have been designed to transfer foreign genes into mammalian cells. The choice of the vector is dependent on the host cell, the level of the desired product formed and safety. Typically an expression vector consists of (i) a constitutive or inducible promoter (ii) a transcription terminator and (iii) a cassette with an origin of replication and a selection marker for vector production in bacteria. Strong constitutive promoters are of

viral genomes (e.g., human cytomegalovirus (HCMV), simian virus 40 and Rous sarcoma virus). Amongst the non-viral promoters the promoter of human elongation factor-1-α (EF-1α) is similar in strength to the HCMV promoter. The desired therapeutic protein gene is isolated as a cDNA, with one intron sequence, which is preferably located between the promoter and the coding sequence of the gene of interest and inserted into the vector at a suitable site. The vector containing the gene insert is then propogated in the bacterial host. The vector is then recovered from the bacteria, linearized and introduced into the mammalian cell by transfection. There are many reagents/methods to effectively mediate gene transfer. Calcium phosphate facilitated transfection, electroporation, lipofection, micro injection, biolistic and polymer mediated transfer are routinely used. For selection of recombinant cell, a second gene is transferred that confers to the recipient cells a selective advantage. The most common selector genes are dihydrofolate reductase (DHFR) and glutamine synthetase (GS). In both cases, selection occurs in the absence of appropriate metabolite (hypoxanthine and thymidine in the case of DHFR and glutamine in the case of GS), thus preventing the growth of non-transformed cells. When the transfected foreign gene is integrated into a "good site" on the chromosome of the host by recombination, the resultant cell line will stably express the desired gene. Selection pressure results in several fold amplification of genes including the integrated genes when high concentrations of respective inhibitors are used (for. e.g., Methotrexate inhibition of DHFR and Methionine sulphoximine inhibition of GS).

There are two main ways of culturing animal cells for the production of recombinant proteins, viz., (a) Adherent cell culture and (b) Suspension culture. In adherent cell culture technology the mammalian cells (e.g., CHO cells) are seeded into roller bottles that are filled to about 25 per cent capacity with the medium and slowly rotated allowing cells to adhere. The rotation provides the continuous contact of the cells with the medium and oxygen is supplied by the "head space" in the bottle. Following attainment of confluency, the product is harvested from the decanted supernatant. The suspension culture method is more widely used for the mass production of recombinant proteins, where the capacity of the mammalian cells for single cell suspension growth is exploited for scalability to very large volumes. The transition of adherent cells to suspension cultivation requires a selection of media formulations. The seeding inoculum begins with a small volume of cell suspension, which is gradually expanded so that sufficient cell numbers are generated for the final production phase. In the suspension culture process the changing composition of the cell medium during the production phase can affect the quality of the product due to degradative enzymes released by the cells as well as the molecular composition of the product due to limitations of nutrients.

General Techniques used in down-stream processing of recombinant proteins

The design of the purification process of a recombinant protein and its scale-up capabilities assumes significant importance since the regulatory authorities would approve the "product by process". Thus, the approved process must be followed even if new developments in separation technologies provide significant advantages.

Liquid chromatography is the core of preparative protein purification and all supplementary procedures like extraction, centrifugation, ultrafiltration, and dialysis serve to prepare the protein solution for chromatography. A series of chromatographic steps usually termed as capture, intermediate purification and polishing, make use of different intrinsic features of proteins, is usually required to achieve sufficient separation of the target from contaminants. Common modes of chromatography include ion exchange chromatography, affinity chromatography, hydrophobic interaction chromatography, gel filtration and reverse phase chromatography. The selection of chromatographic procedures and their arrangements in a suitable order are based on the properties of the recombinant protein particularly its tolerance to organic solvents, its susceptibility to denaturation due to changes in pH, temperature, degradation and oxidation.

In the case of secreted recombinant proteins, downstream processing starts with the collection of the fermentation supernatant after separating the cell mass while if the recombinant protein is intracellular, the process begins with the extraction of soluble proteins from the cell mass. In the former case the target protein may be highly dilute with large amounts of inorganic salts and media proteins such as fetal calf serum, yeast extract and other growth promoting substances, which may limit the choice of the capture step. In the case of the target protein expressed as an intracellular protein, the process of extraction from the cell mass is employed, which leads to a highly complex mixture of recombinant and host biomolecules including host proteins, nucleic acids, lipids, carbohydrates and other cell components. In addition to commonly employed technologies such as continuous centrifugation, tangential flow filtration and fast flow chromatography two novel techniques viz.; affinity tags and expanded bed adsorption techniques are gaining importance in the purification protocols for recombinant proteins. Affinity tags are short peptide sequences genetically fused to a target protein. A peptide of six consecutive histidine residues (His-6-tag) is the most widely used tag peptide, which is fused either at the N- or C-terminal of the protein of interest. This tag facilitates the purification of the protein by immobilized metal ion (generally Nickel) affinity column (IMAC). For this technique, a metal chelating ligand, e.g., iminodiacetic acid or nitrilotriacetic acid is immobilized on a chromatographic matrix

and charged with transition metal ions leaving one or more coordinating sites free for interaction with the analyte. Proteins with the His-6-tag will bind to the ligand tightly to allow quantitative capture of the target molecule from a complex feed stream. The bound target protein is recovered by step-gradient elution using imidazole or by acidification. A potential problem is the presence of leached metal ions in the eluate, which must be removed in a subsequent step of purification. His tag facilitated purification process has yielded therapeutic recombinant proteins without affecting the folding, bioactivity and biodistribution profiles.

Expanded bed adsorption (EBA) is another novel technique, which is employed for preparative protein purification when large volumes of particulate raw materials are involved in the initial purification step. EBA combines the advantages of batch- or fluidized bed methods with superior adsorption characteristics of packed bed columns by a novel design of matrix particles. With the exception of gel filtration, all modes of biochromatography are compatible for EBA. However, ion-exchange media are most extensively used.

Post-Translational Modifications of Proteins

The primary structure of proteins, which is the linear sequence of amino acids, is encoded by the gene sequence of the cell. However, proteins undergo a variety of modifications subsequent to their synthesis. Enzymes within the cell mediate these modifications and such modifications are attributed to certain properties, such as solubility, transportability, resistance to proteolysis, folding, receptor binding, signal transduction etc., in the native milieu of the cell. However, with the advent of production of proteins for therapeutic applications in heterologous expression systems, post-translational modifications (PTMs) have assumed special significance from the point of view of setting specifications and quality assurance. Proteins undergo a broad range of PTMs; the most common of them are listed below.

Post-Translational Modifications of Proteins

i) Acylation
ii) Phosphorylation
iii) Sulphonation
iv) Proteolysis (terminal or domain deletion)
v) Glycosylation (N-linked, O-linked)
vi) Aggregation
vii) High order structural change (conformational change or denaturation)
viii) Amidation or deamidation
ix) Carbamylation
x) Carboxylation
xi) Formylation
xii) Formation of gamma carboxyglutamic acid
xiii) Methylation
xiv) Succinimide forms
xv) Aspartate isomerization
xvi) Disulfide linkage
xvii) Oxidation

Amongst these only a few are relevant from the perspective of therapeutic recombinant proteins. Glycosylation represents the most important PTM since a great diversity exists among different expression systems. For example, prokaryotic expression systems result in aglycosylated proteins often resulting in insoluble inclusion bodies. Yeasts on the other hand, add carbohydrate side chains of high mannose content, CHO and murine cells can also add sugars not normally found in human proteins some of them are known to be immunogenic.

Posttranslational modifications of recombinant therapeutic proteins are significant in terms of potency, stability and toxicity including immunogenecity of the biopharmaceutical product/s. This emphasizes the need for inclusion in the pharmacopoeial monographs, meticulous description of specifications and rigorous analytical data to characterize each biopharmaceutical product.

The mere demonstration that variations in the post translational modifications are found in recombinant glycoproteins produced in different expression systems, does not automatically negate the biological efficacy or the safety of the product. In many instances it has been clearly demonstrated through clinical trials that the products are safe and efficacious (e.g. recombinant factor VIII produced by BHK cells and CHO cells have different glycosylation profiles but both are safe and efficacious). Therefore, comparability of the same protein produced by different processes has to be governed by a pragmatic approach. However, the secretory pathway of *Pichia pastoris* has been genetically re-engineered to perform sequential glycosylation reactions that mimic early processing of N-glycans in humans.

Glycosylation of Proteins

More than one third of the biopharmaceticals currently in clinical use have an absolute requirement for glycosylation and hence they are produced in eukaryote expression systems. Oligosaccharides are attached co-translationally through specific aspargine (N-linked) or serine or threonine residues (O-linked). While the consensus sequence for N-glycosylation has been shown to be Asn-x-ser/Thr as an essential but not sufficient sequence, there is no such consensus sequence known for O-glycosylation sites. In some cases of recombinant proteins, glycosylation may not be essential for therapeutic application, but as pharmaceutical products, glycosylated preparations have been found to have lowered protein

aggregation and better thermal stability which are desired properties.

Immunogenicity is yet another parameter, which is a matter of concern with regard to recombinant therapeutic proteins, produced in different expression systems.

γ-Carboxylation and β-hydroxylation of Proteins

These are particularly found in proteins involved in the process of Blood Coagulation. An enzyme, carboxylase, converts target glutamic acid residues in the protein to γ-carboxyglutamic acid residues, while hydroxylase enzyme catalyses the conversion of either the target aspartic acid or the target aspargine residues to their corresponding β-hydroxy derivatives (β-hydroxyaspartate and β-hydroxyaspargine respectively

O-sulfonation

Tyrosine O-sulfonation is a post-translational modification limited to a few proteins. Tyrosine O-sulfonation is catalyzed by two enzymes, viz., tyrosylprotein sulfotransferases (TPST-1 and TPST-2) that transfer the sulfuryl ($-SO_3^-$) group from phosphoadenosylphosphosulphate to certain tyrosyl residues in the protein. Tyrosine sulfation occurs in all mammalian cell lines but not in yeasts and prokaryotes. The functional importance of tyrosine sulfation is presumed to be in protein-protein interactions.

Amidation

Modification of the carboxy terminal of proteins and peptides with an amide group is widely found in vertebrate and invertebrate proteins and peptides but not in yeasts and prokaryotes. Bioactive peptides such as vasopressin, oxytocin, gastrin, calcitonin, substance P and Neuropeptide Y are amidated at their C-terminal. Although amidation is of widespread occurrence in mammalian bioactive peptides and polypeptides, the biological significance of amidation is less understood. It is presumed that this PTM may contribute to stability and in increasing the hydrophobicity of the peptide to facilitate binding to its receptor. Most bioactive peptides are chemically synthesized and subsequently amidated enzymatically using α-amidating enzyme.

Covalent modification of Therapeutic Proteins

Many therapeutic recombinant proteins are characterized by low protease stability, quick renal excretion and high immunogenicity. Covalent modification of proteins is a process, which to some extent, if not all overcomes problems. Covalent conjugation of polyethylene glycol (PEG) to therapeutic proteins is one of the most important techniques (Molecule Altering Structural Chemistry (MASC)), employed for the development of second-generation biopharmaceuticals. PEgylated proteins have been shown to be less susceptible to proteolytic degradation, less immunogenic due to masking of epitopes and have reduced renal clearance rates. Early procedures of PEgylation resulted in mixtures of PEG substituted positional isomers. But later developments in the chemistry of ligation which includes different coupling techniques using different activated poly ethylene glycols and introduction of linkers between PEG and the protein targets, have resulted in site-specific PEgylation. Several PEGylated biopharmaceuticals have received regulatory approvals, which include PEG-IFN, PEG-human growth hormone and PEG-granulocyte macrophage colony-stimulating factor. In all cases PEGylation increased product plasma half-life which resulted in reduced frequency of administration.

Proteins by nature are heterogeneous but in the context of recombinant DNA derived protein products produced in various systems add to more complexities. In the chain of processes starting from cloning of the gene to the production of therapeutic protein, there could be many products formed such as truncated polypeptides, differently folded proteins, scrambled disulfide linkages, varying extents and different pattern of glycosylation. In addition, there could be process related contaminants resulting in impurities in the final product. Proteins have pleomorphic physiological effects. Thus, although the recombinant therapeutic protein is optimized for one biological activity, it may have a deleterious effect on another activity, which may result in adverse effects. Therefore, pharmacopoeial monographs include precise specifications for each formulation of therapeutic proteins, and analytical characterization.

Specifications

They comprise ICH recommendations of characterization parameters that include appearance, identity, quantity, and purity including impurity profile, stability, and biological potency. These parameters that are numerical limits are established by a list of tests, analytical procedures, and other criteria, to which a drug substance or drug product should conform. Conformity of each batch of production to "specification test accept-criteria" is an important part of the batch quality control release (certificate of analysis, COA). The specifications are developed in the early part of process and product development, scale up, non-GMP manufacture, and cGMP manufacture for clinical trials wherein the active drug is fully characterized for its physiochemical properties, biological activity, immunochemical properties, purity, and quantity. The acceptance criteria are related to the analysis method chosen and established during the development process

Identity

An important part of the overall characterization program for a recombinant protein is the identity tests confirming molecular

weight, isoelectric point, primary structure, higher order structures (secondary, tertiary and quaternary) and possible posttranslational modifications. Protein structure and function are closely related. Even minor deviations in the three-dimensional conformation or in the posttranslational modifications (e.g., glycosylation, phosphorylation, or acylation pattern) may result in altered biological activity or in an adverse imunogenic/allergic response. During the drug development phase extensive characterizations are carried out in order to confirm the chemical and physical properties of the recombinant product with its natural counterpart. Only a minority of the identity tests will be used for batch releases that are specified in the relevant monographs.

The standard methods for determination of molecular weight (MW) are Electrospray Mass Spectra (EMS), Matrix Assisted Laser Desorption/Ionization (MALDI)-Time of Flight (TOF), High Performance Size Exclusion Chromatography (HP-SEC), and Analytical Ultracentrifugation (AUC). Various sedimentation velocity measurements (sedimentation velocity, difference sedimentation, and sedimentation equilibrium) are used to gain information on shape and conformation. This is a useful check of homogeneity.

The isoelectric point (pI) is the pH level where the protein has no net charge. The standard methods for determination of pI are Isoelectric focusing in polyacrylamide gels (IEF) and Capillary Electrophoresis (CE)-IEF. The separation principle is based on charge heterogeneity caused by differences in amino acid residue charges. The pI may also be determined by means of 2D-electrophoresis, where molecules are separated according to pI and MW.

The primary structure provides information on the amino acid sequence of the protein. For most proteins a primary structure analysis comprises N-terminal sequencing by Edman degradation, C-terminal analysis, and peptide mapping followed by High Performance Reversed Phase Chromatography (HP-RPC) purification and subsequent determination of the MW of the fragments by mass spectrometry. The amino acid sequence is often supported by total amino acid analysis, comparison of the cloned gene sequence, and the molecular weight determination. Comparative fingerprints between the natural and the recombinant protein are also used to confirm primary structure identity.

The secondary structure provides information on disulfide bond arrangement, α-helix, and β-sheet content. The standard methods for determination of disulfide arrangements are peptide mapping by HP-RPC or Sodium dodecyl sulphate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE). Structural information is obtained by means of fluorescence, far UV circular dichroism, Raman scattering, and infrared absorption using Fourier Transformed Infra Red Spectroscopy (FTIR). The spectroscopic methods are very powerful when used for comparison analysis with the natural counterpart. The confirmation of correct disulfide linkage is usually carried out on appropriate enzymatic or chemical digests of the target protein followed by HP-RPC purification of the fragments and subsequent mass analysis using ESI-MS or MALDI-TOF.

The tertiary structure is the three-dimensional structure of the molecule. The standard methods for tertiary structure determination are NMR (in solution), X-ray diffraction using crystals at high atomic resolution (< 3Å), and near UV circular dicroism. The latter method is very powerful for comparison analysis with the natural counterpart.

The quaternary structure of a protein represents the interaction between individual polypeptide chains (Subunits) resulting in larger assemblies. The standard methods for determination of quaternary structure are HP-SEC, Raman scattering, and light scattering, useful for determination of large macromolecular assemblies.

Posttranslational modifications include chemical modifications of side groups (e.g., oxidation of

Met, de-amidation of Asn of Gln), phosphorylation, glycosylation, fatty acid acylation, farnesylation, sialic acid capping, N-methylation, and acetylation. Typical standard methods for the detection of posttranslational modifications are HP-RPC, HP-IEC, or mass spectrometry. In mass spectrometry, the MW of the molecule can be determined with high accuracy and precision (better than 0.01%). This performance is normally sufficient to identify modifications such as missing residues or additional groups. Peptide mapping, using specific enzymes and subsequent HP-RPC purification of the fragments prior to MW detection is also used, for example, for determination of glycosylation patterns. Heterologous glycosylated products are often identified by their isoelectric focusing slab gel pattern or more rarely by 2D-electrophoresis. A thorough carbohydrate structural analysis may include glycosylation site(s), carbohydrate chain structure, the oligosaccharide pattern, and the content of neutral sugars, amino sugars, and sialic acids.

The extinction coefficient can be determined from a known protein quantity (protein concentration) and the absorbance at 277 to 280 nm. In chromatographic evaluation, the target protein retention time using HP-IEC or HP-RPC can be used as an identity marker. For biomolecular interaction analysis, the method uses surface plasmon resonance to detect biomolecular interactions.

Biological Activity

The biological activity (Potency) describes the ability of the drug substance to achieve a defined biological effect. Examples of procedures used to measure the biological activity include

animal based biological assays; cell culture-based biological assays, biochemical assays, and ligand binding assays. A biological assay may be replaced by physicochemical tests provided sufficient information and correlation between the bioassay and the said tests can be demonstrated and there exists a well-established manufacturing history (ICH Harmonized Tripartite Guideline. Specifications: Test Procedures and Acceptance Criteria for Biotechnological Products (Q6B).

Structural variants of therapeutic proteins in general and recombinant proteins in particular which include isoforms (e.g., variations in post translational modifications, varying degrees of processing of C- and N- terminal substitutions etc) often show variations in biological activity. Thus, the batch release of the product should be shown to possess the expected degree of biological activity using relevant bioassays. In order to use the same dosing protocol as a reference medicinal product, the content and specific activity of the clinically comparable product should be compared to that of one or more reference medicinal products, or to an international reference standard to which the reference product is calibrated. (WHO Guideline for abbreviated licensing pathways for certain therapeutic proteins)

Purity

Protein purity has been historically linked to the specific biological activity in terms of units of biological activity per mass unit of the product. The purest product is that of the highest specific biological activity. In contrast to drugs based on small molecules, which could be controlled on the drug product level, protein-based pharmaceuticals are closely linked to the process itself due to the complexity of the active pharmaceutical ingredient and the lack of proper characterization of the final product. With the introduction of recombinant technology and modern analytical methods, a much better drug substance/product characterization has become possible resulting in the well-characterized protein concept and the widespread use of comparability studies. The importance of a stronger focus on presence of adventitious agents and specific impurities are also recognized, as the presence of even minor amounts of toxic, immunogenic, or adventitious compounds proved to have severe side effects. The acceptable level of impurities depends on the nature of the drug product and the dose.

Impurity Profile

Product related impurities

Des-amido Forms. Des-amido forms are target protein derivatives in which one or several of the glutaminyl or asparagyl amino acid residues are converted to the corresponding acids (glutamyl and asparagyl). Des-amido forms are detected by analytical HP-IEC, HP-RPC, native PAGE, IEF, MS, or CE. The content accepted depends on the nature of the drug product and the dose.

Oxidized Forms. Oxidized forms are target protein derivatives in which one or several Met, Cys, His, Trp, or Tyr residues have been oxidized. The oxidation of cystinyl residues results in formation of a disulfide bond (cystinyl residue). Oxidized forms are detected by analytical HP-IEC, HP-RPC, native PAGE, IEF, MS, or CE. The content accepted depends on the nature of the drug produet and the dose.

Scrambled Forms. Scrambled forms are target protein molecules with a disulfide bond pattern different from that of the native molecule. Scrambled forms are typically formed during *in vitro* folding of proteins, but disulfide bond shuffling at neutral pH or above also occurs. This requires studies on control of protein stability during downstream processing. The formation of scrambled forms is closely linked to the folding procedure and is protein specific, which are detected by analytical HP-IEC, HP-RPC, CE, or peptide mapping. The content accepted depends on the nature of the drug product and the dose.

Cleaved Forms. Cleaved forms are those where a peptide bond is cleaved, resulting in loss of a N- or C-terminal site or where an internal peptide bond is cleaved while at the same time the resulting fragments are kept together by means of disulfide bonds. These forms are detected by N- and C- terminal amino acid analysis.

Aggregates. Aggregates are target protein derivatives in which two or more molecules are linked together either by covalent inter-disulfide bonds or by hydrophobic interaction. Target protein aggregates are formed as a result of hydrophobic intermolecular reactions or because of intermolecular disulfide bond formation under oxidizing conditions. Aggregates are very often antigenic, resulting in formation of antibodies against the target protein. Proteins exposed to even mildly denaturing conditions may partially unfold, resulting in the exposure of hydrophobic residues to the aqueous-solvent, favoring aggregation. It is generally believed that the aggregation process is controlled by the initial dimerization step in a second order reaction. Consequently, high protein concentrations will increase the aggregation rate. Intermolecular disulfide bond formation between cystinyl residues takes place at alkaline pH under oxidizing conditions. Proteins with reactive free thiol groups should be purified under reducing conditions (typically 1 to 10 mM reducing agent) in the presence of EDTA. Even proteins with disulfide bonds may participate in intermolecular disulfide bond reactions due to disulfide bond shuffling at neutral and alkaline pH. The aggregation reaction based on intermolecular disulfide bond formation is prevented at pH < 6 and under reducing conditions. The hydrophobic aggregation compounds,

enzymes, detergents, and stabilizers must be accounted for and their removal validated. Large amounts of hydrophobic reagents may affect hydrophobic interaction chromatography. Analytical HP-IEC, HP-RPC, native PAGE, IEF, MS, or CE detects oxidized forms. The content accepted depends on the nature of the drug product and the dose. Non-reducing 1D-SDS, HP-SEC, MS, or CE can detect disulfide-based aggregates. Hydrophobic aggregates may be detected by HP-SEC. The content accepted depends on the nature of the drug product and the dose as specified in the product monographs.

Process Related Impurities

Pyrogens and Endotoxins

Pyrogens are a group of chemically diverse substances, including endotoxins of bacteria and debris of dead bacterial cells that cause fever and shock in severe cases. The most important pyrogenic substances in pharmaceutical industry are bacterial endotoxins. Endotoxins come from Gram-negative bacteria (e.g., *E. coli*), if it is used as the expression system. Presence of endotoxins indicates bacterial contamination in raw materials, columns, water, and buffers. There are two methods of detection: the pyrogen test, which is based upon the measurement of body temperature of rabbits before and after injection of the specimen, and the Limulus Amebocyte lysate (LAL) test, which is based upon the clotting reaction of an enzyme complex of cells of the horseshoe crab together with bacterial endotoxins (*in vitro* test).

Nucleic acids

Nucleic acid contamination comes from host cell DNA/RNA or retroviral RNA. Nucleic acids are detected by monitoring absorption of light at 260 nm. The residual content in drug substance (DS) or drug product (DP) is usually measured by PCR or amplification techniques. The maximum allowable content of nucleic acid per dose remains under continuous evaluation by regulatory agencies. "Lot-to-lot" testing for DNA content in biological products produced in cell lines should be performed and lot release limits established which reflects a level of purity that can be achieved reasonably and consistently.

Host cell proteins

Host cell proteins (HCP) come from the host organism and constitute a major purification problem due to variability structure and surface properties. The amount released into the culture medium depends on the expression system used, the culture conditions and the process related cell lysis. Extraneous proteins may be introduced in the downstream process also. Several analytical methods have been used to monitor HCP including SDS-PAGE, 2D-electrophoresis (IEF in combination with SDS-PAGE), Western blot (WB), and immunoassays. However, Generic HCP assays are preferred for lot release. If the assays are based on competently produced antibodies raised against cell lysates, the quality may be adequate

Viruses

Virus contamination comes from the host cell, the culture medium, and infections during manufacture. The host cell may contain a genomic virus or virus vectors used to transform the cell line. The type of viral genome or vector depends on the cell line history. Chronic or latent viruses may be present in continuous cell lines, and the retroviruses associated with continuous cell lines are non-infectious, but oncogenic. Epstein-Barr virus or Sendai virus is often used for cell transformations. Contaminants such as BVDV, IBR, reovirus, PI-3, bovine leukemia virus, and bovine polyoma virus should be expected from serum-supplemented media. The cell line history reveals all information on the origin and identity of the cell line and the host genome vectors used to establish the cell line

Prions

Prions come from transmissible spongiform encephalopathies (TSE), The major source of contamination of a recombinant product is the use of animal-derived raw materials, which could harbor bovine prions (BSE agent). Currently, there are no assays that are sensitive or specific enough to test raw materials or sources, and the only reliable prevention is to include barriers, such as avoidance of animal or human raw materials (e.g., trypsin, serum, transferin, bovine/human serum albumin, protein supplements, peptones).

Microbial agents including Mycoplasma

Microbial agents and fungi come from infection of the bioreactor during cell culture. Other sources are contaminated water, buffers, raw materials, chromatographic columns, and equipment. Fermentation and cell culture bioreactors are prone to microbial infections. Viable cells can be identified by spread out of the cell suspension or sample solution on agar plates.

Mycoplasma

Mycoplasmas have for long been recognized as a contaminant of continuous cell cultures caused by an infection of the cell line or bioreactor. Working in closed systems under GMP will reduce the *risk of infection.* The end of production test includes screening for mycoplasmas. Mycoplasmas are difficult to detect, the only reliable way of demonstrating infection is by agar plating, fluorescent dying of DNA, or by PCR

Quantity

Protein content

Quantitative estimation of protein is measured by several methods (*Kjeldahl analysis, Lowry assay, Biuret assay,*

Bicinchoninic acid (BCA) assay, Bradford assay, UV absorbance and amino acid analysis). It is desirable to confirm the quantity by more than one method.

Stability

Formulation development of biopharmaceutical therapeutic protein will provide a final dosage form that offers *ex vivo* stability during processing, handling, and long-term storage under specified conditions (e.g., temperature, humidity etc). It is also expected to provide adequate *in vivo* bioavailability that meets the desired pharmacokinetic/pharmacodynamic (PK/PD) properties. Therapeutic Proteins do not survive terminal sterilization procedures commonly employed for small therapeutic molecule. Therefore, formulation components and excipients should also undergo microbiological tests including analysis for endotoxins and pyrogens in addition to biological, chemical and physical functions.

Erythropoietin Concentrated Solution

APPRLICDSR	VLERYLLEAK	EAENITTGCA
EHCSLNENIT	VPDTKVNFYA	WKRMEVGQQA
VEVWQGLALL	SEAVLRGQAL	LVNSSQPWEP
LQLHVDKAVS	GLRSLTTLLR	ALGAQKEAIS
PPDAASAAPL	RTITADTFRK	LFRVYSNFLR
GKLKLYTGEA	CRTGD	

Structure of Erythropoietin Mol. Wt. 30,600 (approx)

Erythropoietin Concentrated Solution contains a family of closely related glycoproteins which are not different from the naturally occurring human erythropoietin (urinary erythropoietin) in terms of the amino acid sequence (165 amino acids) and average glycosylation pattern, at a concentration of 0.5 mg per ml to 10 mg per ml. It has a potency of not less than 100000 IU per mg of active substance.

Production

Erythropoietin is produced in rodent cells *in vitro* by a method based on recombinant DNA technology. All batches are tested as described below, prior to release (unless exemption has been granted by the competent authority).

Host cell-derived proteins (HCP)

The limit is as prescribed by WHO.

Host cell-derived proteins. This must be routinely monitored using a scientifically accepted method that demonstrates:

1. The assay is sensitive (a useful target for the limit of detection is 1 to 100 ppm)
2. The assay is specific for the HCP (defined by proprietary detection reagents such as antibodies elicited against process-specific contaminating HCP) consistently found in the product from a specific manufacturing / purification process.

Limits are approved by the competent authority.

Host cell and vector-derived DNA

The limit is as prescribed by the competent authority.

Description. A clear and colourless solution.

Identification

A. It gives the appropriate response when examined using the conditions described under Assay.

B. Determine by isoelectric focusing (2.4.33).

Test solution. Dilute, the preparation in *water,* if necessary and desalt the preparation, using a membrane filtration system suitable for desalting proteins. Make up the volume to the original volume with *water.*

Reference solution. Dissolve erythropoietin RS in water to produce a solution containing 1mg per ml and desalt as above.

The isoelectric focusing procedure may be carried out using a 0.5 mm thick polyacrylamide slab gel containing ampholytes covering the pH of range 3 to 10, prepared as follows.

Mix 9 g of *urea*, 6.0 ml of *30 per cent acrylamide/bisacrylamide solution,* 1.05 ml of pH 3 to 5 ampholyte, 0.45 ml of pH 3 to 10 ampholyte and 13.5 ml of *water*. Degas the mixture . Add 15 µl of *tetramethylethylene diamine* and 0.3 ml of a 100 g per litre freshly prepared solution of *ammonium persulphate.*

Pour into a suitable gel cassette, with approximate dimensions of 15 cm x 15 cm x 0.05 cm. Insert a suitable sample well comb and allow to polymerize.

Use the anode solution the *anolyte for isoelectric focusing at pH 3 to 5* and the cathode solution the *catholyte for isoelectric focusing pH 3 to 5.* Allow prefocusing to take place for 1 hour at a constant power of 10 W, with maximum voltage and current settings of 2000 V and 100 mA, respectively.

Dilute the test solution to 0.5 mg per ml with *water.* Apply to the gel 10 µl of each solution. Carry out focusing for a further 30 minutes at the same power supply settings. Take the gel out of the focusing chamber, and transfer the proteins onto a membrane suitable for immobilization of proteins (such as Polyvinylidene Fluoride), using commercially available

electrotransfer equipment and following the manufacturer's instructions. After electrotransfer, incubate the membrane in a neutral isotonic buffer containing a suitable blocking agent (for example, 50 g per litre of dried milk), for 1 hour, followed by incubation in the same blocking solution with a suitable dilution of a polyclonal anti-erythropoietin antibody. Detect the erythropoietin-bound antibody using a suitable enzyme- or radiolabelled antibody (for example, an alkaline phosphatase-conjugated second antibody). The precise details of blocking agents, concentrations and incubation times should be optimized using the principles set out in Immunochemical methods.

The test is not valid unless the distribution of bands in the electrophoretogram obtained with the reference solution contains at least 6 well separated bands. If necessary, the voltage settings and duration may be altered to optimize the separation of the isoforms.

In the electrophoretogram obtained with the test solution, the pH range of the bands observed corresponds to that of the electrophoretogram obtained with the reference solution. The predominant bands correspond to isoforms 4, 5, 6 and 7. Additional, fainter bands corresponding to isoforms 1, 2, 3 and 8 may also be present. Other bands are present in no more than trace amounts.

C. Determine by capillary electrophoresis (capillary zone electrophoresis) (2.4.32).

All the solutions should be filtered through a 0.45 µm membrane filter before use.

Test solution. Dilute the substance under examination with *water* or concentrate it to obtain a concentration of 1 mg per ml. Desalt 0.25 ml of the solution by passage through a micro-concentrator cartridge provided with a membrane with a molecular mass cut-off not more than 10000. Add 0.2 ml of *water* to the sample and desalt again. Repeat the desalting procedure once more. Dilute the sample with *water*, determine its protein concentration as described under Tests and adjust to a concentration of approximately 1 mg per ml with *water*.

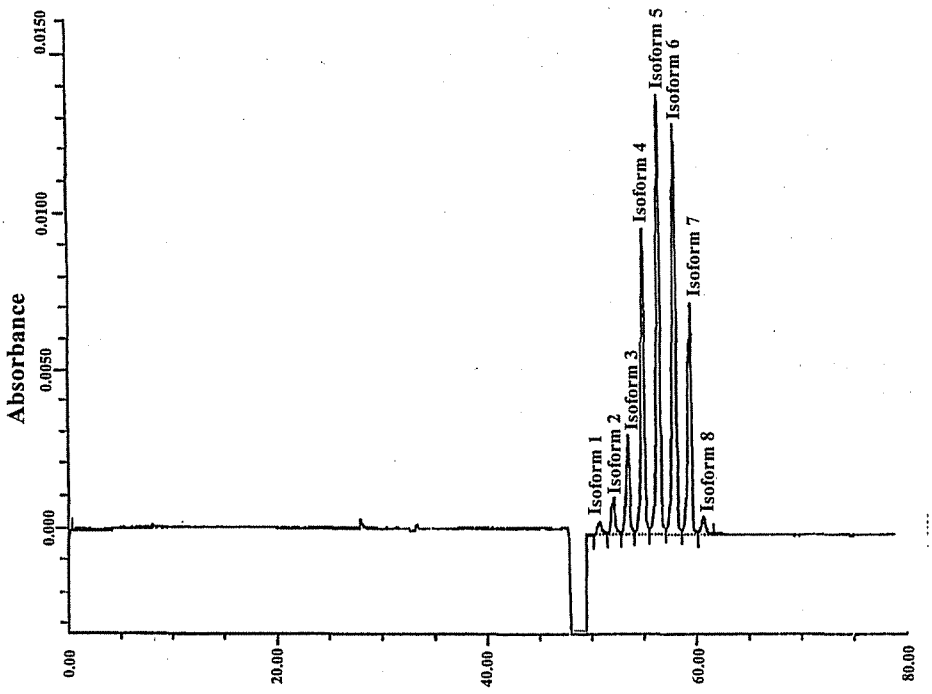
Reference solution. Dissolve *erythropoietin RS* in *water* to produce a solution containing 1 mg per ml. Desalt the sample as described for the test solution.

Capillary system

- material. uncoated fused silica,
- size. effective length = about 100 cm, internal diameter = 50 µm,
- temperature. 35°,
- spectrophotometer set at 214 nm,
- injection. under pressure or vacuum.

CZE buffer concentrate (0.1 M sodium chloride, 0.1 M tricine, 0.1 M sodium acetate). Dissolve 0.584 g of *sodium chloride*, 1.792 g of *tricine* and 0.820 g of *anhydrous sodium acetate* in *water* and dilute to 100.0 ml with the same solvent.

1 M putrescine solution. Dissolve 0.882 g of *putrescine* in 10 ml of *water*. Distribute in 0.5 ml aliquots.



Reference electropherogram of Erythropoietin

CZE buffer. (0.01 M tricine, 0.01 M sodium chloride, 0.01 M sodium acetate, 7 M urea, 2.5 mM putrescine). Dissolve 21.0 g of *urea* in 25 ml of *water* by warming in a water-bath at 30°. Add 5.0 ml of *CZE buffer concentrate* and 125 ml of *1 M putrescine solution*. Dilute to 50.0 ml with *water*. Using *dilute acetic acid*, adjust the pH to 5.55 at room temperature and filter through a 0.45 µm membrane filter.

Set the auto sampler to store the samples at 4° during analysis.

Preconditioning of the capillary. Rinse the capillary for 60 minutes with *0.1 M sodium hydroxide* filtered through a 0.45 µm membrane filter and for 60 minutes with *CZE buffer*. Apply voltage for 12 hours (20 kV).

Between-run rinsing. Rinse the capillary for 10 minutes with *water*, for 5 minutes with *0.1 M sodium hydroxide* filtered through a 0.45 µm membrane filter and for 10 minutes with *CZE buffer*.

Migration. Apply a field strength of 143 V/cm (15.4 kV for capillaries of 107 cm total length) for 80 min, using *CZE buffer* as the electrolyte in both buffer reservoirs.

System suitability. In the electropherogram obtained with the reference solution, a pattern of well-separated peaks corresponding to the peaks in the *reference electropherogram of erythropoietin RS* is seen, and the largest peak is atleast 50 times greater than the baseline noise. If necessary, adjust the sample load to give peaks of sufficient height. Identify the peaks corresponding to isoforms 1 to 8. The peak corresponding to isoform 8 is detected; the resolution between the peaks corresponding to isoforms 5 and 6 is not less than 1. Repeat the separation at least 3 times. The baseline is stable, showing little drift, and the distribution of peaks is qualitatively and quantitatively similar to the distribution of peaks in the *reference electropherogram of erythropoietin RS*. The relative standard deviation of the migration time of the peak corresponding to isoform 2 is less than 2 per cent.

Identify the peaks corresponding to isoforms 1 to 8 in the electropherogram obtained with the test solution by comparison with the electropherogram obtained with the reference solution. Calculate the percentage content of each isoform from the corresponding peak area. The percentages are within the following ranges:

Isoform Number	Content (per cent)
1	0–15
2	0–15
3	1–20
4	10–35
5	15–40
6	10–35
7	5–25
8	0–15

D. Immunoblotting. Determine by electrophoresis (sodium dodecyl sulphate polyacrylamide gel electrophoresis) (SDS-PAGE) (2.4.12).

Use a 1-mm thick spacer with six-well comb, a well capacity of 45 l and suitable plate size e.g. 100 x 120 mm

Assemble the gel casting as recommended by the manufacturer.

Use the composition of gel given below:

— Separating gel 12 per cent

— Add the reagents into a clean glass/plastic container in the same sequence as given in the table below:

Solutions	For 7 ml (for 1 gel)	For 14 ml (for 2 gels)
Water	2.16 ml	4.32 ml
Acrylamide (30 per cent)	2.8 ml	5.6 ml
1.5 M Tris-Cl pH.8.8	1.75 ml	3.4 ml
20 per cent SDS	35 ml	70 ml
Ammonium persulphate (10 per cent w/v)	70 ml	140 ml
TEMED	3 ml	6 ml

Mix the above contents by swirling gently (do not mix vigorously) in the container and pour into the casting unit using a 1-ml pipette to 1 to 1.5 cm from the top edge of the plate. After addition of *ammonium persulphate* and *TEMED*, mix the solution and pour quickly (in less than 1 minute) or it will begin to polymerize. Pour 200 to 1000 µl of *water-saturated butanol* on the top of separation gel. Keep aside for at least 45 minutes for polymerization at room temperature.

Casting the Stacking gel: 4 per cent

Decant the water-saturated butanol and rinse the separating gel with *water*. (If the gel has not polymerized and flows out, discard and prepare fresh). Place the comb in position above the separating gel. Prepare the stacking mix by adding the reagents in the same sequence as given in the table below:

Solutions	For 5 ml (1 gel)	For 10 ml (2 gels)
Water	1.8 ml	3.6 ml
Acrylamide (30 per cent)	0.6 ml	1.2 ml
0.5 M Tris Cl pH. 6.8	2.5 ml	5.0 ml
20 per cent SDS	25 µl	50 µl
Ammonium persulphate (10 per cent w/v) (APS)	50 µl	100 µl
TEMED	5 µl	10 µl

Mix the above ingredients by swirling gently and pour over the separating gel. Avoid bubbles. Keep aside for at least 45 minutes for polymerization at room temperature.

Preparation of samples and loading

Samples include:

1.	*Test sample*	1.0 µg
2.	*Reference standard RS*	1.0 µg
3.	Prestained Marker	20 µl

Test solution. Take X µl of the test sample and add an equal volume of *2X non-reducing sample buffer* where,

X (µl) = 1 µg per (concentration of the test sample in mg per ml)

Reference solution. Dilute a part of 1mg per ml *reference standard RS* stock 10 times to get a final concentration of 0.1 mg per ml with *water* as follows:

To 10 µl of 1 mg per ml *reference standard RS* solution add 90 ml *water.*

To 10 µl of 0.1 mg per ml *reference standard RS* solution add 10 ml of 2X *non-reducing sample buffer.*

Prestained marker. Take 20 ml of prestained marker, reconstituted as recommended by the manufacturer.

Loading of samples.

Boil the samples for two minutes, centrifuge, bring to room temperature and load the entire volume on the gel.

Sample No.	Samples	Amount of protein loaded/well (µg)	Total Vol. to be loaded. (µl)
1	Test Sample	1	–
2	Reference	1	20
3	Marker	–	20

Running the gel.

1. Fix the gel apparatus in the running unit according to the manufacturer's instructions.
2. Chill the *1X running buffer* to 4° for at least 1 hour.
3. Pour *1X running buffer* in the upper as well as lower chambers of the running unit.
4. Set parameters on the Power pack as follows:
   - — Constant Voltage: 130 V
   - — Max Current: 200 mA.
5. Run until dye front just goes out at the gel bottom (usually about 1.5 hrs)

Transfer, blotting and development of membrane:

1. Disassemble the running unit according to the manufacturer's instructions.
2. Cut Nitrocellulose Membrane (NCM) having the same dimensions as of the gel.
3. Equilibrate the membrane in chilled *transfer buffer* for 15 – 20 minutes.
4. Equilibrate the gel in chilled *transfer buffer* for 15 – 20 minutes.
5. Soak the nylon pads in *transfer buffer* and place on the pad on the cathode.
6. Take 5 sheets of a suitable filter paper, cut to the size of the gel and soak in chilled *transfer buffer*. Place them on the nylon pad placed on cathode plate.
7. Carefully place the equilibrated gel on the filter papers.
8. Place the equilibrated membrane onto the gel.
9. Roll a clean glass rod dipped in *transfer buffer* on top of the membrane to get rid of any air bubbles trapped between the gel and the membrane.
10. Place 5 sheets of a suitable filter paper soaked in chilled transfer buffer over the membrane. Once again roll the glass rod to remove any air bubbles.
11. Close the cassette.
12. Place the cassette in the running unit.
13. Connect the electrodes with the Power pack and set following parameters for transfer:
    - — Current: 200 mA
    - — Voltage: 100 V
    - — Time: 1 hour.
14. After the transfer is over, disassemble the cassette.
15. Transfer the membrane to the *blocking solution.* Incubate the membrane in the *blocking buffer* for 1 hour with gentle shaking at room temperature.
16. Discard the *blocking buffer.* Add 25 ml of *rabbit anti r-Hu EPO antibody* (Primary antibody) solution.
17. Incubate for 1hour with gentle agitation at room temperature.
18. Wash the membrane with *1 X transfer buffer saline* with change of buffer in-between at room temperature with gentle agitation (3 times X 5 minutes).
19. Discard the *transfer buffer saline.* Add 25 ml of *goat anti-rabbit ALP conjugated antibody* (secondary antibody) solution. Incubate for 1 hour with gentle shaking at room temperature.
20. Wash the membrane with *1 X transfer buffer saline* (3 times X 5 minutes)
21. Develop the color by adding 5 ml of the *substrate solution* (Usually it takes 15 – 20 minutes).
22. Stop the reaction by pouring off the *substrate solution* and washing it with water before the color of the background gets dark.
23. Scan the blot while it is wet.
24. Air dry the blot and preserve it.

The molecular mass markers are resolved on the membrane into discrete bands with a linear relationship between distance migrated and logarithm$_{10}$ of the molecular mass.

To evaluate the linear relationship between the distance migrated and logarithm$_{10}$ of the molecular mass, calculate

a) log molecular weights corresponding to marker bands
b) migration distance of protein band
c) plot (a) vs (b) and perform linear regression analysis

The graph should be linear

The single broad band of the test solution and of *reference standard RS* match in position and intensity.

Peptide mapping (2.3.47).

Test solution. Dilute the substance under examination in *tris-acetate buffer solution pH 8.5* to a concentration of 1.0 mg per ml. Equilibrate the solution in *tris-acetate buffer solution pH 8.5* using a suitable procedure (such as dialysis against *tris-acetate buffer solution pH 8.5*, or membrane filtration using the procedure described under Identification C, but reconstituting the desalted sample with *tris-acetate buffer solution pH 8.5*). Transfer the dialyzed solution to a polypropylene centrifuge tube. Freshly prepare a solution of *trypsin for peptide mapping* at a concentration of 1 mg per ml in *water*, and add 5 ml to 0.25 ml of the dialysed solution. Cap the tube and place in a water-bath at 37° for 18 hours. Remove the sample from the water-bath and stop the reaction immediately by freezing.

Reference solution. Dissolve the contents of a vial of *erythropoietin RS* in 0.25 ml of *water*. Prepare as for the test solution, ensuring that all procedures are carried out simultaneously, and under identical conditions.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with butylsilyl silica gel (5-10 µm),
- mobile phase: A. 0.06 per cent v/v solution of *trifluoroacetic acid*,
  B. to 100 ml of *water* add 0.6 ml of *trifluoroacetic acid* and dilute to 1000 ml with *acetonitrile*,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 214 nm,
- injection volume. 50 µl.

Time (in min)	Flow rate (ml/min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0–10	0.75	100	0
10–125	0.75	100 →39	0 → 61
125–135	1.25	39 →17	61→83
135–145	1.25	17 → 0	83 →100
145–150	1.25	100	0

Equilibrate at initial conditions for at least 15 minutes. Carry out a blank run using the above-mentioned gradient.

The chromatogram obtained with each solution is qualitatively similar to the *reference chromatogram of erythropoietin RS digest*.

The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

F. Determine by *N*-terminal sequence analysis

The first 15 amino acids are: Alanine - Proline - Proline - Arginine - Leucine - Isoleucine - (no recovered peak) - Aspartic acid - Serine - Arginine - Valine - Leucine - Glutamic acid - Arginine - Tyrosine.

Perform the Edman degradation using an automated solid-phase sequencer, operated in accordance with the manufacturer's instructions.

Desalt the equivalent of 50 µg of erythropoietin. For example, dilute a volume of the substance under examination containing 50 µg of the active substance in 1 ml of a 0.1 per cent v/v solution of *trifluoroacetic acid.* Pre-wash C18 reverse-phase sample preparation cartridge according to the instructions supplied by the manufacturer and equilibrate the cartridge in a 0.1 per cent v/v solution of *trifluoroacetic acid.* Apply the sample to the cartridge, and wash successively with a 0.1 per cent v/v solution of *trifluoroacetic acid* containing 0 per cent, 10 per cent and 50 per cent v/v of *acetonitrile* according to the manufacturer's instructions. Lyophilise the 50 per cent v/v *acetonitrile* eluate.

Redissolve the desalted sample in 50 µl of a 0.1 per cent v/v solution of *trifluoroacetic acid* and couple to a sequencing cartridge using the protocol provided by the manufacturer. Run 15 sequencing cycles, using the reaction conditions for proline when running the second and third cycles.

Identify the phenylthiohydantoin (PTH)-amino acids released at each sequencing cycle by reverse-phase liquid chromatography. The procedure may be carried out using the column and reagents recommended by the manufacturer of the sequencing equipment for the separation of PTH-amino-acids.

The separation procedure is calibrated using:

— the mixture of PTH-amino acids provided by the manufacturer of the sequencer, with the gradient conditions adjusted as indicated to achieve optimum resolution of all amino acids,
— a sample obtained from a blank sequencing cycle obtained as recommended by the equipment manufacturer.

Tests

Protein. 80 per cent to 120 per cent of the stated amount.

Test solution. Dilute the substance under examination in a 0.4 per cent w/v solution of *ammonium hydrogen carbonate or the appropriate blank solution.*

When examined in the range 250 nm to 400 nm (2.4.7), the solution shows an absorption maximum between 276 nm and 282 nm. After correction of any light scattering measured up to 400 nm, calculate the content of erythropoietin taking the specific absorbance to be 7.43.

Dimers and related substances of higher molecular mass

A. Determine by size-exclusion chromatography (2.4.16).

Test solution. Dilute the substance under examination in the mobile phase to obtain a concentration of 0.2 mg per ml.

Reference solution. To 0.02 ml of the test solution add 0.98 ml of the mobile phase (2 per cent).

Chromatographic system

- a stainless steel column 60 cm x 7.5 mm, packed with hydrophilic silica gel, of a grade suitable for fractionation of globular proteins in the molecular mass range of 20 000 to 200 000,
- mobile phase: Dissolve 1.15 g of *anhydrous disodium hydrogen phosphate*, 0.2 g of *potassium dihydrogen phosphate* and 23.4 g of *sodium chloride* in 1 litre of *water (1.5 mM potassium dihydrogen phosphate, 8.1 mM disodium hydrogen phosphate, 0.4 M sodium chloride, pH 7.4)*; adjusted to pH to 7.4, if necessary,
- flow rate. 0.5 ml per minute,
- spectrophotometer at 214 nm,
- injection volume. 100 µl.

Inject the test solution and the reference solution. Continue the chromatography for 1 hour. The area of the principal peak in the chromatogram obtained with the reference solution is 1.5 to 2.5 per cent of the area of the principal peak in the chromatogram obtained with the test solution. The total area of any peaks eluted before the principal peak is not more than the area of the principal peak in the chromatogram obtained with the reference solution (2 per cent).

B. Determine by electrophoresis (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12).

Casting the gels

— Use a 1-mm thick spacer with eight-well comb.

— The well capacity is 65 µl.

— Assemble the gel-casting unit according to the manufacturer's instructions.

— Use the assembly of suitable plate size e.g. 160 x 160 mm.

a) Casting the separating gel: 12 per cent

Add the reagents into a clean glass/plastic container in the same sequence as given in the table below:

Solutions	For 20 ml (1 gel)	For 40 ml (2 gels)
Water	6.17 ml	12.34 ml
Acrylamide (30 per cent)	8.0 ml	16.0 ml
1.5 M Tris Cl pH. 8.8	5.0 ml	10.0 ml
20 per cent SDS	100 µl	200 µl
Ammonium persulphate (10 per cent w/v) (APS)	200 µl	400 µl
TEMED	8 µl	16 µl

Mix the above ingredients by swirling gently and pour.

Mix the above contents by swirling gently (do not mix vigorously) in the container and pour into the casting unit using a 1-ml pipette till 1 - 1.5 cm from the top edge of the plate.

After addition of *ammonium persulphate* and *TEMED,* the solution should be mixed and poured quickly (less than 1 minute) or it will begin to polymerize.

Pour 200 – 1000 µl of *water-saturated butanol* on the top of the separation gel.

Keep aside for at least 45 minutes for polymerization at room temperature (RT).

b) Casting the Stacking gel: 4 per cent

Decant the *water-saturated butanol* and rinse the separating gel with *water.* (If the gel has not polymerized and flows out, discard and prepare fresh)

Place the comb in position above the separating gel.

Prepare the stacking mix by adding the reagents in the same sequence as given in the table below:

Solutions	For 5 ml (1 gel)	For 10 ml (2 gels)
Water	1.8 ml	3.6 ml
Acrylamide (30 per cent)	0.6 ml	1.2 ml
0.5 M Tris Cl pH. 6.8	2.5 ml	5.0 ml
20 per cent SDS	25 µl	50 µl
Ammonium persulphate (10 per cent w/v) (APS)	50 µl	100 µl
TEMED	5 µl	10 µl

Mix the above contents by swirling gently and pour over the separating gel. Avoid bubbles.

Keep aside for at least 45 minutes for polymerization at room temperature.

Preparation of samples

Samples include:

1.	Test sample	10 µg
2.	*Reference standard RS*	10 µg
3.	Marker for non-reducing gel	20 µl

Test solution. To a volume containing 10 µg of protein add an equal volume of *2X non-reducing sample buffer.*

Reference solution. Take 10 µl of the *reference standard RS* from 1mg per ml stock in a micro-centrifuge tube and add 10 µl of *2X non-reducing sample buffer.*

Molecular weight marker. Take 20 µl of low molecular weight markers for SDS-PAGE, which is reconstituted according to the manufacturer's instruction:

Sample loading

Keep all the samples in a boiling water-bath for 2 minutes. Centrifuge, bring to room temperature and load the entire volume on to the gel.

Sample No.	Samples	Amount of protein loaded/well (µg)	Total Vol. to be loaded. (µl)
1	Test Sample	1	–
2	Reference	1	20
3	Marker	–	20

Running the gel

Fix the gel apparatus in the running unit as given in the instruction manual of the manufacturer.

Chill *the 1X running buffer* to 4° for at least 1 hour.

Pour *1X running buffer* in the upper as well as lower chambers of the running unit.

Set parameters on the Power pack as follows:

Constant Voltage: 130 V

Max Current: 200 mA.

Run until the dye front reaches the bottom of the gel (usually about 5.0 hrs. The dye front should have run at least 80 per cent of the gel).

Staining of Gel

Stop the run. Disassemble the casting unit and transfer the gel carefully into a staining tray. Do not touch the gel with naked hands; wear gloves while handling the gel.

Detect proteins in the gel by commassie stain.

Scanning

Scan and save the image of the stained gel.

Gel drying

The stained gel is placed in between two cellophane sheets and clamped with the gel dryer frames (taking care that no air bubbles are present in between the two cellophane sheets).

The set up is placed in the gel dryer apparatus and left at least 2 hrs for drying.

The dye front is run for at least 80 per cent of the total gel length. Molecular weight markers are resolved on the gel into discrete bands, with a linear relationship between distance migrated and logarithm_{10} of the molecular mass.

To evaluate the linear relationship between distance migrated and logarithm_{10} of the molecular mass, calculate

a) log molecular weights corresponding to marker bands

b) migration distance of protein bands

c) plot (a) vs (b) and perform linear regression analysis.

The graph should be linear.

The single diffuse band of the test solution and of the reference solution match in position and intensity.

Sialic acids. Not less than 10 mol of Sialic acids (calculated as *N*-acetylneuraminic acid) per mole of erythropoietin, determined in the following manner.

Test solution (a). Dilute the preparation under examination in the mobile phase used in the test for dimers and related substances of higher molecular mass to obtain a concentration of 0.3 mg per ml.

Test solution (b). To 0.5 ml of test solution (a) add 0.5 ml of the mobile phase used in the test for dimers and related substances of higher molecular mass.

Reference solution (a). Dissolve a suitable amount of *N-acetylneuraminic acid* in *water* to produce a solution containing 0.1 mg per ml.

Reference solution (b). To 0.8 ml of reference solution (a) add 0.2 ml of *water.*

Reference solution (c). To 0.6 ml of reference solution (a) add 0.4 ml of *water.*

Reference solution (d). To 0.4 ml of reference solution (a) add 0.6 ml of *water.*

Reference solution (e). To 0.2 ml of reference solution (a) add 0.8 ml of *water.*

Reference solution (f). Use *water*

Carry out the test in triplicate. Transfer 100 ml each of the test and reference solutions to 10-ml glass test tubes. To each tube add 1.0 ml of *resorcinol reagent.* Stopper the tubes and incubate at 100° for 30 minutes. Cool on ice. To each tube, add 2.0 ml of a mixture of 12 volumes of *butanol* and 48 volumes of *butyl acetate.* Mix vigorously, and allow the 2 phases to separate. Ensuring that the upper phase is completely clear, remove the upper phase, taking care to exclude completely any of the lower phases. Measure the absorbance of all samples at 580 nm (2.4.7).

Using the calibration curve generated by the reference solutions, determine the content of sialic acids in each of the two test solutions and calculate the mean. Calculate the

number of moles of sialic acids per mole of erythropoietin assuming that the relative molecular mass of erythropoietin is 30,600 and that the relative molecular mass of *N*-acetylneuraminic acid is 309.

System suitability. The individual replicates agree to within ±10 per cent of each other; the value obtained from reference solution (a) is between 1.5 and 2.5 times that obtained with test solution (a).

Bacterial endotoxins (2.2.3). Not more than 20 Endotoxin Units in the volume that contains 100 000 IU of erythropoietin.

Assay. The activity of the preparation is compared with that of *erythropoietin RS* and expressed in International Units (IU).

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error of the estimated potency ($P = 0.95$) are not less than 64 per cent and not more than 156 per cent of the stated potency.

Determine by Method A or Method B.

A. *In polycythaemic mice*

The activity of the preparation is estimated by examining, under given conditions, its effect in stimulating the incorporation of ^{59}Fe into circulating red blood cells of mice made polycythaemic by exposure to reduced atmospheric pressure.

The following schedule, using treatment in a hypobaric chamber, has been found to be suitable.

Induce polycythaemia in female mice of the same strain, weighing 16 g to 18 g. Place the mice in a hypoxic chamber and reduce the pressure to 0.6 atmospheres. After 3 days at 0.6 atmospheres, further reduce the pressure to 0.4 to 0.5 atmospheres and maintain the animals at this pressure for a further 11 days (the partial vacuum is interrupted daily for a maximum of 1 hour at about 11:00 a.m., in order to clean the cages and feed the animals). At the end of the specified period, return the mice to normal atmospheric conditions. Randomly distribute the mice into cages, each containing 6 animals, and mark them.

Test solution (a). Dilute the substance under examination in *phosphate-albumin buffered saline pH 7.2* to obtain a concentration of 0.2 IU per ml.

Test solution (b). Mix equal volumes of test solution (a) and *phosphate-albumin buffered saline pH 7.2*.

Test solution (c). Mix equal volumes of test solution (b) and *phosphate-albumin buffered saline pH 7.2*.

Reference solution (a). Dissolve erythropoietin *RS* in *phosphate-albumin buffered saline pH 7.2* to obtain a concentration of 0.2 IU per ml.

Reference solution (b). Mix equal volumes of reference solution (a) and *phosphate-albumin buffered saline pH 7.2*.

Reference solution (c). Mix equal volumes of reference solution (b) and *phosphate-albumin buffered saline pH 7.2*.

Radiolabelled ferric [^{59}Fe] chloride solution, concentrated. Use a commercially available solution of [^{59}Fe] ferric chloride (approximate specific activity: 100-1000 MBq per mg of Fe).

Radiolabelled [^{59}Fe] ferric chloride solution. Dilute the concentrated radiolabelled [^{59}Fe] ferric chloride solution in *sodium citrate buffer solution pH 7.8* to obtain a solution with an activity of 3.7×10^4 Bq per ml.

The concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

3 days after returning the animals to atmospheric pressure, inject each animal subcutaneously with 0.2 ml of one of the solutions. The 6 animals in each cage must each receive one of the 6 different treatments (3 test solutions and 3 reference solutions), and the order of injection must be separately randomised for each cage. A minimum of 8 cages is recommended. 2 days after injection of the test or reference solution, inject each animal intraperitoneally with 0.2 ml of radiolabelled [^{59}Fe] ferric chloride solution. The order of the injections must be the same as that of the erythropoietin injections, and the time interval between administration of the erythropoietin and the radiolabelled ferric chloride solution must be the same for each animal. After a further 48 hours, anaesthetise each animal by injection of a suitable anaesthetic, record body weights and withdraw blood samples (0.65 ml) into haematocrit capillaries from the bifurcation of the aorta. After determining the packed cell volume for each sample, measure the radioactivity.

Calculate the response (percentage of iron-59 in total circulating blood) for each mouse using the expression:

$$\frac{A_s \times M \times 7.5}{A_t \times V_s}$$

Where, A_s = radioactivity in the sample,
A_t = total radioactivity injected,
7.5 = total blood volume as per cent body weight,
M = body weight, in grams,
V_s = sample volume.

Calculate the potency by the usual statistical methods for a parallel line assay. Eliminate from the calculation any animal where the packed cell volume is less than 54 per cent, or where the body weight is more than 24 g.

B. *In normocythaemic mice*

The assay is based on the measurement of stimulation of reticulocyte production in normocythaemic mice.

Test solution (a). Dilute the substance under examination in *phosphate-albumin buffered saline pH 7.2* to obtain a concentration of 80 IU per ml.

Test solution (b). Mix equal volumes of test solution (a) and *phosphate-albumin buffered saline pH 7.2.*

Test solution (c). Mix equal volumes of test solution (b) and *phosphate-albumin buffered saline pH 7.2.*

Reference solution (a). Dissolve *erythropoietin RS* in *phosphate-albumin buffered saline pH 7.2* to produce a solution containing 80 IU per ml.

Reference solution (b). Mix equal volumes of reference solution (a) and *phosphate-albumin buffered saline pH 7.2.*

Reference solution (c). Mix equal volumes of reference solution (b) and *phosphate-albumin buffered saline pH 7.2.*

The exact concentrations of the test solutions and reference solutions may need to be modified, based on the response range of the animals used.

At the beginning of the assay procedure, randomly distribute mice of a suitable age and strain (8-week old B6D2F1 mice are suitable. Other strains like Swiss Albino, Balb/C of suitable age can also be used) into 6 cages. A minimum of 8 mice per cage is recommended. Inject each animal subcutaneously with 0.5 ml of the appropriate treatment (one solution per cage) and put the animal in a new cage. Combine the mice in such a way that each cage housing the treated mice contains one mouse out of the 6 different treatments (3 test solutions and 3 reference solutions, 6 mice per cage). 4 days after the injections, collect blood samples from the animals and determine the number of reticulocytes using the following procedure.

The volume of blood, dilution procedure and fluorescent reagent may need to be modified to ensure maximum development and stability of fluorescence.

Colorant solution, concentrated. Use a solution of thiazole orange suitable for the determination of reticulocytes. Prepare at a concentration twice that necessary for the analysis.

Proceed with the following dilution steps. Dilute whole blood 500-fold in the buffer used to prepare the colorant solution. Dilute this solution 2-fold in the concentrated colorant solution. After staining for 3-10 min, determine the reticulocyte count microfluorometrically in a flow cytometer. The percentage of reticulocytes is determined using a biparametric histogram: number of cells/red fluorescence (620 nm).

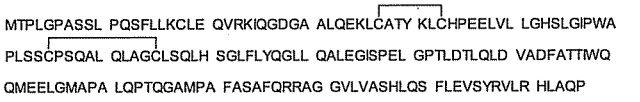
Calculate the potency by the usual statistical methods for a parallel line assay.

Storage. Store in an airtight container at a temperature below –20°. Avoid repeated freezing and thawing.

Labelling. The label states (1) the erythropoietin content in mg per ml; (2) the protein content in mg per ml; (3) the name and the concentration of any other excipients.

Filgrastim Concentrated Solution

Granulocyte Colony Stimulating Factor Solution



$C_{845}H_{1339}N_{223}O_{243}S_9$ Mol. Wt. 18799

Filgrastim Concentrated Solution is a solution of a protein having the structure of the granulocyte colony-stimulating factor (G-CSF) produced and secreted by various human blood cell types. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes. It is produced by a method based on rDNA technology, using bacteria as host cells.

Filgrastim Concentrated Solution contains not less than 0.9 mg per ml, and not less than 1.0×10^8 IU of filgrastim per mg of protein.

Prior to release, the following tests are carried out on each batch of the final bulk product, unless the regulatory authority has granted exemption.

Description. A clear, colourless to slightly yellowish liquid.

Identification

A. It shows the biological activity as decribed under Assay.

B. Determine by isoelectric focusing (2.4.33).

In the test for impurities with charges different from that of filgrastim the principal band in the electropherogram obtained with the test solution is similar in position to the principal band in the electropherogram obtained with the reference solution.

C. In the test for impurities of molecular masses higher than that of filgrastim, the retention time, of the principal peak obtained with the test solution is similar to that of the principal peak obtained with the reference solution.

D. In the test for impurities with molecular masses differing from that of rG-CSF under both reducing and non-reducing conditions, the principal band in the electropherogram obtained with test solution (a) is similar in position to the principle band in the electropherogram obtained with reference solution (a).

E. Determine by peptide mapping (2.3.47).

Test solution. Introduce 50 µl of a *0.05 M sodium phosphate buffer pH 8.0* into a polypropylene tube. Add a volume of the substance under examination corresponding to 25 µg of protein, add 25 µl of a 0.1 mg per ml solution of Glu-C2 protease; dilute to 1 ml with *water,* stopper the tube and incubate at

about 37° for 18 hours. Add 125 µl of a 7.64 per cent w/v solution of *guanidine hydrochloride* and mix well. Add 10 µl of a 1.542 per cent w/v solution of *dithiothreitol* and mix well. Place the capped tube in boiling *water* for 1 minute. Allow to cool to room temperature.

Reference solution. Prepare at the same time and in the same manner as for the test solution but use *G-CSF RS* instead of the test preparation under examination.

Chromatographic system

- a stainless steel column 10 cm x 2 mm, packed with octadecylsilyl silica gel (5 µm) with a pore size of 20 nm,
- column temperature. 60°,
- mobile phase: A. Dilute 0.5 ml of *trifluoroacetic acid* to 950 ml with *water* add 50 ml of *acetonitrile* and mix,
  B. Dilute 0.5 ml of *trifluoroacetic acid* to 50 ml with *water*; add 950 ml of *acetonitrile,*
- flow rate. 0.2 ml per minute,
- A linear gradient programme using the conditions given below,
- spectrophotometer set at 215 nm,
- injection volume. 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0-8	97-94	3-6
8-25	94-66	6-34
25-40	66-10	34-90
40-45	10	90
45-46	10-97	90-3
45-65	97	3

Equilibrate the column at the initial conditions for at least 30 minutes.

Inject the test solution and the reference solution.

The chromatograms obtained with the reference solution and the test solutions are qualitatively similar.

The profile of chromatogram obtained with the reference solution and the test solution corresponds to that of the chromatogram obtained with the reference solution.

Tests

Impurities with molecular masses differing from that of Filgrastim. Determine by electrophoresis (sodium dodecyl sulphate polyacrylamide gel electrophoresis) (2.4.12) under both reducing and non-reducing conditions.

Resolving gel. 13 per cent Acrylamide
Sample buffer A.
Sample buffer B (reducing conditions).

Test solution (a). Dilute the preparation under examination with sample buffer A to obtain a protein concentration of 100 µg per ml.

Test solution (b). To 0.20 ml of test solution (a) add 0.20 ml of sample buffer A.

Test solution (c). Dilute 0.20 ml of test solution (b) to 1.0 ml with sample buffer A.

Test solution (d). Dilute 0.20 ml of test solution (c) to 1.0 ml with sample buffer A.

Test solution (e). To 0.20 ml of test solution (d) add 0.20 ml of sample buffer A.

Reference solution. Solution of molecular mass markers suitable for calibrating SDS-polyacrylamide gels in the range of 14.4-94 kDa.

Sample treatment: boil for 5 minutes.

Apply 20 µl of each reduced and non-reduced solutions to separate gels.

Detection: Silver staining as described below.

Immerse the gel for 30 minutes in a mixture of 10 volumes of *acetic acid*, 40 volumes of *water* and 50 volumes of *methanol.* Transfer the gel to 5 per cent *methanol* and shake for 5 minutes.

Repeat this washing step thrice. Replace the 5 per cent v/v solution of *methanol* with 0.2 g per litre *sodium thiosulphate.* Wash the gel thrice in *water* for 30 seconds each. Transfer the gel to a 0.2 per cent w/v solution of *silver nitrate.*

This solution is prepared immediately before use. Place the gel on shaker for 25 minutes. Wash the gel for 1 minute. Repeat this washing step thrice. Transfer the gel into a mixture containing 30 g per litre solution of *sodium carbonate*, 0.05 per cent v/v solution of *formaldehyde* and 0.2 g per litre solution of *sodium thiosulphate* in *water*. Protein bands become visible during this step. Keep the gel in the solution until sufficiently stained and then stop the staining by soaking the gel in a 14 g per litre solution of *disodium edetate.*

The test is not valid unless the proteins of the molecular weight marker are distributed along 80 per cent of the gel and over the required separation range (the range covering the product and its dimer or the product and its related impurities); a band is seen in the electropherogram obtained with test solution (e), and a gradation of intensity is seen in the electropherograms obtained with solutions (a) to (e).

In the electropherogram obtained with test solution (a) no band is more intense that the principal band in the electropherogram obtained with reference solution (f).

Impurities with charges differing from that of Filgrastim. Determine by isoeloelectric focusing (2.4.33).

Test solution. Dilute the preparation under examination to produce a solution containing 0.3 mg per ml.

Reference solution (a). A solution of *filgrastim RS* containing 0.3 mg per ml.

Reference solution (b). A solution of *filgrastim RS* containing 0.03 mg per ml.

Reference solution (c). Use an isoelectric point (pI) calibration solution, in the pI range of 2.5-6.5, prepared according to manufacturer's instructions.

Focusing:
- pH gradient. 4.5 - 8.0,
- catholyte. *1 M sodium hydroxide*,
- anolyte: *0.04 M glutamic acid* in a 0.0025 per cent v/v solution of *phosphoric acid*,
- Application 20 µl.

Detection. Proceed as described in Isoelecric Focusing (2.4.33).

Detect the product and its dimer or the product and its related impurities.

In the electropherogram obtained with reference solution (c), relevant isoelectric point markers are distributed along the entire length of the gel.

In the electropherogram obtained with reference solution (a), the pI of the principal band is 5.7-6.3.

No band is more intense that the principal band in the electropherogram obtained with reference solution (b).

Related Proteins. Determine by liquid chromatography (2.4.14).

Test solution. Dilute the preparation under examination with mobile phase A to obtain a concentration of 0.3 mg per ml.

Reference solution (a). Dilute *G-CSFRS* with the same mobile phase to obtain a concentration of 0.3 mg per ml.

Reference solution (b). To 570 µl of reference solution (a), add 6.8 µl of a 0.45 per cent v/v solution of *hydrogen peroxide*; mix and incubate at 25° for 1hour, then add 2.5 mg of *methionine RS.*

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm) and a 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (20 µm),
- column temperature. 65°,
- mobile phase: A. dilute 1 ml of *trifluoroacetic acid* to 500 ml with *water* and add 499 ml of *acetonitrile*,
  B. dilute 1 ml of *trifluoroacetic acid* to 950 ml with *acetonitrile* and add 49 ml of *water*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 215 nm,
- injection volume. 50 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0 – 4	92	8
4 – 19	92 → 72	8 → 28
19 – 19.1	72 → 0	28 → 100
19.1 – 21	0	100
21 – 21.1	0 → 92	100 → 8
21.1 – 25	92	8

Inject the test solution and reference solutions (a) and (b).

Relative retention with reference to filgrastim (retention time = about 12 minutes.): oxidized filgrastim 2 = about 0.95.

The profile of the chromatogram obtained with reference solution (b) is similar to that of the chromatogram of oxidized filgrastim supplied with *filgrastim RS*. The chromatogram shows two peaks corresponding to oxidized filgrastim 1 and oxidized filgrastim 2 that elute before the principal peak, the second peak not being completely separated from the principal peak.

In the chromatogram obtained with the test solution, the area of any peak other than the principal peak is not greater than 2.0 per cent of the total area of all the peaks. The sum of the areas of any peaks other than the principal peak is not greater than 3.5 per cent of the total area of all of the peaks.

Dimers and Related Substance of Higher Molecular Mass. Determine by size-exclusion chromatography (2.4.16).

Solution A. Dissolve 4.1 g of *sodium acetate* in 400 ml of *water*, adjust to pH 4.0 with *acetic acid* and dilute to 500 ml with *water.*

Test solution. Dilute the preparation under examination with solution A to obtain a concentration of 0.4 mg per ml.

Reference solution. Dilute *filgrastim RS* with solution A to obtain a concentration of 0.4 mg per ml.

Resolution solution. Mix a sample of the reference solution for about 30 seconds using a vortex mixer.

Chromatographic system
- a stainless steel column 30 cm x 7.8 mm, packed with hydrophilic silica gel, of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 10,000 to 5,00,000,
- column temperature. 30°,
- mobile phase: dissolve 7.9 g of *ammonium hydrogen carbonate* in 1000 ml of *water* and adjust to pH 7.0 with *phosphoric acid*; dilute to 2000 ml with *water*,
- flow rate. 0.5 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume. 20 µl.

Relative retention times with reference to filgrastim monomer (retention time=about 19 minutes): aggregates = about 0.60; filgrastim oligomer 1= about 0.75; filgrastim oligomer 2= about 0.80; filgrastim dimer=about 0.85.

Inject the resolution solution. The retention time of filgrastim monomer is 17 minutes to 20 minutes. The resolution between the peaks due to filgrastim dimer and filgrastim monomer is not less than 4.0.

Calculate the content of dimer, oligomers and aggregates. The sum of the peaks with retention times less that that of the principal peak is not more than 2.0 per cent.

Bacterial endotoxins (2.2.3). Not more than 20 Endotoxin Units per mg of protein.

Assay. A. *Protein* - Determine by liquid chromatography (2.4.14) as described under the test for Related proteins.

Inject the test solution and reference solution (a).

Calculate the content of filgrastim.

B. *Potency*- Determination of the biological activity of rG-CSF concentrated solution is based on the stimulation of NFS-60 cells (murine myeloblastic cell line) or any other suitable myeloblastic cell line by rG-CSF.

The following method uses the conversion of tetrazolium bromide (MTT or any other suitable dye) as a staining method. Alternative methods of quantifying cell proliferation, such as measurement of intracellular ATP by luciferase bioluminescence have also been found suitable, and may be used as the assay readout, subject to appropriate validation.

NFS-60 cells (murine myeloblastic cell line) or any other suitable myeloblastic cell line are incubated with varying dilution of test and reference preparations of rG-CSF. They are then incubated with a solution of MTS, MTT, XTT or any other suitable dye. This cytochemical stain is converted by cellular dehydrogenases to a purple formazan product. The formazan is then measured spectrophotometrically. The potency of the test preparation is determined by comparison of the dilutions of the test preparation with the dilutions of the appropriate International Standard of rG-CSF or with a reference preparation calibrated in International Units, which yield the same response (50 per cent maximal stimulation).

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International standard is stated by the World Health Organization.

Add 50 µl of the dilution medium to all wells of a 96 –well microtitre plate. Add an additional 50 µl of this solution to the wells designed for blanks. Add 50 µl of each solution to be tested in triplicate (test preparation and reference preparation at a concentration of about 800 IU per ml, plus a series of 10 twofold dilutions to obtain a standard curve). Prepare a suspension of NFS-60 cells (murine myeloblastic cell line) or any other suitable myeloblastic cell line containing $7x10^5$ cells per ml immediately before use, add *2-mercaptoethanol* to a final concentration of 0.1 mM, and add 50 µl of the prepared cell suspension to each well, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36° to 38° for a minimum of 24 hours in a humidified incubator using 6 ± 1 per cent CO_2. 20 µl of a 5.0 g per litre sterile solution of *tetrazolium bromide* to each well and re-incubate for 4 hours. Estimate the quantity of formazan produced using a microtitre well plate reader at 490 nm.

Analyze the data by fitting a sigmoidal dose-response curve to the data obtained and by using a suitable statistical method, for example the 4-parameter or parallel line models.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P=0.95) of the estimated potency are not less than 74 per cent and not more than 136 per cent of the stated potency.

Storage. Store protected from light in a refrigerator (2° to 8°).

Labeling. The label states the content, in mg of protein per ml; the potency, in IU per mg of protein.

Interferon Alfa-2 Concentrated Solution

CDLPQTHSLG SRRTLMLLAQ MRX$_1$ISLFSCL KDRHDFGFPQ
EEFGNQFQKA ETIPVLHEMI QQIFNLFSTK DSSAAWDETL
LDKFYTELYQ QLNDLEACVI QGVGVTETPL MKEDSILAVR
KYFQRITLYL KEKKYSPCAW EVVRAEIMRS FSLSTNLQES
LRSKE

alfa-2a: $C_{860}H_{1353}N_{227}O_{255}S_9$ Mol. Wt.19,241

alfa-2b: $C_{860}H_{1353}N_{229}O_{255}S_9$ Mol. Wt.19,269

Interferon alfa-2 concentrated solution is a solution of an rDNA derived therapeutic protein which exhibits non-specific antiviral activity, at least in homologous cells. Interferon alfa-2 concentrated solution also exerts antiproliferative and immunomodulator activity. Two different types of alfa-2 interferon, varying in the amino acid residue at position 23, are found and are named as alfa-2a and alfa-2b.

Designation	Residue at position 23 (X_1)
alfa-2a	Lys
alfa-2b	Arg

This monograph applies to interferon alfa-2a and 2b concentrated solutions.

Interferon alfa-2 concentrated solution contains not less than 1.4×10^8 IU per mg of protein and not less than 2×10^8 IU of Interferon alfa-2 per ml.

Production

Interferon alfa-2 concentrated solution is produced by a method based on recombinant DNA technology using bacteria as host cells. It is produced under controlled conditions designed to minimise microbial contamination of the product.

Interferon alfa-2 concentrated solution complies with the following additional requirements.

Host-cell-derived proteins

The limit is approved by WHO guidelines.

Host-cell- or vector-derived DNA

The limit is approved by WHO guidelines.

Description. A clear,colourless or slightly yellowish liquid.

Identification

A. It shows the expected biological activity as described under Assay for potency.

B. Determine by isoelectric focusing (2.4.33).

Test solution. Dilute the preparation under examination with *water* to obtain a solution containing 0.5 mg protein per ml.

Reference solution. A 0.5 mg per ml solution of *interferon alfa-2 RS* in *water.*

Isoelectric point calibration solution pI range 3.0 to 10.0. Prepare and use according to the manufacturer's instructions.

Isoelectric focusing is carried out using either horizontal electrophoresis system or by vertical electrophoresis system as per the procedure described below or by any appropriate validated method.

Horizontal Electrophoresis

Select and use a suitable horizontal isoelectric focusing apparatus with facility for connecting a circulating bath chiller capable of maintaining 10°. Select gels for isoelectric focusing with a pH gradient from 3.5 to 9.5.

Use *phosphoric acid* as anode solution (98 g per litre *phosphoric acid*) and *1 M sodium hydroxide* as the cathode solution. Using filter paper apply 15 µl of the test solution and the reference solution to the gel close to the cathode.

Start the isoelectric focusing at 1500 V and 50 mA. Turn off the power after 30 minutes. Remove the application filters and reconnect the power supply for 1 hour. Keep the power constant during the focusing process.

Immerse the gel in a solution containing 115 g per litre of *trichloroacetic acid* and 34.5 g per litre of *sulphosalicylic acid* in *water* and agitate the container gently for 60 minutes.

Prepare a mixture of 32 volumes of *glacial acetic acid*, 100 volumes of *ethanol* and 268 volumes of *water*. Transfer the gel to the mixture and soak for 5 minutes.

Immerse the gel for 10 minutes in a staining solution prewarmed to 60°.The staining solution is prepared by adding 1.2 g per litre of *acid blue 83* to the mixture of *glacial acetic acid*, *ethanol* and *water.*

Wash the gel several times to destain with the mixture of *glacial acetic acid*, *ethanol* and *water* and keep the gel in this mixture for about 12-24 hours until the background is clear.

Add *glycerol*, 10 per cent v/v to the mixture of *glacial acetic acid*, *ethanol* and *water*. Soak the gel for 1 hour in the solution.

The principal bands of the electropherogram obtained with the test solution correspond in position to the principal bands of the electropherogram obtained with the reference solution. Plot the migration distances of the isoelectric point markers versus their isoelectric points and determine the isoelectric points of the principal components of the test solution and the reference solution. They do not differ by more than 0.2 pI unit. The test is not valid unless the isoelectric point markers are distributed along the entire length of the gel and the isoelectric points of the principal bands in the electropherogram obtained with the reference solution are between 5.8 and 6.3.

C. Examine the electropherograms obtained under reducing conditions in the test for impurities of molecular masses differing from that of interferon alfa-2. The principal band in the electropherogram obtained with test solution (a) corresponds in position to the principal band in the electropherogram obtained with reference solution (a).

D. Determine by peptide mapping (2.3.47).

Test solution. Dilute the preparation under examination with *water* to produce a solution containing 0.5 mg protein per ml. Transfer 25 µl to a microfuge tube of 1.5 ml capacity. Add 1.6 µl of *1 M phosphate buffer solution pH 8.0*, 2.8 µl of a freshly prepared 1.0 mg per ml solution of *trypsin* in *water* (suitable for peptide mapping) and 3.6 µl of *water* and mix vigorously. Cap the tube and place it in a water-bath at 37° for 18 hours. Add 100 µl of a 573 g per litre solution of *guanidine hydrochloride* and mix well. Add 7 µl of 154.2 g per litre solution of *dithiothreitol* and mix well. Place the capped tube in boiling water for 1 minute and cool to room temperature.

Reference solution. A 0.5 mg per ml solution of the appropriate *interferon alfa-2 RS* prepared at the same time and in the same manner as the test solution.

Chromatographic system

- a stainless steel column 100 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) with a pore size of 30 nm,
- mobile phase: A.1 ml of *trifluoroacetic acid* dilute to 1000 ml with *water*,

B. Add 1 ml of *trifluroacetic acid* to 100 ml of *water* and dilute to 1000 ml with *acetonitrile,*

- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 214 nm,
- injection volume. 100 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Comment
0.8	100	0	isocratic
8.68	100 → 04	0 → 60	linear gradient
68.72	40	60	isocratic
72.75	40 → 100	60 → 0	linear gradien
75.08	100	0	re-equilibration

Equilibrate the column with mobile phase A for at least 15 minutes maintaining the temperature of the column at 30°.

Inject the test solution and the reference solution. The chromatogram obtained with each solution should be qualitatively similar to the chromatogram of interferon alfa-2. The profile of the chromatogram obtained with the test solution should also correspond to that of the chromatogram obtained with the reference solution.

Tests

Impurities of molecular masses differing from that of interferon alfa-2. Determine by electrophoresis (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12). The test is performed under both reducing and non-reducing conditions, using resolving gels of 14 per cent *acrylamide* and silver staining as the detection method.

Sample buffer (non-reducing conditions). Mix equal volumes of *water* and *concentrated SDS-PAGE sample buffer.*

Sample buffer (reducing conditions). Mix equal volumes of *water* and concentrated *SDS-PAGE sample buffer for reducing conditions* containing *2-mercaptoethanol* as the reducing agent.

*Test solution (a).*Dilute the preparation under examination in the sample buffer to obtain a solution containing a concentration of 0.5 mg protein per ml.

Test solution (b). Dilute 0.2 ml of test solution (a) to 1 ml with the sample buffer.

Reference solution (a). Prepare a 0.625 mg per ml solution of the appropriate *interferon alfa-2 RS* in the sample buffer.

Reference solution (b). Dilute 0.2 ml of reference solution (a) to 1 ml with the sample buffer.

Reference solution (c). Dilute 0.2 ml of reference solution (b) to 1 ml with the sample buffer.

Reference solution (d). Dilute 0.2 ml of reference solution (c) to 1 ml with the sample buffer.

Reference solution (e). Dilute 0.2 ml of reference solution (d) to 1 ml with the sample buffer.

Reference solution (f). Use a solution of molecular mass standards suitable for calibrating SDS-PAGE gels in the range 14 kDa to 99 kDa.

Place the test and reference solutions, contained in covered test-tubes, on a water-bath for 2 minutes.

Apply 10 µl of reference solution (f) and 50 µl of each of the other solutions to the stacking gel wells. Perform the electrophoresis under the conditions recommended by the manufacturer of the equipment. Detect proteins in the gel by silver staining.

The test is not valid unless (1) the validation criteria are met; (2) a band is seen in the electropherogram obtained with reference solution (e); (3) a gradation of intensity of staining is seen in the electropherograms obtained, respectively, with test solution (a) and test solution (b) and with reference solutions (a) to (e).

The electropherogram obtained with test solution (a) under reducing conditions may show, additional bands but no such band should be more intense than the band obtained with reference solution (d). Further, not more than 3 such bands should be more intense than the principal band obtained with reference solution (e).

The electropherogram obtained with test solution (a) under non-reducing conditions may show, in addition to the principal band, less intense bands with molecular masses higher than the principal band. No such band is more intense than the principal band in the electropherogram obtained with reference solution (d) and not more than three such bands are more intense than the principal band in the electropherogram obtained with reference solution (e).

Related proteins. Determine by liquid chromatography (2.4.14).

0.25 per cent w/w hydrogen peroxide solution. Dilute *hydrogen peroxide solution* with *water* to obtain 0.25 per cent w/w solution.

Test solution. Dilute the preparation under examination with *water* to obtain a solution containing 0.5 mg protein per ml.

Reference solution. To a volume of the test solution, add a suitable volume of the 0.25 per cent *hydrogen peroxide solution* to give a final hydrogen peroxide concentration of 0.005 per cent, and allow to stand at room temperature for 1 hour, to generate about 5 per cent oxidised interferon. Add 12.5 mg of *L-methionine* per ml of the solution. Allow to stand at room temperature for 1 hour. Store the solutions for not longer than 24 hours at a temperature of 2° to 8°.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) with a pore size of 30 nm,
- mobile phase: A. To 700 ml of *water* add 2 ml of *trifluoroacetic acid* and 300 ml of *acetonitrile*,
  B. To 200 ml of *water* add 2 ml of *trifluoroacetic acid* and 800 ml of *acetonitrile*,
- flow rate. 1 ml per minute,
- a linear gradient programme using the conditions given below,
- spectrophotometer set at 210 nm,
- injection volume. 100 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Comment
0.1	72	28	isocratic
1.5	72 → 67	28 → 33	linear gradient
5 – 20	67 → 63	33 → 37	linear gradient
20 – 30	63 → 57	37 → 43	linear gradient
30 – 40	57 → 40	43 → 60	linear gradient
40 – 42	40	60	isocratic
42 – 50	40 → 72	60 → 28	linear gradient
50 – 60	72	28	re-equilibration

Equilibrate the column with the mobile phases in the initial gradient ratio for at least 15 minutes.

Inject alternatively the test solution and the reference solution.

Interferon alfa-2 elutes at a retention time of about 20 minutes in the chromatogram. With the reference solution a peak related to oxidized interferon appears at a retention time of about 0.9 relative to the principal peak.

The test is not valid unless the resolution between the peaks corresponding to oxidised interferon and interferon is at least 1.0. Consider only the peaks whose retention time is 0.7 to 1.4 relative to that of the principal peak.

In the chromatogram obtained with the test solution, the area of any peak, apart from the principal peak, is not greater than 3.0 per cent of the total area of all of the peaks. The sum of the areas of any peaks other than the principal peak is not greater than 5.0 per cent of the total area of all of the peaks.

Bacterial endotoxins (2.2.3). Not more than 100 Endotoxin Units per mg of protein.

Assay

A. Protein

Test solution. Dilute the preparation under examination with *water* to obtain a concentration of about 0.5 mg of interferon alfa-2 per ml.

Reference solutions. Prepare a stock solution of 0.5 mg per ml of *bovine albumin*. Prepare eight dilutions of the stock solution containing between 3 µg per ml and 30 µg per ml of *bovine albumin*.

Prepare 30-fold and 50-fold dilutions of the test solution.

Prepare a mixture of 2.0 ml of a 2.0 per cent w/v solution of *copper sulphate* in *water*, 2.0 ml of a 4.0 per cent w/v solution of *sodium tartrate* in *water* and 96.0 ml of a 4.0 per cent w/v solution of *sodium carbonate* in *0.2 M sodium hydroxide*.

Add 1.25 ml of the above mixture to the test-tube containing 1.5 ml of *water* to prepare the blank, 1.25 ml to the test tube containing different dilutions of the sample and 1.25 ml to the test tube with the reference solution.

Mix after each addition and after approximately 10 minutes, add to each test-tube 0.25 ml of a mixture of equal volumes of *water* and *phosphomolybdotungstic reagent*. Mix after each addition. After 30 minutes, measure the absorbance of each solution at 750 nm (2.4.7) using the blank as the compensation liquid.

Draw a calibration curve from the absorbances of the eight reference solutions and the corresponding protein contents and read from the curve the content of protein in the test solution.

B. Potency

The potency of interferon alfa-2 is estimated based on its ability to protect cells against a viral cytopathic effect compared to the protection accorded by an appropriate International Standard of human recombinant interferon alfa-2 or of a reference preparation calibrated in International Units.

The International Unit is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Carry out the assay by a suitable method, based on the following design.

Use, an established cell line sensitive to the cytopathic effect of a suitable virus, responsive to interferon.

The following cell cultures and virus have shown to be suitable: MDBK cells (ATCC No. CCL22), or Mouse L cells (NCTC clone 929; ATCC No.CCL 1) as the cell culture and vesicular stomatitis virus VSV, Indiana strain (ATCC No. VR-158) as the infective agent; or human diploid fibroblast FS-71 cells responsive to interferon as the cell culture, and encephalomyocarditis virus (ATCC No.VR-129B) as the infective agent.

Incubate in at least three groups, cells with three or more different concentrations of the preparation under examination and one with the reference preparation in a microtitre plate. Include appropriate controls of untreated cells in each group.

Choose the concentrations of the preparations such that the lowest concentration produces some protection and the largest concentration produces less than maximal protection against the viral cytopathic effect.

Add the cytopathic virus after the cells have established to all wells except the control wells.

Determine the cytopathic effect of virus quantitatively and calculate the potency of the preparation to be examined by the usual statistical methods for a parallel line assay.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits of the estimated potency (P= 0.95) are not less than 64 per cent and not more than 156 per cent of the stated potency.

Storage. Store protected from light, at or below –20°.

Labelling. The label states (1) The type of interferon (alfa-2a or alfa-2b); (2) the type of production.

Streptokinase Bulk Solution

Streptokinase Bulk Solution is a fibrinolytic enzyme present in certain strains of haemolytic Streptococcus group C. It has the property of combining with human plasminogen to form plasminogen activator. Streptokinase is also produced by a method based on recombinant DNA technology using bacteria or suitable genetically engineered host cells.

Streptokinase Bulk Solution has a potency of not less than 96,000 IU per mg of protein.

Production

If intended for use in the manufacture of parenteral preparations, the method of manufacture is validated to demonstrate that the product, if tested, would comply with the following test.

Abnormal toxicity

Inject into each mouse a quantity of the preparation under examination (diluted, if necessary, with water for injections) containing 50,000 IU of streptokinase activity in 0.5 ml, the injection lasting 15 to 20 seconds.

Description. A clear, colourless liquid.

Identification

A. Place 0.5 ml of *citrated human plasma* in a haemolysis tube maintained in a water-bath at 37°. Add 0.1 ml of a dilution of the preparation under examination containing 10,000 IU of streptokinase activity per ml in *phosphate buffer pH 7.2* and 0.1 ml of a solution of *human thrombin RS* containing 20 IU per ml in *phosphate buffer pH 7.2*. Mix immediately. A clot forms and lyses within 30 minutes. Repeat the procedure using *citrated bovine plasma*. The clot does not lyse within 60 minutes.

B. Dissolve 0.6 g of *agar* in 50.0 ml of *barbitone buffer pH 8.6*, heating until a clear solution is obtained. Use glass plates 50 mm square (transparency mounts) free from traces of grease. Using a pipette, apply to each plate 4 ml of the agar solution. Maintain the plates horizontal. Allow to cool. Pierce a cavity 6 mm in diameter in the centre of the agar and an appropriate number of cavities (not exceeding 6) at distances of 11 mm from the central cavity. Remove the residual agar from the cavities using a cannula connected to a vacuum pump. Using pipettes graduated in microlitres, place in the central cavity about 80 µl of goat or rabbit antistreptokinase serum containing 10,000 units of antistreptokinase activity per ml; place in each of the surrounding cavities about 80 µl of a dilution of the preparation under examination containing 125,000 IU of streptokinase activity per ml. Allow the plates to stand in a humidified tank for 24 hours. Only one precipitation arc appears and it is well defined and localised between the application point of the serum and each cavity containing the solution of the preparation under examination.

Tests

pH (2.4.24). 6.8 to 7.5, determined in a solution prepared by diluting the preparation under examination in *carbon dioxide-free water* to produce a solution containing 5000 IU of streptokinase activity per ml.

Streptodornase. Not more than 10 IU of streptodornase activity per 100,000 IU of streptokinase activity.

Test solution. Dilute the preparation under examination in *imidazole buffer pH 6.5* to obtain a solution containing 150,000 IU of streptokinase activity per ml.

Reference solution. Dissolve in *imidazole buffer pH 6.5* a reference preparation of streptodornase, calibrated in International Units against the International Standard of streptodornase, to obtain a solution containing 20 IU of streptodornase activity per ml. The equivalence in International Units of the International Standard is stated by the regulatory authority.

To each of 8 numbered centrifuge tubes, add 0.5 ml of a 0.1 per cent solution of *sodium deoxyribonucleate* in *imidazole buffer pH 6.5*. To tube number 1 and tube number 2 add 0.25 ml of *imidazole buffer pH 6.5*, 0.25 ml of the test solution and, immediately, 3.0 ml of 2.5 per cent w/v of *perchloric acid*. Mix, centrifuge at about 3000 rpm for 5 minutes and measure the absorbances of the supernatant liquids at 260 nm (2.4.7), using as the compensation liquid a mixture of 1.0 ml of *imidazole buffer pH 6.5* and 3.0 ml of 2.5 per cent w/v solution of *perchloric acid* (absorbances A1 and A2). To the other 6

tubes (numbers 3 to 8) add 0.25 ml, 0.25 ml, 0.125 ml, 0.125 ml, 0 ml and 0 ml respectively of *imidazole buffer solution pH 6.5*; add to each tube 0.25 ml of the test solution and 0 ml, 0 ml, 0.125 ml, 0.125 ml, 0.25 ml and 0.25 ml respectively of the reference solution. Mix the contents of each tube and heat at 37° for 15 minutes. To each tube add 3.0 ml of 2.5 per cent w/v of *perchloric acid*, mix and centrifuge. Measure the absorbances of the supernatant liquids at 260 nm (2.4.7), using the compensation liquid described above (absorbances A3 to A8). The absorbances comply with the following test.

$$(A3+A4)-(A1+A2)<\frac{(A5+A6+A7+A8)}{2}-(A3+A4)$$

Streptolysin

In a haemolysis tube, use a quantity of the preparation under examination containing 500,000 IU of streptokinase activity and dilute to 0.5 ml with a mixture of 1 volume of *phosphate buffer pH 7.2* and 9 volumes of a 0.9 per cent w/v solution of *sodium chloride*. Add 0.4 ml of a 2.3 per cent w/v solution of *sodium thioglycollate*. Heat in a water-bath at 37° for 10 minutes. Add 0.1 ml of a solution of a reference preparation of human antistreptolysin O containing 5 IU per ml. Heat at 37° for 5 minutes. Add 1 ml of rabbit erythrocyte suspension. Heat at 37° for 30 minutes. Centrifuge at about 1000 rpm. In the same manner, prepare a haemolysis tube in which the solution of the preparation under examination has been replaced by 0.5 ml of a mixture of 1 volume of *phosphate buffer pH 7.2* and 9 volumes of a 0.9 per cent w/v solution of *sodium chloride*. Measure the absorbances of the supernatant liquids at 550 nm (2.4.7). The absorbance of the test solution is not more than 50 per cent than that of the reference solution.

Protein. Determine the nitrogen content (2.3.30).

1 mg of N is equivalent to 6.25 mg of protein.

Potency

The potency of streptokinase is determined by comparing its capacity to activate plasminogen to form plasmin with the same capacity of a reference preparation of streptokinase calibrated in International Units; the formation of plasmin is determined using a suitable chromogenic substrate.

The International Unit is the activity of a stated amount of the International Standard for streptokinase. The equivalence in International Units of the International Standard is stated by the regulatory authority.

Reference and test solutions. Prepare 2 independent series of 4 dilutions of each of the substance under examination and of the reference preparation of streptokinase in *tris (hydroxymethyl) aminomethane sodium chloride buffer pH 7.4*, in the range of 0.5-4.0 IU per ml. Prepare and maintain all solutions at 37°.

Substrate solution. Mix 1.0 ml of *tris (hydroxymethyl) aminomethane buffer pH 7.4* with 1.0 ml of chromophore substrate. Add 5 µl of a 10 per cent w/v solution of *polysorbate 20*. Keep at 37° in a water-bath. Immediately before commencing the activation assay, add 45 µl of a 1 mg per ml solution of human plasminogen.

Analyse each streptokinase dilution, maintained at 37°, in duplicate. Initiate the activation reaction by adding 60 µl of each dilution to 40 µl of substrate solution. For blank wells, use 60 µl of *tris (hydroxymethyl) aminomethane sodium chloride buffer solution pH 7.4* instead of the reference and test solutions. Allow the reaction to proceed at 37° for 20 minutes and read the absorbance at 405 nm (2.4.7). If a suitable thermostated plate reader is available, this may be used to monitor the reaction. Alternatively, it may be necessary to stop the reaction after 20 minutes using 50 µl of a 50 per cent v/v solution of *glacial acetic acid*. Best results are obtained when the absorbance for the highest streptokinase concentration is between 0.1 and 0.2 (after blank subtraction). If necessary, adjust the time of incubation in order to reach this range of absorbances.

Calculate the regression of the absorbance on log concentrations of the solutions of the substance under examination and of the reference preparation of streptokinase and calculate the potency of the substance under examination using the usual statistical methods for parallel-line assays.

The estimated potency is not less than 90 per cent and not more than 111 per cent of the stated potency. The confidence limits (P = 0.95) of the estimated potency are not less than 80 per cent and not more than 125 per cent of the stated potency.

Streptokinase Bulk Solution intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Less than 0.02 Endotoxin Unit per 100 IU of streptokinase activity.

Storage. Store protected from light and at a temperature of about - 20°. If it is intended for the manufacture of parenteral preparations, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of International Units of streptokinase activity per mg, calculated on the dried basis; (2) the name and quantity of any added substance; (3) where applicable, that the substance is free from bacterial endotoxins; (4) where applicable, that the substance is suitable for use in the manufacture of parenteral preparations.

VETERINARY PRODUCTS

General Monographs

Monographs

Veterinary Preparations

General Requirements

The general requirements relating to a specific type of dosage form of an active pharmaceutical ingredient or ingredients, that have been given in the chapter on General Monographs on Dosage Forms of Active Pharmaceutical Ingredients apply to all veterinary dosage forms or preparations of the type defined. However, a valid interpretation of the appropriateness of a test or requirement should be done in the context of the monograph as a whole and of the relevant General Notices.

The requirement for compliance with the tests given under each dosage form or preparation is indicated in each monograph of a drug product or preparation under the heading 'Other tests'. These tests are mandatory and are additional to the tests given in the individual monograph.

Dip Concentrates

Dip concentrates are preparations for the prevention and treatment of ectoparasitic infestations of animals. They contain one or more medicaments, usually in the form of wettable powders, pastes or solutions from which diluted suspensions or emulsions are prepared by appropriate dilution with the recommended liquid. The diluted preparations are applied by complete immersion of the animal or by spraying, as appropriate. They contain suitable antimicrobial preservatives.

Labelling. The label states (1) the name(s) and proportion(s) of medicament(s); (2) the name and proportion of any added antimicrobial preservative; (3) the name and quantity of the diluent and the manner of preparing the diluted dip solution or spray; (4) any special precautions to be taken for use of the preparation; (6) the storage conditions; (6) the date after which the preparation is not intended to be used.

If the preparation contains an organophosphorus compound the label also states (1) that the preparation contains an organophosphorus compound; (2) and special precautions on the use of the preparation.

Intramammary Infusions

Intramammary Infusions for Veterinary Use; Intramammary Injections.

Intramammary Infusions are sterile products intended for injection into the mammary gland through the teat canal. They are solutions, emulsions or suspensions or semi-solid preparations containing one or more active ingredients in a suitable vehicle. They may contain stabilizing, emulsifying, suspending and thickening agents. If a sediment is formed in a suspension, it is readily dispersible on shaking. In emulsions, phase separation may occur but this is readily miscible on shaking.

There are two main types of Intramammary Infusions. One is intended for administration to lactating animals as qualified by the term Lactating Cow/Buffalo and the other, qualified as Non-lactating or Dry Cow/Buffalo, is intended for administration to animals at the end of lactation or during the non-lactating period for the prevention or treatment of infection during the dry period.

Intramammary Infusions are prepared by dissolving or suspending the sterile medicaments in the sterilized vehicle using aseptic precautions, unless a process of terminal sterilisation is employed.

Containers. Intramammary Infusions are usually supplied in single dose containers for administration into a single teat canal of an animal. If supplied in multiple dose containers, aqueous preparations contain an antimicrobial preservative in adequate concentration except when the preparation itself has antimicrobial properties. The containers are made as far as possible from materials that meet the requirements for Parenteral Preparations intended for use in human beings.

The containers are sealed so as to exclude micro-organisms and each container is fitted with a smooth, tapered nozzle to facilitate the introduction of the infusion into the teat canal. The containers are sterilised and filled aseptically unless the preparation is subjected to a process of terminal sterilisation.

Tests

Sterility. Intramammary Infusions comply with the test for sterility (2.2.11), using Method A or B, as appropriate, using the contents of 10 containers mixed thoroughly before use in the test. Use for each medium 0.5 to 1.0 g or 0.5 to 1.0 ml, as appropriate, of the mixed sample.

Storage. Store in sterile, single dose or multiple dose, tamper-evident containers.

Labelling. The label states (1) the strength in terms of the weight or the number of Units of activity of the active ingredient(s) or that may be expressed from the container using normal techniques; (2) whether the preparation is intended for use in lactating cow/buffalo or in dry or non-lactating cow/buffalo; (3) for Intramammary Infusions (Non-lactating or Dry Cow/Buffalo), that the preparation is not intended for use in lactating animals; (4) in the case of infusions in multiple dose containers, the name of any added antimicrobial preservative.

Premixes

Premixes are mixtures of one or more active ingredients with suitable bases intended for mixing with feedstuffs before administration to the animals. They are used to dilute medicament(s) with the feed and are usually issued as pellets, granules or powders. If issued as granules, these are free-flowing and free from aggregates. Suitable precautions are taken during manufacture for ensuring that the premix is homogeneous.

Unless otherwise stated in the individual monograph, the concentration of the premix in medicated feedstuffs is not less than 0.5 per cent.

Tests

Loss on drying (2.4.19). Not more than 15.0 per cent, determined on 3 g by drying in an oven at 105° for 2 hours.

Labelling. The label states (1) the strength in terms of the amount of active ingredient(s) as a percentage; (2) the category of animal for which the premix is intended to be used; (3) the directions for the preparation of the medicated feed; (4) where applicable, the minimum interval between the stoppage of feeding of the diluted premix and the slaughter of the animal for human consumption; (5) any special precautions to be taken for use of the premix; (6) the storage conditions; (7) the date after which the preparation is not intended to be used.

Veterinary Aerosols

Veterinary Sprays

Veterinary Aerosols are solutions, suspensions or emulsions of one or more active ingredients intended for use by external application. They may contain auxiliary substances such as solvents, solubilising agents, emulsifying agents and suspending agents. They are delivered in the form of an aerosol by the actuation of an appropriate valve or by means of a suitable atomizing device that is either an integral part of the container or is supplied separately.

They may be presented in special containers under pressure of a gas and contain propellants or mixtures of propellants. The medicaments are released from the container in the form of an aerosol upon actuation of an appropriate valve.

Veterinary Aerosols supplied in special pressurized containers comply with the appropriate requirements for Inhalation Preparations. The following requirements also apply for any veterinary aerosol that is the subject of an individual monograph.

Containers. A suitable atomizing device may form part of the container or is supplied separately.

Labelling. The label states (1) that the aerosol is intended for external use only; (2) the instructions for use; (3) any special precautions in the use of the preparation.

Veterinary Diagnostics

Veterinary Diagnostics are antigenic materials of bacterial or viral origin employed for various tests. These will also include polyclonal or monoclonal antibodies. The preparations are examined for their purity at various critical stages of production. The diagnostic kits may be prepared using bacterial or viral antigens and antisera.

Tests

Veterinary Diagnostics, reconstituted where necessary, comply with the following tests unless otherwise stated in the individual monograph.

Identification

Unless otherwise stated in the individual monograph, Veterinary Diagnostics give specific reaction when injected into the skin of a healthy white guinea-pig or rabbit that has not been previously treated with any material that will interfere with the test but fails to produce this reaction when mixed with a sufficient quantity of the specific antitoxin or antiserum.

Sterility. Unless otherwise stated, Veterinary Diagnostics comply with the test for sterility (2.2.11), except that in the case of preparations containing living bacteria there may be growth of the organism from which the diagnostic was prepared.

Use suitable solid media for streaking the preparation under examination and incubate at 32° to 37° for 72 hours for detecting bacteria and at 20° to 25° for 72 hours for detecting fungi. The media selected will depend upon the nature of the product to be tested. The contents of each randomly selected sealed container of the preparation under examination or portions or dilutions thereof, as appropriate, are used for the test.

Other tests to determine the nature and identity of contaminating microorganisms, if any, detected during the test include examination for mobility of the organisms, fermentation reactions, thermo-agglutination tests and dye inhibitor tests (in the case of Brucella cultures).

Unless otherwise stated in the monograph, the preparation passes the test if no growth of microorganisms, other than those from which the veterinary diagnostic was prepared, is observed in any of the media during the incubation period. Repeat the tests if growth of organisms, other than those from which the veterinary diagnostic was prepared, is observed. The vaccine passes the test if no growth of microorganisms,

other than those from which the diagnostic was prepared, is observed in any of the media. The preparation fails the test if growth of a microorganism that was seen after the first test, other than those from which the veterinary diagnostic was prepared, is observed. If growth of a different microorganism is observed, the test may be repeated a second time. The preparation passes the test if no growth of a microorganism, other than those from which the veterinary diagnostic was prepared, is observed in any of the media.

The number of containers recommended to be drawn by the manufacturer for performing the test for sterility depends on the environmental conditions of manufacture, the volume of preparation per container and any other special considerations applicable to the preparation concerned. For preparations intended for veterinary use, 1 per cent of the containers in a batch, with a minimum of three and a maximum of ten, is considered a suitable number assuming that the preparation has been manufactured under appropriately validated conditions designed to exclude contamination.

Storage. Store protected from light in a refrigerator (2° to 8°) unless otherwise stated in the individual monograph.

Labelling. The label states (1) the name and quantity of any antibacterial substance added; (2) for a dried preparation, the nature and quantity of the liquid to be used for reconstitution.

Veterinary Oral Liquids

Veterinary oral liquids intended for administration in large animals may also be called Drenches.

Veterinary Oral Powders

Veterinary Oral Powders are intended for oral administration, usually after dilution in drinking water or the feed. They may be in the form of soluble or wettable powders.

Labelling. The label states (1) for single dose containers, the name and quantity of active medicament(s) per container; (2) for multiple dose containers, the name and quantity of active medicament(s) by weight; (3) the name of any added antimicrobial preservative(s); (4) the directions for use of the preparation.

Veterinary Parenteral Preparations

Veterinary Parenteral Preparations prepared with oily vehicles are not meant for intravenous administration but are suitable for intramuscular or subcutaneous use.

Veterinary Parenteral Preparations comply with the appropriate requirements for Parenteral Preparations (Injections) that are given in the chapter on General Monographs on Dosage Forms of Active Pharmaceutical Ingredients.

Veterinary Tablets

Veterinary tablets are usually solid, circular cylinders the end surfaces of which are flat or biconvex and the edges of which are bevelled except that those weighing 5 g of more may be elongated or biconical.

Tests

Disintegration (2.5.1). The test may have to be suitably modified in the case of large tablets; the discs may have to be omitted because they would otherwise be dislodged from the disintegration tubes. It may also be necessary to adjust the volume of the disintegration medium so that the tablet does not break the surface of the medium at the top of the up-stroke, care being taken to apply the minimum practical volume of liquid for this purpose. For certain tablets where the diameter of the tablet may not permit adequate movement of the disintegration medium, the apparatus and the method should be suitably modified.

Veterinary Vaccines

Vaccines for Veterinary Use

Vaccines are a heterogeneous class of medicinal products containing immunogenic substances capable of inducing specific, active and protective host immunity against infectious diseases. They may be prepared from bacteria, viruses, parasites or other organisms or their toxins. Vaccines may contain live attenuated or avirulent microorganisms or these may consist of killed or inactivated microorganisms. Some vaccines consist of antigenic fractions or substances produced by the same pathogenic organisms but rendered harmless whilst retaining their immunogenecity. Vaccines may be prepared from one species or from two or more species of microorganisms.

Vaccines may be prepared by the method described in the individual monograph or by any other appropriate method provided the identity of the antigens is maintained and the preparations are free from microbial contamination and extraneous agents. Suitable adjuvants may be added during the preparation of the vaccines. The addition of antibiotics during the manufacturing process is normally restricted to cell culture fluids and other media, egg inocula and material harvested from skin or other tissues. A suitable bactericide may be added to sterile and inactivated vaccines. The final products are distributed aseptically into sterile

containers that are then sealed to exclude extraneous microorganisms. Unless otherwise indicated in the monograph, the final vaccine may be filled into single dose or multiple dose containers; however, inactivated vaccines in multiple dose containers must invariably contain a bactericide.

Bacterial vaccines. Bacterial vaccines are either suspensions of live or killed bacteria or sterile antigenic extracts or derivatives of bacteria pathogenic to animals. They may be simple vaccines prepared from one species or may be combined or polyvalent vaccines prepared by blending two or more simple vaccines from different species or strains. Bacterial vaccines may be prepared from cultures grown on suitable solid or liquid media. The identity, antigenic potency and purity of each bacterial culture must be carefully controlled.

Bacterial vaccines are suspensions of varying degrees of opacity in colourless or slightly coloured liquids or they may be freeze-dried so that the water content is not more than 3.0 per cent w/w unless otherwise stated in the individual monograph. They may be standardised in terms of international opacity units or, where appropriate, by numbers of live or killed bacteria determined by viable count or by direct cell count.

Live bacterial vaccines. Live bacterial vaccines are prepared from avirulent or attenuated strains of the specific bacteria that are capable of stimulating immune response against pathogenic strains of the same or of antigenically related species of bacteria.

Inactivated bacterial vaccines. Inactivated bacterial vaccines are either prepared from bacteria or their immunogenic components that have been inactivated in a suitable way that they retain adequate immunogenicity.

Bacterial toxoids. Bacterial toxoids are prepared from toxins by diminishing their toxicity to a very low level or by completely eliminating it by physical or chemical means whilst retaining adequate immunising potency. The toxins are obtained from selected strains of specific microorganisms, grown in suitable media devoid of agents capable of inducing undesirable immunological reactions in animals. Bacterial toxoids may be liquid or may be prepared by adsorbing on suitable agents such as aluminium phosphate, aluminium hydroxide or any other suitable adsorbents. Bacterial toxoids are clear or slightly opalescent liquids, colourless or slightly yellow. Adsorbed toxoids may be white or greyish-white suspensions or paleyellow liquids with sediment at the bottom of the container. Freeze-dried preparations are greyish-white or yellowish-white powders or pellets.

Viral vaccines. Viral vaccines are suspensions of viruses or preparations obtained from tissues or blood of animals artificially infected with viruses pathogenic to animals or from cultures in fertile eggs, or from cell or tissue cultures, they may be live, inactivated / killed and may be freeze-dried.

Live viral vaccines. Live viral vaccines are prepared using avirulent or attenuated strains of the specific viruses that are capable of stimulating immunogenic response against pathogenic strains of the same or of antigenically related viruses.

Inactivated viral vaccines. Inactivated viral vaccines contain viruses that have been inactivated by suitable chemical or physical means in such a way that the preparations retain adequate immunogenicity or they are suspensions of immunogenic components of such viruses.

Combined vaccines. Combined vaccines consist of two or more antigens, combined by the manufacturer at the final formulation stage or mixed immediately before administration. Such vaccines are intended to protect against either more than one disease, or against one disease caused by different strains or serotypes of the same organism.

Stability. Stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf life.

Adjuvants. Substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine.

Tests

Vaccines comply with the tests prescribed in the individual monographs including, where applicable, the following:

Aluminium (2.3.9). Where an aluminium adsorbent has been used in the vaccine, not more than 1.25 mg of aluminium (Al) per single dose, unless otherwise stated.

Calcium (2.3.11). Where a calcium adsorbent has been used in the vaccine, not more than 1.3 mg of calcium (Ca) per single dose, unless otherwise stated.

Formaldehyde (2.3.20). Where formaldehyde has been used in the preparation of the vaccine, not more than 0.2 g/l of free formaldehyde is present in the final product, unless otherwise stated.

Phenol (2.3.36). Where phenol has been used in the preparation of the vaccine, not more than 2.5 g/l is present in the final product, unless otherwise stated.

Water (2.3.43). For freeze-dried vaccines, not more than 3.0 per cent, unless otherwise stated.

Thiomersal (2.2.12) (2.3.48). Where thiomersal has been used in the preparation of the vaccine, not more than 0.02 per cent w/v.

Extraneous pathogens. Unless otherwise stated in the individual monograph, live viral vaccines other than those intended for poultry comply with the following test. The mixture obtained after neutralisation of the vaccine with specific

antiserum does not cause cytopathic effects in cell cultures known to be sensitive to agents pathogenic for the species in which the vaccine is intended to be used.

Sterility (2.2.11). Unless otherwise stated in the individual monograph, use method A. Incubate the media for not less than 14 days at 30° to 37° in the test for detecting bacteria and at 20° to 25° in the test for detecting fungi. However, for live bacterial vaccines growth of the organism from which the vaccine was prepared is permitted.

The number of containers to be drawn for the test should be 1 per cent of the containers in a batch, with a minimum of 3 and a maximum of 10, assuming that the preparation has been manufactured under appropriately validated conditions designed to exclude contamination.

Safety test. Unless otherwise stated in the individual monograph, vaccines other than live viral vaccines intended for poultry comply with the following test.

Inject at least 2 healthy, susceptible animals of one of the species in which the vaccine is intended to be used by the route recommended by the manufacturer for field use. The quantity to be injected in each animal is twice the appropriate vaccinating dose. Observe the animals for not less than 7 days. No animal exhibits an abnormal reaction.

Abnormal toxicity. Where stated in the individual monograph vaccines comply with the following test.

Inject 0.5 ml subcutaneously into each of five mice and 2 ml intraperitoneally into each of two guinea pigs. If the vaccine being examined contains an adjuvant, inject 2 ml of the vaccine subcutaneously into each guinea pig. Observe the animals for 7 days. None of the animals shows significant local or systemic reaction. If one animal dies or shows signs of ill health during the observation period repeat the test. None of the animals of the second group dies or shows signs of ill health. This test may be omitted if a safety test is carried out on animals of the species for which the vaccine is intended.

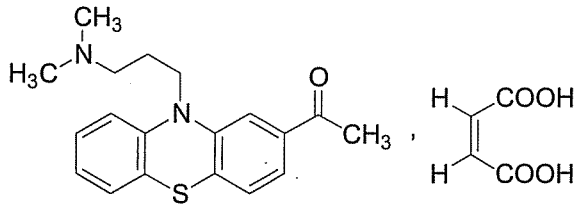
Potency. Determine the potency of the vaccine using the method described in the individual monograph. The vaccine complies with the level of immune response specified in the monograph. A combined vaccine complies with the level specified in the respective monographs for each individual component. If the immunogenicity test (potency test) has been performed with satisfactory results on representative batch of live vaccine from the same seed lot, it may be omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot.

Storage. Store protected from light in a refrigerator (2° to 8°), unless otherwise directed. Do not freeze. Store freeze-dried vaccines at a temperature not exceeding 20°.

Labelling. The label states (1) the potency of the preparation; (2) the route of administration and dose; (3) the date up to which the product is expected to remain within specifications; (4) the storage conditions.

NON BIOLOGICAL VETERINARY MONOGRAPHS

Acepromazine Maleate



$C_{19}H_{22}N_2OS, C_4H_4O_4$ Mol. Wt. 442.5

Acepromazine Maleate is 2-acetyl-10-(3-dimethylaminopropyl)phenothiazine hydrogen maleate.

Acepromazine Maleate contains not less than 98.5 per cent and not more than 101.0 per cent of $C_{19}H_{22}N_2OS,C_4H_4O_4$, calculated on the dried basis.

Category. Sedative; preanaesthetic.

Dose. *Farm animals*. By intramuscular or slow intravenous injection, the equivalent of 50 to 100 mg of acepromazine per kg of body weight. *Dogs and cats*. Orally, the equivalent of 1 to 3 mg of acepromazine per kg of body weight; by intramuscular or intravenous injection, the equivalent of 125 to 250 mg of acepromazine per kg of body weight.

(Each 135 mg of acepromazine maleate is approximately equivalent to 100 mg of acepromazine).

Description. A yellow, crystalline powder.

Identification

Tests B and D may be omitted if tests A, C, E and F are carried out. Tests A and E may be omitted if tests B, C, D and F are carried out.

NOTE — Carry out the tests in subdued light.

A. Dissolve 20 mg in 2 ml of *water*, add 3 ml of *2 M sodium hydroxide*, extract with 5 ml of *cyclohexane* and remove the solvent under reduced pressure. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acepromazine RS* or with the reference spectrum of acepromazine.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M hydrochloric acid*, exhibits maxima at about 244 nm and 280 nm; absorbance at about 244 nm, about 1.1 and at about 280 nm, about 0.82.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)*, 2 volumes of *diethylamine* and 6 to 8 volumes of *2-phenoxyethanol*. Shake and use the supernatant liquid.

Test solution. Dissolve 0.2 g of the substance under examination in 100 ml of *dichloromethane*.

Reference solution. A 0.2 per cent w/v solution of *acepromazine maleate RS* in *dichloromethane*.

Impregnate the dry plate by placing it in a tank containing a shallow layer of a mixture of 85 volumes of *acetone*, 10 volumes of *2-phenoxyethanol* and 5 volumes of *polyethyleneglycol 300* so that the plate dips about 5 mm below the surface of the liquid and allow the impregnating solvent to ascend almost to the top. Use the plate immediately after removing it from the tank. Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution. A secondary spot due to maleic acid is also observed in both chromatograms. Spray the plate with *ethanolic sulphuric acid (10 per cent v/v)*. The spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

D. Dissolve 5 mg in 2 ml of *sulphuric acid*; a yellow colour is produced which changes to deep orange on warming for 2 minutes.

E. Dissolve 0.2 g in a mixture of 3 ml of *water* and 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Discard the ether extracts. Add 2 ml of *bromine solution* to the aqueous solution, warm in a water-bath for 10 minutes, heat to boiling, cool and add 0.25 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish-black colour is produced on heating for 15 minutes in a water-bath.

F. Melting range (2.4.21). 136° to 139°.

Tests

pH (2.4.24). 4.0 to 4.5, determined in a 1.0 per cent w/v solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 95 volumes of *methanol* and 5 volumes of *diethylamine*.

Mobile phase. A mixture of 75 volumes of *hexane*, 17 volumes of *2-butanol* and 8 volumes of *diethylamine*.

Test solution. Dissolve 0.2 g of the substance under examination in 10 ml of the solvent mixture.

Reference solution. A 0.01 per cent w/v solution of the substance under examination in the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm.

Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18) Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 0.1 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.4 g, dissolve in 50 ml of *acetic anhydride*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.04425 g of $C_{19}H_{22}N_20S,C_4H_4O_4$.

Storage. Store protected from moisture.

Acepromazine Injection

Acepromazine Maleate Injection

Acepromazine Injection is a sterile solution of Acepromazine Maleate in Water for Injections.

Acepromazine Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of acepromazine, $C_{19}H_{22}N_2OS$.

Usual strengths. The equivalent of 200 mg of acepromazine in 20 ml and of 20 mg of acepromazine in 10 ml.

Identification

NOTE — Carry out the tests in subdued light.

A. To a volume containing 20 mg of acepromazine, add 2 ml of *water* and 3 ml of *2 M sodium hydroxide*, extract with two quantities, each of 5 ml, of *cyclohexane* and remove the solvent under reduced pressure. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acepromazine RS* or with the reference spectrum of acepromazine.

B. To 5 mg of the residue obtained in test A, add 2 ml of *sulphuric acid*; a yellow colour is produced which changes to deep orange on warming for 2 minutes.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)*, 2 volumes of *diethylamine* and 6 to 8 volumes of *2-phenoxyethanol*. Shake and use the supernatant liquid.

Test solution. Extract a volume containing 20 mg of acepromazine with two quantities, each of 5 ml, of *dichloromethane* and use the combined extracts.

Reference solution. A 0.2 per cent w/v solution of *acepromazine maleate RS* in *dichloromethane*.

Impregnate the dry plate by placing it in a tank containing a shallow layer of a mixture of 85 volumes of *acetone*, 10 volumes of *2-phenoxyethanol* and 5 volumes of *polyethyleneglycol 300* so that the plate dips about 5 mm below the surface of the liquid and allow the impregnating solvent to ascend almost to the top. Use the plate immediately after removing it from the tank. Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution. A secondary spot due to maleic acid is also observed in both chromatograms. Spray the plate with *ethanolic sulphuric acid (10 per cent v/v)*. The spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

D. To a volume containing 25 mg of acepromazine add 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Discard the ether extracts. Add 2 ml of *bromine solution* to the aqueous solution, warm in a water-bath for 10 minutes, heat to boiling, cool and add 0.25 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish-black colour is produced on heating for 15 minutes in a water-bath.

Tests

pH (2.4.24). 4.5 to 5.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing 40 mg of acepromazine add 5 ml of *1 M sodium hydroxide* and extract with three or more quantities, each of 50 ml, of *dichloromethane* until the dichloromethane extract is colourless. Wash the extracts with the same 10 ml of *water* and filter through a plug of absorbent cotton previously moistened with *dichloromethane*. Evaporate the combined extracts to dryness, dissolve the residue in 15 ml of *acetic anhydride*. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.006529 g of $C_{19}H_{22}N_2OS$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of acepromazine.

Acepromazine Tablets

Acepromazine Maleate Tablets

Acepromazine Tablets contain not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of acepromazine, $C_{19}H_{22}N_2OS$.

Usual strengths. 10 mg; 25 mg.

Identification

NOTE — Carry out the tests in subdued light.

A. To a quantity of the powdered tablets containing 20 mg of acepromazine add 2 ml of *water* and 3 ml of *2 M sodium hydroxide*. Extract with two quantities, each of 5 ml, of *cyclohexane* and remove the solvent under reduced pressure. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *acepromazine RS* or with the reference spectrum of acepromazine.

B. To 5 mg of the residue obtained in test A add 2 ml of *sulphuric acid*; a yellow colour is produced which changes to deep orange on warming for 2 minutes.

C. Determine by thin-layer chromatography (2.4.17), coating the plate with *kieselguhr G*.

Mobile phase. A mixture of 100 volumes of *light petroleum (40° to 60°)*, 2 volumes of *diethylamine* and 6 to 8 volumes of *2-phenoxyethanol*. Shake and use the supernatent liquid.

Test solution. Extract a quantity of powdered tablets containing 20 mg of acepromazine with two quantities, each of 5 ml, of *dichloromethane* and use the combined extracts.

Reference solution. A 0.2 per cent w/v solution of *acepromazine maleate RS* in *dichloromethane*.

Impregnate the dry plate by placing it in a tank containing a shallow layer of a mixture of 85 volumes of *acetone*, 10 volumes of *2-phenoxyethanol* and 5 volumes of *polyethyleneglycol 300* so that the plate dips about 5 mm below the surface of the liquid and allow the impregnating solvent to ascend almost to the top. Use the plate immediately after removing it from the tank. Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution. A secondary spot due to maleic acid is also observed in both chromatograms. Spray the plate with *ethanolic sulphuric acid (10 per cent v/v)*. The spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

D. Dissolve a quantity of the powdered tablets containing 25 mg of acepromazine as completely as possible in a mixture of 3 ml of *water* and 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Discard the ether extracts. Add 2 ml of *bromine solution* to the aqueous solution, warm in a water-bath for 10 minutes, heat to boiling, cool and add 0.25 ml of a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a bluish-black colour is produced on heating for 15 minutes in a water-bath.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Solvent mixture. 95 volumes of *methanol* and 5 volumes of *diethylamine*.

Mobile phase. A mixture of 75 volumes of *hexane*, 17 volumes of *2-butanol* and 8 volumes of *diethylamine*.

Test solution. Shake a quantity of the powdered tablets containing 50 mg of acepromazine with 10 ml of *dichloromethane*, filter, evaporate to dryness and dissolve the residue in 5 ml of *methanol* containing 0.5 per cent v/v of *strong ammonia solution*.

Reference solution. Dilute 1 ml of the test solution to 100 ml with *methanol* containing 0.5 per cent v/v of *strong ammonia solution*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

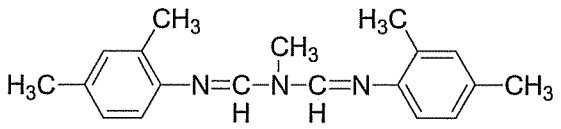
Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 60 mg of acepromazine, add 5 ml of *water* and extract with three or more quantities, each of 50 ml, of *dichloromethane* until the dichloromethane extract is colourless. Wash the extracts with the same 10 ml of *water* and filter through a plug of absorbent cotton previously moistened with *dichloromethane*. Evaporate the combined extracts to dryness, dissolve the residue in 15 ml of *acetic anhydride*. Titrate with *0.02 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.02 M perchloric acid* is equivalent to 0.006529 g of $C_{19}H_{22}N_2OS$.

Labelling. The label states the strength in terms of the equivalent amount of acepromazine.

Amitraz



$C_{19}H_{23}N_3$ Mol. Wt. 293.41

Amitraz is *N,N*-di-(2,4-xylyliminomethyl)methylamine.

Amitraz contains not less than 95.0 per cent and not more than 101.5 per cent of $C_{19}H_{23}N_3$, calculated on the anhydrous basis.

Category. Acaricide.

Dose. The usual recommended concentration in the dip-bath may vary between 0.005% and 0.1 per cent w/v, depending upon the host and parasite. *Cows, buffaloes, sheep and goats*. 0.025 per cent w/v.

Description. A white to buff powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amitraz RS* or with the reference spectrum of amitraz.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254*.

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 30 volumes of *ethyl acetate* and 20 volumes of *triethylamine*.

Test solution (a). Dissolve 1 g of the substance under examination in 10 ml of *toluene*.

Test solution (b). Dissolve 20 mg of the substance under examination in 10 ml of *toluene*.

Reference solution (a). A 0.20 per cent w/v solution of *amitraz RS* in *toluene*.

Reference solution (b). A 0.030 per cent w/v of *2,4-dimethylaniline* in *toluene*.

Impregnate the plate to a depth of about 3.5 cm with a solution prepared by dissolving 35 g of *acetamide* in 100 ml of *methanol*, adding 100 ml of *triethylamine* and diluting to 250 ml with *methanol*, before standing it in a stream of cold air for about 30 seconds. Immediately apply to the plate, at a level 1 cm below the top of the impregnated zone, 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with test solution (a) is not more intense than the spot in the chromatogram obtained with reference solution (a). Expose the plate to the vapours of *hydrochloric acid* until the plate smells strongly of acid. Expose to the vapours of nitrogen dioxide (prepared by the action of *nitric acid* on *granulated zinc*) for 10 minutes, remove the excess of nitrogen dioxide with air and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in a 50 per cent v/v solution of *methanol*. Any secondary spot corresponding to 2,4-dimethylaniline in the chromatogram obtained with test solution (a) is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b).

Water (2.3.43). Not more than 0.1 per cent, determined on 5 g and using *anhydrous pyridine* in place of *anhydrous methanol*.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Assay. Determine by gas chromatography (2.4.13).

Internal Standard Solution. A 1.0 per cent v/v solution of *squalane* in *methyl acetate*.

Test solution (a). Dissolve 0.8 g of the substance under examination in 100 ml of *methyl acetate*.

Test solution (b). Dissolve 0.8 g of the substance under examination in 100 ml of the internal standard solution.

Reference solution. A 0.8 per cent w/v solution of *amitraz RS* in the internal standard solution.

Chromatographic system

- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w methyl silicone gum or fluid (such as OV-1 or OV-101),
- temperature:
  column. 250°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{19}H_{23}N_3$.

Storage. Store in containers which may contain paraformaldehyde packed in separate sachets as stabiliser.

Liquid Amitraz Dip Concentrate

Liquid Amitraz Dip Concentrate contains Amitraz in a suitable emulsifiable vehicle. It may contain a suitable stabilising agent.

Liquid Amitraz Dip Concentrate contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amitraz, $C_{19}H_{23}N_3$.

Usual strength. 12.5 per cent w/v.

Identification

A. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

B. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with reference solution (b).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 30 volumes of *ethyl acetate* and 20 volumes of *triethylamine.*

Test solution (a). Dilute the dip concentrate with *toluene* to obtain 5.0 per cent w/v of Amitraz.

Test solution (b). Dilute the dip concentrate with *toluene* to obtain 0.2 per cent w/v of Amitraz.

Reference solution (a). A 0.20 per cent w/v solution of *amitraz RS* in *toluene.*

Reference solution (b). A 0.030 per cent w/v of *2,4-dimethylaniline* in *toluene.*

Impregnate the plate to a depth of about 3.5 cm in a solution prepared by dissolving 35 g of *acetamide* in 100 ml of *methanol*, adding 100 ml of *triethylamine* and diluting to 250 ml with *methanol*, before standing it in a stream of cold air for about 30 seconds. Immediately apply to the plate, at a level 1 cm below the top of the impregnated zone, 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with the reference solution (a). Expose the plate to the vapours of *hydrochloric acid* until the plate smells strongly of acid. Expose to the vapours of nitrogen dioxide (prepared by the action of *nitric acid* on *granulated zinc*) for 10 minutes, remove the excess of nitrogen dioxide with air and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in a 50 per cent v/v solution of *methanol.* Any secondary spot corresponding to 2,4-dimethylaniline in the chromatogram obtained with test solution (a) is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b).

Water (2.3.43). Not more than 0.15 per cent w/v, determined in 5 ml of the dip concentrate and using *anhydrous pyridine* in place of *anhydrous methanol.*

Other tests. Complies with the tests stated under Dip Concentrates.

Assay. Determine by gas chromatography (2.4.13).

Internal Standard Solution. A 1.0 per cent v/v solution of *squalane* in *methyl acetate.*

Test solution (a). Dissolve an accurately measured volume of the dip concentrate containing 80 mg of Amitraz in 10 ml of *methyl acetate.*

Test solution (b). Dissolve an accurately measured volume of the dip concentrate containing 80 mg of Amitraz in 10 ml of the internal standard solution.

Reference solution. A 0.8 per cent w/v solution of *amitraz RS* in the internal standard solution.

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w methyl silicone gum or fluid (such as OV-1 or OV-101),
- temperature:
  column. 250°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{19}H_{23}N_3$.

Amitraz Dip Concentrate Powder

Amitraz Dip Concentrate Powder consists of Amitraz mixed with suitable wetting, dispersing and suspending agents. It may contain a suitable stabilising agent.

Amitraz Dip Concentrate Powder contains not less than 92.0 per cent and not more than 108.0 per cent of the stated amount of amitraz, $C_{19}H_{23}N_3$.

Usual strengths. 25 per cent w/w and 50 per cent w/w.

Identification

A. Shake a quantity of the powder containing 0.1 g of Amitraz with 10 ml of *acetone* for 5 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amitraz RS* or with the reference spectrum of amitraz.

B. In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (a).

C. In the Assay, the principal peak in the chromatogram obtained with the test solution (b) corresponds to the peak in the chromatogram obtained with the reference solution.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel HF254.*

Mobile phase. A mixture of 50 volumes of *cyclohexane*, 30 volumes of *ethyl acetate* and 20 volumes of *triethylamine.*

Test solution (a). The supernatant liquid obtained by shaking a quantity of the powder containing 0.5 g of Amitraz with 10 ml of *toluene* for 5 minutes and centrifuging the suspension.

Test solution (b). The supernatant liquid obtained by shaking a quantity of the powder containing 20 mg of Amitraz with 10 ml of *toluene* for 5 minutes and centrifuging the suspension.

Reference solution (a). A 0.20 per cent w/v solution of *amitraz RS* in *toluene.*

Reference solution (b). A 0.030 per cent w/v of *2,4-dimethylaniline* in *toluene.*

Impregnate the plate to a depth of about 3.5 cm in a solution prepared by dissolving 35 g of *acetamide* in 100 ml of *methanol,* adding 100 ml of *triethylamine* and diluting to 250 ml with *methanol,* before standing it in a stream of cold air for about 30 seconds. Immediately apply to the plate, at a level 1 cm below the top of the impregnated zone, 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution (a) is not more intense than the spot in the chromatogram obtained with the reference solution (a). Expose the plate to the vapours of *hydrochloric acid* until the plate smells strongly of acid. Expose to the vapours of nitrogen dioxide (prepared by the action of *nitric acid* on *granulated zinc*) for 10 minutes, remove the excess of nitrogen dioxide with air and spray with a 0.5 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride* in a 50 per cent v/v solution of *methanol.* Any secondary spot corresponding to 2,4-dimethylaniline in the chromatogram obtained with test solution (a) is not more intense than the corresponding spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Dip Concentrates.

Assay. Determine by gas chromatography (2.4.13).

Internal Standard Solution. A 1.0 per cent v/v solution of *squalane* in *methyl acetate.*

Test solution (a). Shake a quantity of the powder containing 80 mg of Amitraz with 10 ml of *methyl acetate,* centrifuge and use the supernatant liquid.

Test solution (b). Shake a quantity of the powder containing 80 mg of Amitraz with 10 ml of the internal standard solution, centrifuge and use the supernatant liquid.

Reference solution. A 0.8 per cent w/v solution of *amitraz RS* in the internal standard solution.

Chromatographic system

- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (80 to 100 mesh) coated with 3 per cent w/w methyl silicone gum or fluid (such as OV-1 or OV-101),
- temperature:
  column. 250°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{19}H_{23}N_3$.

Ampicillin and Cloxacillin Intramammary Infusion (Lactating Cow/Buffalo)

Ampicillin Sodium and Cloxacillin Sodium Intramammary Infusion (LC/B)

Ampicillin and Cloxacillin Intramammary Infusion (Lactating Cow/Buffalo) is a sterile suspension of Ampicillin Sodium and Cloxacillin Sodium in a suitable vehicle containing suitable suspending agents.

Ampicillin and Cloxacillin Intramammary Infusion (Lactating Cow/Buffalo) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of each of ampicillin, $C_{16}H_{19}N_3O_4S$, and cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strength. The equivalent of 75 mg of ampicillin and 200 mg of cloxacillin.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 10 volumes of *butyl acetate,* 6 volumes of *glacial acetic acid,* 1 volume of *1-butanol* and 2 volumes of solution A (see below).

Test solution. Extract a quantity of the infusion containing 50 mg of ampicillin with three successive quantities, each of 15 ml, of *light petroleum (120° to 160°).* Discard the extracts, wash the residue with 10 ml of *ether* and dry in a current of air. Dissolve the residue in 50 ml of *phosphate buffer pH 7.0,* shake well, filter and use the filtrate.

Reference solution. A 0.12 per cent w/v solution of *ampicillin trihydrate RS* in *phosphate buffer pH 7.0.*

Impregnate the plate by spraying it with a 0.1 per cent w/v solution of *disodium edetate* in a 5 per cent w/v solution of *sodium dihydrogen phosphate* (solution A), allow the plate to dry in air and heat it at 105° for 1 hour. Apply to the plate 1 µl of each solution. After development, dry the plate in air and heat at 150° for 10 to 15 minutes and spray with a mixture of 100 volumes of *starch mucilage,* 6 volumes of *glacial acetic acid* and 2 volumes of a 1 per cent w/v solution of *iodine* in a 4 per cent w/v solution of *potassium iodide.* The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silanised silica gel GF254.*

Mobile phase. A mixture of 70 volumes of *0.05 M potassium hydrogen phthalate*, 30 volumes of *acetone* and 1 volume of *formic acid* that has been adjusted first to pH 6.0 with *5 M sodium hydroxide* and then to pH 9.0 with *0.1 M sodium hydroxide.*

Test solution. Extract a quantity of the infusion containing 130 mg of cloxacillin with three successive quantities, each of 15 ml, of *light petroleum (120° to 160°).* Discard the extracts, wash the residue with 10 ml of *ether* and dry in a current of air. Dissolve the residue in 50 ml of *phosphate buffer pH 7.0*, shake well, filter and use the filtrate.

Reference solution. A 0.28 per cent w/v solution of *cloxacillin sodium RS* in *phosphate buffer pH 7.0.*

Apply to the plate 1 µl of each solution. After development, dry the plate in air and heat at 150° for 10 to 15 minutes and spray with a mixture of 100 volumes of *starch mucilage*, 6 volumes of *glacial acetic acid* and 2 volumes of a 1 per cent w/v solution of *iodine* in a 4 per cent w/v solution of *potassium iodide.* The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

C. Extract a quantity containing 50 mg of ampicillin with three successive quantities, each of 15 ml, of *light petroleum (120° to 160°).* Discard the extracts, wash the residue with 10 ml of *ether* and dry the residue at 55°. The residue produces an intense, persistent yellowish orange colour when introduced into a non-luminous flame on a platinum wire moistened with *hydrochloric acid.*

Tests

Water (2.3.43). Not more than 1.0 per cent , determined on 1.5 g using a mixture of 70 volumes of *dichloromethane* and 30 volumes of *anhydrous methanol* as the solvent.

Other tests. Complies with the tests stated under Intramammary Infusions.

Assay. Weigh and mix the contents of 10 containers. Weigh accurately a quantity of the mixed contents containing 50 mg of ampicillin and extract with three successive quantities, each of 15 ml, of *light petroleum (120° to 160°)* previously saturated with ampicillin sodium and cloxacillin sodium. Discard the extracts, wash the residue with *ether* previously saturated with ampicillin sodium and cloxacillin sodium, dry in a current of air, dissolve in *water* and dilute to 100.0 ml with *water.* Centrifuge and use the clear supernatant liquid (solution B).

For ampicillin — Dilute 2.0 ml of solution B to 50.0 ml with *buffered cupric sulphate solution pH 5.2*, transfer 10.0 ml of the resulting solution to a stoppered test-tube and heat in a water-bath at 75° for 30 minutes. Cool to room temperature rapidly, dilute to 20.0 ml with *buffered cupric sulphate solution pH 5.2* and measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7), using as the blank a solution prepared by diluting 2.0 ml of solution B to 100.0 ml with *buffered cupric sulphate solution pH 5.2.*

Calculate the content of $C_{16}H_{19}N_3O_4S$ in a container of average content from the absorbance obtained by carrying out the procedure simultaneously using 2.0 ml of a solution prepared by dissolving 60 mg of *ampicillin trihydrate RS* in 100.0 ml of *water*, diluting to 50.0 ml with *buffered cupric sulphate solution pH 5.2* and beginning at the words "transfer 10.0 ml....." .

For cloxacillin — Dilute 2.0 ml of solution B to 100.0 ml with *1 M hydrochloric acid.* Measure the absorbance of the resulting solution at 20° after exactly 12 minutes at the maximum at about 350 nm (2.4.7), using *1 M hydrochloric acid* as the blank. Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in a container of average content from the absorbance obtained by carrying out the procedure simultaneously using 2.0 ml of a solution prepared by dissolving 0.14 g of *cloxacillin sodium RS* in 100.0 ml of *water.*

Labelling. The label states the quantity of Ampicillin Sodium in terms of the equivalent amount of ampicillin and the quantity of Cloxacillin Sodium in terms of the equivalent amount of cloxacillin.

Ampicillin and Cloxacillin Benzathine Intramammary Infusion (Dry Cow/ Buffalo)

Ampicillin and Cloxacillin Benzathine Intramammary Infusion (Dry Cow/Buffalo) is a sterile suspension of Ampicillin Trihydrate and Cloxacillin Benzathine in a suitable vehicle containing suitable suspending agents.

Ampicillin and Cloxacillin Benzathine Intramammary Infusion (Dry Cow/Buffalo) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of ampicillin, $C_{16}H_{19}N_3O_4S$, and cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strength. The equivalent of 250 mg of ampicillin and 500 mg of cloxacillin.

Identification

A. Extract a quantity containing 250 mg of ampicillin with three quantities, each of 15 ml, of *light petroleum (120° to 160°).* Discard the extracts, wash the residue with 10 ml of *ether* and dry in a current of air. Shake with 10 ml of *dichloromethane* and filter. Keep both the residue and the filtrate.

Wash the residue with two quantities, each of 5 ml, of *dichloromethane* and dry in a vaccum desiccator.

On the residue determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ampicillin trihydrate RS* or with the reference spectrum of ampicillin trihydrate.

B. Wash the filtrate with two quantities, each of 5 ml, of *water*, dry the dichloromethane layer with *anhydrous sodium sulphate*, filter and dilute the filtrate to 20 ml with *dichloromethane*.

On the filtrate determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin benzathine RS* or with the reference spectrum of cloxacillin benzathine.

Tests

Water (2.3.43). Not more than 3.0 per cent, determined on 1.5 g using a mixture of 70 volumes of *dichloromethane* and 30 volumes of *anhydrous methanol* as the solvent.

Other tests. Complies with the tests stated under Intramammary Infusions.

Assay. Weigh and mix the contents of 10 containers. Weigh accurately a quantity of the mixed contents containing 60 mg of ampicillin and extract with three quantities, each of 15 ml, of *light petroleum (120° to 160°)* previously saturated with *ampicillin trihydrate* and *cloxacillin benzathine*. Discard the extracts, wash the residue with *ether* previously saturated with *ampicillin trihydrate* and *cloxacillin benzathine*, dry in a current of air, dissolve in 50 ml of *methanol* and dilute to 100.0 ml with *water*. Centrifuge and use the clear supernatant liquid (solution A).

For ampicillin — Dilute 2.0 ml of solution A to 50.0 ml with *buffered cupric sulphate solution pH 5.2*, transfer 10.0 ml to a stoppered test-tube and heat in a water-bath at 75° for 30 minutes. Cool to room temperature rapidly, dilute to 20.0 ml with *buffered cupric sulphate solution pH 5.2* and measure the absorbance of the resulting solution at the maximum at about 320 nm (2.4.7), using as the blank the unheated buffered solution of the infusion.

Calculate the content of $C_{16}H_{19}N_3O_4S$ in a container of average content from the absorbance obtained by carrying out the procedure simultaneously using 2.0 ml of a solution prepared by dissolving 70 mg of *ampicillin trihydrate RS* in 100.0 ml of a 50 per cent v/v solution of *methanol*, diluting to 50.0 ml with *buffered cupric sulphate solution pH 5.2*, and beginning at the words "transfer 10.0 ml.....".

For cloxacillin — Dilute 2.0 ml of solution A to 100.0 ml with *1 M hydrochloric acid* and measure the absorbance of the resulting solution at 20° after exactly 12 minutes at the maximum at about 350 nm, (2.4.7), using *1 M hydrochloric acid* as the blank. Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in a container of average content from the absorbance obtained by carrying out the procedure simultaneously using 2.0 ml of a solution prepared by dissolving 0.165 g of *cloxacillin benzathine RS* in 100.0 ml of a 50 per cent v/v solution of *methanol*.

Labelling. The label states the strength of Ampicillin Trihydrate in terms of the equivalent amount of ampicillin and that of Cloxacillin Benzathine in terms of the equivalent amount of cloxacillin.

Ampicillin Veterinary Oral Powder

Ampicillin TrihydrateVeterinary Oral Powder

Ampicillin Veterinary Oral Powder is a mixture of Ampicillin Trihydrate and Lactose or other suitable diluent.

Ampicillin Veterinary Oral Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of ampicillin, $C_{16}H_{19}N_3O_4S$.

Usual strength. The equivalent of 10 per cent w/w of ampicillin.

Description. A fine granular powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

NOTE — Prepare the solutions immediately before use.

Mobile phase. A mixture of 10 volumes of *butyl acetate*, 6 volumes of *glacial acetic acid*, 2 volumes of a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0* and 1 volume of *1-butanol*.

Test solution. Shake a quantity of the powder containing 0.1 g of ampicillin with 50 ml of *phosphate buffer pH 7.0* for 15 minutes, filter and use the filtrate.

Reference solution. A 0.2 per cent w/v solution of *ampicillin trihydrate RS* in *phosphate buffer pH 7.0*.

Impregnate the dry plate by placing it in a tank containing a shallow layer of a 0.1 per cent w/v solution of *disodium edetate* in *mixed phosphate buffer pH 4.0*, allowing the solvent to ascend to the top, removing the plate from the tank and allowing the solvent to evaporate. Use the plate with the flow of the mobile phase in the direction in which impregnation was carried out. Before use heat the plate at 100° for 1 hour and allow to cool. Apply to the plate 1 µl of each solution. After development, dry the plate in air and spray with a mixture of 100 volumes of a 1 per cent w/v solution of *starch*, 6 volumes of *glacial acetic acid* and 2 volumes of a 1 per cent w/v

solution of *iodine* in a 4 per cent w/v solution of *potassium iodide*. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

B. To a quantity of the powder containing 10 mg of ampicillin add sufficient *water* to produce 10 ml, shake for 15 minutes and filter. Place 0.1 ml of a 0.1 per cent w/v solution of *ninhydrin* on a filter paper, dry at 105°, superimpose 0.1 ml of the solution of the preparation under examination, heat for 5 minutes at 105° and allow to cool; a mauve colour is produced.

C. Suspend a quantity of the powder containing 10 mg of ampicillin in 1 ml of *water* and add 2 ml of a mixture of 2 ml of *potassium cupri-tartrate solution* and 6 ml of *water*; a magenta-violet colour is immediately produced.

Tests

Uniformity of weight. When supplied in containers intended for use on one occasion, complies with the test for Uniformity of weight described under Parenteral Preparations (Powders for Injection).

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. Determine by liquid chromatography (2. 4.14).

NOTE — Prepare the solutions immediately before use.

Solvent mixture. Mix 10 ml of *1 M monobasic potassium phosphate* and 1 ml of *1 M acetic acid* and dilute to 1000 ml with *water*.

Test solution. Dissolve an accurately weighed quantity of powder containing about 100 mg of ampicillin in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). Weigh accurately a suitable quantity of *ampicillin RS,* dissolve in the solvent mixture by shaking and mixing if necessary, with the aid of ultrasound to obtain a solution having a known concentration of about 1 mg per ml.

Reference solution (b). Dissolve *caffeine* in reference solution (a) to obtain a solution containing about 0.12 mg per ml.

Chromatographic system

- a stainless steel column 30 cm x 4.0 mm, packed with octadecylsilane bonded to porous silica or ceramic microparticles (5 µm),
- mobile phase: a mixture of 90 volumes of *water,* 8 volumes of *acetonitrile,* 1 volume of *1 M monobasic potassium phosphate* and 1 volume of *1 M acetic acid,*
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume.20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the caffeine and ampicillin peaks is not less than 2.0. The relative retention time with reference to caffeine for ampicillin is about 0.5.

Inject reference solution (a). The test is not valid unless the capacity factor is not more than 2.5, the tailing factor is not more than 1.4 and the relative standard deviation for replicate injections is not more than 2.0 per cent.

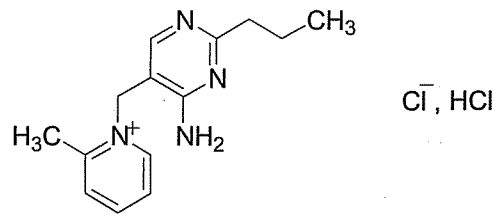
Inject the test solution and reference solution (a).

Calculate the content of $C_{16}H_{19}N_3O_4S$ in oral powder.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the equivalent concentration of ampicillin.

Amprolium Hydrochloride



$C_{14}H_{19}ClN_4,HCl$ Mol. Wt. 315.2

Amprolium Hydrochloride is hydrochloride salt of 1-[(4-amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride.

Amprolium Hydrochloride contains not less than 97.5 per cent and not more than 101.0 per cent of $C_{14}H_{19}ClN_4,HCl$, calculated on the dried basis.

Category. Coccidiostat.

Dose. *Poultry*. Usual recommended concentration in feed as preventive against coccidiosis infection, about 0.0125 per cent (between 100 and 125 g per tonne of feed).

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *amprolium hydrochloride RS* or with the reference spectrum of amprolium hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M hydrochloric acid* exhibits maxima at about 246 nm and 262 nm; absorbance at about 246 nm, about 0.84 and at about 262 nm, about 0.80.

C. To 1 mg add 5 ml of *naphthalenediol reagent*; a deep violet colour is produced.

D. Gives the reactions of chlorides (2.3.1).

Tests

Picoline. Dissolve 1.5 g in 30 ml of *water* in a distillation flask, add 20 ml of a saturated solution of *potassium carbonate sesquihydrate*, connect the flask to a ground-glass aerator extending to the bottom of a 100-ml graduated cylinder containing 50 ml of *0.05 M hydrochloric acid* and pass air, which has previously been passed through *sulphuric acid* and glass wool, through the system for 60 minutes. To 5 ml of the hydrochloric acid solution add sufficient *0.05 M hydrochloric acid* to produce 200 ml. Absorbance of the resulting solution at about 262 nm (2.4.7), not more than 0.52.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying to constant weight at 100° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.3 g, dissolve in 20 ml of *anhydrous glacial acetic acid*, add 10 ml of *mercuric acetate solution*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01577 g of $C_{14}H_{19}ClN_4,HCl$.

Amprolium Hydrochloride and Ethopabate Premix

Amprolium Hydrochloride and Ethopabate Premix contains Amprolium Hydrochloride and Ethopabate.

Amprolium Hydrochloride and Ethopabate Premix contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of amprolium hydrochloride, $C_{14}H_{19}ClN_4,HCl$ and of ethopabate, $C_{12}H_{15}NO_4$.

Usual strength. 25 per cent w/w of Amprolium Hydrochloride and 1.6 per cent w/w of Ethopabate.

Identification

A. Shake a quantity containing 20 mg of Amprolium Hydrochloride with 90 ml of *methanol* and filter. Add 5 ml of the filtrate to 5 ml of *naphthalenediol reagent*; a deep violet colour is produced.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

NOTE — Prepare the solution immediately before use.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Shake continuously for 10 minutes a quantity containing 10 mg of Ethopabate with 25 ml of *acetone* that has been warmed to 50°, filter and use the filtrate.

Reference solution. A 0.04 per cent w/v solution of *ethopabate RS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with the reference solution.

Tests

pH (2.4.24). 2.5 to 4.0, determined in a 25 per cent w/v slurry in *carbon dioxide-free water*.

Other tests. Complies with the tests stated under Premixes.

Assay. *For amprolium hydrochloride* — Weigh accurately a quantity containing 50 mg of Amprolium Hydrochloride, shake continuously for 20 minutes with 100.0 ml of a mixture of 2 volumes of *methanol* and 1 volume of *water* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with the methanol-water mixture. To 4.0 ml of the resulting solution add 10.0 ml of *naphthalenediol reagent*, allow to stand for 20 minutes and measure the absorbance of the resulting solution at the maximum at about 520 nm (2.4.7), using as the blank a solution obtained by mixing 4.0 ml of a mixture of 2 volumes of *methanol* and 1 volume of *water* with 10.0 ml of *naphthalenediol reagent* and allowing to stand for 20 minutes. Calculate the content of $C_{14}H_{19}ClN_4,HCl$ from the absorbance obtained by carrying out the procedure simultaneously, using 4.0 ml of a 0.0025 per cent w/v solution of *amprolium hydrochloride RS* in a mixture of 2 volumes of *methanol* and 1 volume of *water* and beginning at the words, "To 4.0 ml of the resulting solution add 10.0 ml of.....".

For ethopabate — Weigh accurately a quantity containing 6 mg of Ethopabate, add 75 ml of *methanol*, shake continuously for 20 minutes, dilute to 100.0 ml with *methanol* and filter. To 10.0 ml of the filtrate add 10 ml of *1 M sodium hydroxide* and evaporate to dryness. Dissolve the residue in 10.0 ml of *water*, heat on a water-bath for 15 minutes, add 10 ml of *2 M hydrochloric acid*, dilute to 100.0 ml with *water* and filter. To 25.0 ml of the filtrate, add 2.5 ml of *2 M hydrochloric acid* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite* prepared immediately before use. Allow to stand for 3 minutes and add 2.0 ml of a freshly prepared 0.5 per cent w/v solution of *ammonium sulphamate*. Allow to stand for 2 minutes, add 5.0 ml of a freshly prepared 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, allow to stand for 10 minutes and dilute to 50.0 ml with *water*. Measure

the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7), using as the blank a solution obtained by repeating the procedure with 25 ml of *water* and beginning at the words "add 2.5 ml of *2 M hydrochloric acid*.....". Calculate the content of $C_{12}H_{15}NO_4$ from the absorbance obtained by carrying out the procedure simultaneously, using 10.0 ml of a 0.006 per cent w/v solution of *ethopabate RS* in *methanol* and beginning at the words "add 10 ml of *1 M sodium hydroxide* and evaporate to dryness.....".

Amprolium, Ethopabate and Sulphaquinoxaline Premix

Amprolium Hydrochloride, Ethopabate and Sulphaquinoxaline Premix

Amprolium, Ethopabate and Sulphaquinoxaline Premix contains Amprolium Hydrochloride, Ethopabate and Sulphaquinoxaline.

Amprolium, Ethopabate and Sulphaquinoxaline Premix contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of amprolium hydrochloride, $C_{14}H_{19}ClN_4,HCl$, of ethopabate, $C_{12}H_{15}NO_4$, and of sulphaquinoxaline, $C_{14}H_{12}N_4O_2S$.

Usual strength. 20 per cent w/w of Amprolium Hydrochloride, 1 per cent w/w of Ethopabate and 12 per cent w/w of Sulphaquinoxaline

Identification

A. Shake a quantity containing 20 mg of Amprolium Hydrochloride with 90 ml of *methanol* and filter. Add 5 ml of the filtrate to 5 ml of *naphthalenediol reagent*; a deep violet colour is produced.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

NOTE — Prepare the solution immediately before use.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Shake continuously for 10 minutes a quantity containing 10 mg of Ethopabate with 25 ml of *acetone* that has been warmed to 50°, filter and use the filtrate.

Reference solution (a). A 0.04 per cent w/v solution of *ethopabate RS* in *acetone*.

Reference solution (b). A 0.4 per cent w/v solution of *sulphaquinoxaline RS* in *acetone*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to the spot in the chromatogram obtained with reference solutions (a) and (b).

Tests

pH (2.4.24). 2.5 to 4.0, determined in a 25 per cent w/v slurry in *carbon dioxide-free water*.

Other tests. Complies with the tests stated under Premixes.

Assay. *For amprolium hydrochloride* — Weigh accurately a quantity containing 50 mg of Amprolium Hydrochloride, shake continuously for 20 minutes with 100.0 ml of a mixture of 2 volumes of *methanol* and 1 volume of *water* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with the methanol-water mixture. To 4.0 ml of the resulting solution add 10.0 ml of *naphthalenediol reagent*, allow to stand for 20 minutes and measure the absorbance of the resulting solution at the maximum at about 520 nm (2.4.7), using as the blank a solution obtained by mixing 4.0 ml of a mixture of 2 volumes of *methanol* and 1 volume of *water* with 10.0 ml of *naphthalenediol reagent* and allowing to stand for 20 minutes. Calculate the content of $C_{14}H_{19}ClN_4,HCl$ from the absorbance obtained by carrying out the procedure simultaneously, using 4.0 ml of a 0.0025 per cent w/v solution of *amprolium hydrochloride RS* in a mixture of 2 volumes of *methanol* and 1 volume of *water* and beginning at the words, "To 4.0 ml of the resulting solution add 10.0 ml of.....".

For ethopabate — Weigh accurately a quantity containing 6 mg of Ethopabate, add 75 ml of *methanol*, shake continuously for 20 minutes, dilute to 100.0 ml with *methanol* and filter. To 10.0 ml of the filtrate add 10 ml of *1 M sodium hydroxide* and evaporate to dryness. Dissolve the residue in 10.0 ml of *water*, heat on a water-bath for 15 minutes, add 10 ml of *2 M hydrochloric acid*, dilute to 100.0 ml with *water* and filter. To 25.0 ml of the filtrate, add 2.5 ml of *2 M hydrochloric acid* and 5 ml of a 0.1 per cent w/v solution of *sodium nitrite* prepared immediately before use. Allow to stand for 3 minutes and add 2.0 ml of a freshly prepared 0.5 per cent w/v solution of *ammonium sulphamate*. Allow to stand for 2 minutes, add 5.0 ml of a freshly prepared 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, allow to stand for 10 minutes and dilute to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7), using as the blank a solution obtained by repeating the procedure with 25 ml of *water* and beginning at the words "add 2.5 ml of *2 M hydrochloric acid*.....". Calculate the content of $C_{12}H_{15}NO_4$ from the absorbance obtained by carrying out the procedure simultaneously, using 10.0 ml of a 0.006 per cent w/v solution of *ethopabate RS* in *methanol* and beginning at the words "add 10 ml of *1 M sodium hydroxide* and evaporate to dryness.....".

For sulphaquinoxaline — Weigh accurately a quantity containing 40 mg of Sulphaquinoxaline, shake continuously

for 10 minutes with a mixture of 75 ml of *water* and 4 ml of *2 M sodium hydroxide*, dilute to 250.0 ml with *water* and centrifuge. To 10.0 ml of the supernatant liquid add 5 ml of *2 M hydrochloric acid* and dilute to 200.0 ml with *water*. To 10.0 ml of the diluted solution add 2.5 ml of *2 M hydrochloric acid* and 5 ml of a freshly prepared 0.1 per cent w/v solution of *sodium nitrite* and allow to stand for 3 minutes. Add 5.0 ml of a freshly prepared 0.5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 2 minutes. Add 5.0 ml of a freshly prepared 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, allow to stand for 10 minutes and dilute to 50.0 ml with *water*. Measure the absorbance of the resulting solution at the maximum at about 540 nm (2.4.7), using as the blank the solution obtained by repeating the procedure with 10 ml of *water* and beginning at the words "add 2.5 ml of *2 M hydrochloric acid*.....". Calculate the content of $C_{14}H_{12}N_4O_2S$ from the absorbance obtained by carrying out the procedure simultaneously, using 10.0 ml of a 0.0008 per cent w/v solution of *sulphaquinoxaline RS* in *0.001 M sodium hydroxide* and beginning at the words "To 10.0 ml of the diluted solution add 2.5 ml of *2 M hydrochloric acid*.....".

Calcium Borogluconate Injection

Calcium Borogluconate Injection is a sterile solution of Calcium Gluconate and Boric Acid in Water for Injections. The solution may contain up to 0.2 per cent w/v of Chlorocresol.

Calcium Borogluconate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of calcium, Ca, and boric acid, H_3BO_3 equivalent to not more than 2.3 times the stated content of calcium.

Category. Hypocalcaemic.

Dose. *All species*. By subcutaneous, intravenous or intraperitoneal injection, the equivalent of 7.5 to 22.5 mg of calcium per kg of body weight.

(Each 100 mg of calcium borogluconate is approximately equivalent to 7.5 mg of calcium).

Usual strength. 25 per cent w/v solution equivalent to 1.9 per cent w/v of calcium (approximately).

Identification

A. Dilute 1 ml with sufficient *water* to produce a solution containing about 0.75 per cent w/v of calcium and add 0.05 ml of *ferric chloride test solution*; an intense yellow or yellowish green colour is produced.

B. Gives the reactions of calcium salts (2.3.1).

C. To 1 ml add 0.15 ml of *sulphuric acid* and 5 ml of *methanol* and ignite; the mixture burns with a flame tinged with green.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a solution diluted if necessary with *carbon dioxide-free water* to produce a solution containing 1.5 per cent w/v of calcium.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For calcium* — Dilute an accurately measured volume containing 45 mg of calcium to about 50 ml with *water*. Titrate with *0.05 M disodium edetate* to within a few ml of the expected end-point, add 4 ml of a 40 per cent w/v solution of *sodium hydroxide* and 10 mg of *calcon mixture* and continue the titration until the colour changes from pink to blue.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002004 g of Ca.

For boric acid — Dilute an accurately measured volume containing 0.1 g of boric acid to 50 ml with *water*, add 3 g of *mannitol* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006183 g of H_3BO_3.

Storage. Store protected from light, at a temperature not exceeding 30°.

Labelling. The label states (1) the strength in terms of the equivalent amount of calcium in a suitable dose-volume; (2) the proportion of boric acid present; (3) the proportion of chlorocresol, if present.

Calcium Magnesium Borogluconate Injection

Calcium Magnesium Borogluconate Injection is a sterile solution of Calcium Gluconate, Boric Acid, Magnesium Hypophosphite and Dextrose in Water for Injections. It may contain upto 0.2 per cent w/v of Chlorocresol.

Calcium Magnesium Borogluconate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of calcium, Ca, of magnesium, calculated as magnesium hypophosphite, $Mg(H_2PO_2)_2,6H_2O$, and of dextrose, $C_6H_{12}O_6$, and the content of boric acid, H_3BO_3, is not more than 2.3 times the stated content of calcium.

Usual strengths. Equivalent to 1.86 per cent w/v; 2.25 per cent w/v; 3.0 per cent w/v of Ca.

Identification

A. Dilute 1 ml with sufficient *water* to produce a solution containing about 0.75 per cent w/v of calcium and add 0.05 ml

of *ferric chloride test solution*; an intense yellow or yellowish green colour is produced.

B. Gives the reactions of calcium salts (2.3.1).

C. Gives the reactions of magnesium salts (2.3.1).

D. To 1 ml add 5 ml of *water*, neutralise to pH 7.0 with *dilute ammonia solution* and add 5 ml of *silver nitrate solution*. A yellow precipitate is produced which does not change colour on boiling but dissolves on addition of *dilute ammonia solution*.

E. To 1 ml add 0.15 ml of *sulphuric acid* and 5 ml of *methanol* and ignite; the mixture burns with a flame tinged with green.

F. To 1 ml add 2 ml of *2 M sodium hydroxide solution* and 0.05 ml of *copper sulphate solution*. The solution is blue and clear. Heat to boiling. A copious red precipitate is produced.

Tests

pH (2.4.24). 3.0 to 4.0, determined in a solution diluted, if necessary, with *carbon dioxide-free water* so as to contain 1.5 per cent w/v of calcium.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per ml.

Assay. *For calcium* — Dilute a volume containing 45 mg of calcium to about 50 ml with *water*. Add 1 ml of *1 M sodium hydroxide solution*. Titrate with *0.05 M disodium edetate* to within a few ml of the expected end-point, add 5 ml of *strong ammonia-ammonium chloride solution* and 10 mg of *calcon mixture* as indicator and continue the titration until the colour changes from pink to blue. Calculate the volume of *0.05 M disodium edetate* consumed by substracting the volume of *0.05 M disodium edetate* consumed in the assay for *magnesium*.

1 ml of *0.05 M disodium edetate* is equivalent to 0.002004 g of Ca.

For magnesium — Dilute a volume containing 10 mg of magnesium to about 50 ml with *water*. Add 1 g of *ammonium chloride* and 1 g of *ammonium oxalate*. Neutralise to litmus paper with *dilute ammonia solution* and add 5 ml in excess. Boil for 5 minutes and allow to stand for 1 hour. Filter and wash the residue with hot *water*. Collect the filtrate and washings and add 5 ml of *strong ammonia-ammonium chloride solution*. Titrate with *0.05 M disodium edetate* using *eriochrome black T mixture* as indicator.

1 ml of *disodium edetate* is equivalent to 0.001216 g of magnesium or 0.0107894 g of magnesium hypophosphite, $Mg(H_2PO_2)_2,6H_2O$.

For boric acid — Dilute a volume containing 0.1 g of boric acid to 50 ml with *water*, add 3 g of *mannitol* and titrate with *0.1 M sodium hydroxide* using *phenolphthalein solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.006183 g of H_3BO_3.

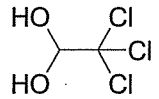
For dextrose — Dilute a volume containing 200 mg of dextrose to 50 ml with *water*; add 30 ml of *0.1 M iodine solution* and 10 ml of a 5 per cent w/v solution of *sodium carbonate* and allow to stand for 20 minutes. Add 15 ml of *1 M hydrochloric acid* and titrate the excess of iodine with *0.1 M sodium thiosulphate solution* using *starch solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M iodine solution* is equivalent to 0.009008 g of dextrose, $C_6H_{12}O_6$.

Storage. Store protected from light.

Labelling. The label states (1) the strength in terms of the equivalent amount of calcium and magnesium in a suitable dose-volume; (2) the proportion of boric acid to calcium; (3) the percentage of any added stabilising agent.

Chloral Hydrate



$C_2H_3Cl_3O_2$ Mol. Wt. 165.4

Chloral Hydrate is 2,2,2-trichloro-1,1-ethanediol.

Chloral Hydrate contains not less than 98.5 per cent and not more than 101.0 per cent of the stated amount of chloral hydrate, $C_2H_3Cl_3O_2$.

Category. Sedative and hypnotic.

Dose. *Cows, buffaloes and horses*. By intravenous injection, 60 to 100 mg per kg of body weight.

Description. A colourless, transparent crystals; odour, pungent.

Identification

A. To 10 ml of a 10 per cent w/v solution in *carbon dioxide-free water* (solution A) add 2 ml of *2 M sodium hydroxide*. The mixture becomes cloudy and when heated gives an odour of chloroform.

B. To 1 ml of solution A add 2 ml of *sodium sulphide solution*; a yellow colour develops which quickly turns reddish brown and may yield a red precipitate on standing.

Tests

pH (2.4.24). 3.5 to 5.5, determined in solution A.

Appearance of solution. Solution A is clear (2.4.1) and colourless (2.4.1).

Heavy metals (2.3.13). 12 ml of a 5 per cent w/v solution in *water*, complies with the limit test for heavy metals, Method D (20 ppm).

Chlorides (2.3.12). Dissolve 2.5 g in 15 ml with *water*; the resulting solution complies with the limit test for chlorides (100 ppm).

Chloral alcoholate. Warm 1 g with 10 ml of *2 M sodium hydroxide*, filter the upper layer and add *0.05 M iodine* dropwise until a yellow colour is produced. Set aside for 1 hour; no yellow precipitate is produced and no smell of iodoform is perceptible.

Assay. Weigh accurately about 4 g, dissolve in 10 ml of *water* and add 40 ml of *1 M sodium hydroxide*. Allow the mixture to stand for exactly 2 minutes and titrate the residual alkali immediately with *0.5 M sulphuric acid* using *phenolphthalein solution* as indicator.

1 ml of *1 M sodium hydroxide* is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

Storage. Store protected from moisture.

Chloramphenicol Injection

Chloramphenicol Injection is a sterile suspension of Chloramphenicol in Water for Injections containing suitable suspending and stabilising agents.

Chloramphenicol Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Usual strengths. 4.5 g in 30 ml; 5.0 g in 50 ml; 11.25 g in 75 ml; 15 g in 100 ml; 20 g in 100 ml.

Identification

Centrifuge a volume containing 0.15 g of Chloramphenicol; wash the residue with water and dry over *self-indicating silica gel* and then for 1 hour at 105°. The dried residue complies with the following tests.

A. Wash 75 mg of the residue with two quantities, each of 10 ml, of *light petroleum (60° to 80°)* and allow to dry. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *chloramphenicol RS* or with the reference spectrum of chloramphenicol.

B. In the test for Related substances, the principal spot in the chromatogram obtained with 1 µl of test solution corresponds to that in the chromatogram obtained with reference solution (a).

C. To 5 ml of a 0.1 per cent w/v *solution* add a few drops of *silver nitrate solution*; no precipitate is produced. Heat about 50 mg with 3 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 15 mg of *decolorising charcoal*, shake and filter. The filtrate gives the reactions of chlorides (2.3.1).

Tests

pH (2.4.24). 3.5 to 6.5.

Consistence. Chloramphenicol Injection containing 150 mg per ml passes readily through a 23G hypodermic needle.

2-Amino-1-(4-nitrophenyl)propane-1,3-diol Determine by liquid chromatography (2.4.14).

Test solution. Dilute the injection with sufficient of the mobile phase to produce a solution containing 0.030 per cent w/v of Chloramphenicol.

Reference solution. A 0.00225 per cent w/v of *2-amino-1-(4-nitrophenyl) propane-1,3-diol RS* in the mobile phase.

Chromatographic system

- a stainless steel column 10 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a filtered and degassed mixture of 85 volumes of *0.012 M sodium pentane- sulphonate*, 15 volumes of *acetonitrile* and 1 volume of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 272 nm,
- injection volume. 20 µl.

Inject alternatively the test solution and the reference solution. In the chromatogram obtained with test solution the area of any peak corresponding to 2-amino-1-(4- nitrophenyl)-propane-1,3-diol is not more than the area of the peak obtained with the reference solution.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *dichloromethane*, 10 volumes of *methanol* and 1 volume of *water*.

Test solution. A 1.0 per cent w/v solution of the dried residue obtained in the test for identification in *acetone*.

Reference solution (a). A 1.0 per cent w/v solution of *chloramphenicol RS* in *acetone*.

Reference solution (b). Dilute 1 ml of reference solution (a) to 200 ml with *acetone*.

Apply to the plate 1 µl and 20 µl of the test solution, 1 µl of reference solution (a) and 20 µl of reference solution (b). After

development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (b).

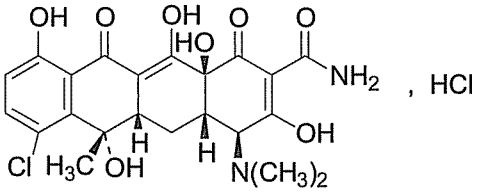
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing 0.75 g of chloramphenicol add sufficient *water* to produce 1000.0 ml and shake until a clear solution is obtained. Dilute 5.0 ml of this solution to 200.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 278 nm (2.4.7). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ in the injection taking 297 as the specific absorbance at 278 nm.

Storage. Store protected from light. Do not freeze.

Labelling. The label states (1) the name of any added suspending agent; (2) that the injection is for intramuscular injection only; (3) the date after which the contents are not intended to be used.

Chlortetracyline Hydrochloride



$C_{22}H_{23}ClN_2O_8,HCl$ Mol. Wt. 515.4

Chlortetracycline Hydrochloride is [4*S*-(4α,4aα,5aα,6β,12aα)]-7-chloro-4- dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide hydrochloride.

Chlortetracycline Hydrochloride contains not less than 89.5 per cent of chlortetracycline hydrochloride and the sum of the contents of chlortetracycline hydrochloride and tetracycline hydrochloride is not less than 94.5 per cent and not more than 100.5 per cent, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. *Horses, calves and pigs*. 10 to 20 mg per kg of body weight daily. *Dogs and cats*. 20 to 50 mg per kg of body weight daily. *Pigs and poultry*. By mass medication, 130 mg per litre of drinking water.

Cows, buffaloes and horses. By intra-uterine insertion, upto 1 g. *Pigs and sheep*. By intra-uterine insertion, upto 0.5 g.

Description. Yellow powder.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

NOTE — Use freshly prepared solution.

Mobile phase. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water*.

Test solution. Dissolve 50 mg of the substance under examination in 100 ml of *methanol*.

Reference solution (a). A 0.05 per cent w/v of *chlortetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *chlortetracycline hydrochloride RS*, *tetracycline hydrochloride RS* and *metacycline hydrochloride RS* in *methanol*.

Adjust the pH of a 10 per cent w/v solution of *disodium edetate* to 8.0 with *10 M sodium hydroxide* and spray this solution evenly on the plate (about 10 ml for a plate of 100 mm by 200 mm size). Allow the plate to dry in a horizontal position for at least 1 hour. Dry the plate in an oven at 100° for 1 hour before use. Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To about 2 mg add 5 ml of *sulphuric acid*; a deep blue colour develops which becomes bluish green. Add the solution to 2.5 ml of *water*; the colour changes to brownish.

C. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 2.3 to 3.3, determined in a 1 per cent w/v solution in *carbon dioxide-free water* prepared by slight heating, if necessary.

Specific optical rotation (2.4.22). –235° to –250°, determined at 20° in a 0.25 per cent w/v solution in *water*, calculated on the anhydrous basis.

Light absorption. When examined at 460 nm of a 0.5 per cent w/v solution in *water* is not more than 0.40.

Related substances. Carry out the method described under Assay injecting test solution, reference solutions (e) and (f). The test is not valid unless the peak in the chromatogram obtained with reference solution (f) is properly integrated. In the chromatogram obtained with test solution the area of the peak corresponding to 4-epichlortetracycline is not more than the area of the peak corresponding to 4-epichlortetracycline in the chromatogram obtained with reference solution (e)

(4 per cent) and the total area of any secondary peaks, other than the peaks due to tetracycline and 4-epichlortetracycline, is not more than 25 per cent of the area of the peak corresponding to 4-epichlortetracycline in the chromatogram obtained with reference solution (e) (1 per cent). Ignore any peak with an area smaller than that of the principal peak in the chromatogram obtained with reference solution (f) (0.1 per cent).

Tetracycline hydrochloride. Not more than 8.0 per cent, calculated on the anhydrous basis and determined as described under the Assay, injecting separately test solution and reference solution (e).

Heavy metals (2.3.13). 0.5 g complies with the limit test for heavy metals, Method D (50 ppm), using 2.5 ml of lead standard solution (10 ppm Pb) as the standard.

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Water (2.3.43). Not more than 2.0 per cent, determined on 0.3 g.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of *0.01 M hydrochloric acid.*

Reference solution (a). A 0.1 per cent w/v solution of *chlortetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (b). A 0.04 per cent w/v of *4-epichlortetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (c). A 0.08 per cent w/v of *tetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (d). Mix 5 ml of reference solution (a) and 10 ml of reference solution (b) and dilute to 25 ml with *0.01 M hydrochloric acid.*

Reference solution (e). Mix 5 ml of reference solution (b) and 5 ml of reference solution (c) and dilute to 50 ml with *0.01 M hydrochloric acid.*

Reference solution (f). Dilute 1 ml of reference solution (c) to 20 ml with *0.01 M hydrochloric acid* and dilute 2.5 ml of this solution to 100 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm × 4.6 mm, packed with octylsilane groups (5 µm),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 450 ml of *dimethyl sulphoxide*, 50 ml of *1 M perchloric acid* and 500 ml of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (d) and adjust the instrument so that the peak heights correspond to at least 50 per cent of the full scale deflection of the recorder. If necessary, adjust the dimethyl sulphoxide content in the mobile phase. The test is not valid unless the resolution factor between the first peak (4-epichlortetracycline) and the second (chlortetracycline) is not less than 2.0 and the symmetry factor for the second peak is not more than 1.3.

Inject reference solution (a). The test is not valid unless the relative standard deviation of the peak area for chlortetracycline hydrochloride is not more than 1.0 per cent. If necessary, adjust the integrator parameters.

Inject alternately the test solution and reference solution (a).

Calculate the content of $C_{22}H_{23}ClN_2O_8,HCl$.

Chlortetracycline Hydrochloride intended for use in the manufacture of Parenteral Preparations without a further appropriate procedure for removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 1.1 EU per mg.

Chlortetracycline Hydrochloride intended for use in the manufacture of Parenteral Preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light. If it is intended for use in the manufacture of Parenteral Preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the date after which the material is not intended to be used; (2) the storage conditions; (3) where applicable, that the material is sterile and free from Bacterial endotoxins.

Chlortetracycline Veterinary Oral Powder

Chlortetracycline Hydrochloride Veterinary Oral Powder; Chlortetracycline Soluble Powder

Chlortetracycline Veterinary Oral Powder is a mixture of Chlortetracycline Hydrochloride and Lactose or other suitable diluent.

Chlortetracycline Veterinary Oral Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of chlortetracycline hydrochloride, $C_{22}H_{23}ClN_2O_8,HCl$

Usual strength. 5.5 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H.*

NOTE — Use freshly prepared solutions.

Mobile phase. A mixture of 59 volumes of *dichloromethane*, 35 volumes of *methanol* and 6 volumes of *water.*

Test solution. The supernatant liquid obtained by extracting a quantity containing 5 mg of Chlortetracycline Hydrochloride with 10 ml of *methanol* and centrifuging.

Reference solution (a). A 0.05 per cent w/v of *chlortetracycline hydrochloride RS* in *methanol.*

Reference solution (b). A solution containing 0.05 per cent w/v each of *chlortetracycline hydrochloride RS*, *tetracycline hydrochloride RS* and *metacycline hydrochloride RS* in *methanol.*

Adjust the pH of a 10 per cent w/v solution of *disodium edetate* to 8.0 with *10 M sodium hydroxide* and spray this solution evenly on the plate (about 10 ml for a plate of 100 mm by 200 mm size). Allow the plate to dry in a horizontal position for at least 1 hour. Dry the plate in an oven at 100° for 1 hour before use. Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To a quantity containing 10 mg of Chlortetracycline Hydrochloride, add 20 ml of warm *ethanol (95 per cent)*, allow to stand for 20 minutes, filter and evaporate to dryness on a water-bath. Dissolve the residue in sufficient *phosphate buffer pH 7.6* to produce a 0.1 per cent w/v solution and heat at 100° for 1 minute; it exhibits a strong blue fluorescence in ultra-violet light.

Tests

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 100.0 ml of *0.01 M hydrochloric acid.*

Reference solution (a). A 0.1 per cent w/v solution of *chlortetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (b). A 0.04 per cent w/v solution of *4-epichlortetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (c). A 0.08 per cent w/v solution of *tetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (d). Mix 5.0 ml of reference solution (a) and 10.0 ml of reference solution (b) and dilute to 25 ml with *0.01 M hydrochloric acid.*

Reference solution (e). Mix 5.0 ml of reference solution (b) and 5.0 ml of reference solution (c) and dilute to 50 ml with *0.01 M hydrochloric acid.*

Reference solution (f). Dilute 1.0 ml of reference solution (c) to 20 ml with *0.01 M hydrochloric acid.* Further, dilute 2.5 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature.35°,
- mobile phase: a mixture of 45 ml of *dimethyl sulphoxide*, 5 ml of *1 M perchloric acid* and 50 ml of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject reference solution (d). Adjust the instrument so that the peak heights correspond to at least 50 per cent of the full scale deflection of the recorder. If necessary, adjust the *dimethyl sulphoxide* content in the mobile phase. The test is not valid unless the resolution factor between the first peak (4-epichlortetracycline) and the second (chlortetracycline) is not less than 2.0 and the symmetry factor for the second peak is not more than 1.3.

Inject reference solution (a). The test is not valid unless the relative standard deviation of the peak area for chlortetracycline hydrochloride is not more than 1.0 per cent.

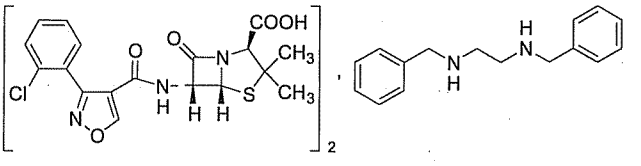
Inject the test solution and reference solution (a).

Calculate the content of $C_{22}H_{23}ClN_2O_8,HCl$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Labelling. The label states the strength in terms of the concentration of Chlortetracycline Hydrochloride

Cloxacillin Benzathine



$(C_{19}H_{18}ClN_3O_5S)_2,C_{16}H_{20}N_2$ Mol. Wt. 1112.1

Cloxacillin Benzathine is *N,N'* -dibenzylethylenediammonium bis-[(6*R*)-6-{3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido}penicillanate].

Cloxacillin Benzathine contains not less than 92.0 per cent of $(C_{19}H_{18}ClN_3O_5S)_2,C_{16}H_{20}N_2$ and not less than 20.0 per cent and not more than 22.0 per cent of benzathine, $C_{16}H_{20}N_2$, both calculated on the anhydrous basis.

Category. Antibacterial.

Dose. Dry cows/buffaloes. By intramammary infusion into each quarter, alone or in combination with Ampicillin Trihydrate, the equivalent of 500 mg of cloxacillin as a single dose.

(Each 1.28 g of cloxacillin benzathine is approximately equivalent to 500 mg of cloxacillin).

Description. A white or almost white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin benzathine RS* or with the reference spectrum of cloxacillin benzathine.

B. Shake 0.1 g with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *dilute potassium dichromate solution*; a golden yellow precipitate is produced.

C. Shake 50 mg with 10 ml of *water* and filter. To 5 ml of the filtrate add a few drops of *silver nitrate solution*; no precipitate is produced. Heat 50 mg with 2 ml of *ethanolic potassium hydroxide solution* on a water-bath for 15 minutes, add 15 mg of *decolorising charcoal*, shake and filter. Acidify the filtrate with *2 M nitric acid*; the solution gives reaction A of chlorides (2.3.1).

Tests

Water (2.3.43). Not more than 5.0 per cent w/w, determined on 0.5 g.

Assay. *For cloxacillin benzathine* — Weigh accurately about 60 mg, add 40 ml of *methanol*, shake to dissolve, add 25 ml of *1 M sodium hydroxide* and allow to stand for 30 minutes. Add 27.5 ml of *1 M hydrochloric acid* and sufficient *water* to produce 100.0 ml, mix, transfer 20.0 ml of the solution to a stoppered conical flask, add 30.0 ml of *0.01 M iodine*, close the flask with a wet stopper and allow to stand for 15 minutes protected from light. Titrate the excess of iodine with *0.02 M sodium thiosulphate*, using *starch mucilage*, added towards the end of the titration, as indicator. Add a further 12 mg of the substance under examination to 10 ml of *water*, swirl to disperse, add 30 ml of *0.01 M iodine* and titrate immediately with *0.02 M sodium thiosulphate*, using *starch mucilage*, added towards the end of the titration, as indicator. The difference between the titrations represents the volume of *0.01 M iodine* equivalent to the total penicillins present.

Calculate the content of $(C_{19}H_{18}ClN_3O_5S)_2,C_{16}H_{20}N_2$ from the difference obtained by carrying out the procedure simultaneously using *cloxacillin benzathine RS*.

For benzathine — Weigh accurately about 1 g, add 30 ml of a saturated solution of *sodium chloride* and 10 ml of *5 M sodium hydroxide*, shake well and extract with four quantities, each of 50 ml, of *ether*. Wash the combined extracts with three quantities, each of 10 ml, of *water*, extract the combined washings with 25 ml of *ether* and add the extract to the main ether solution. Evaporate the ether solution to low volume, add 2 ml of *ethanol* and evaporate to dryness. To the residue add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using 0.1 ml of *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01202 g of $C_{16}H_{20}N_2$.

Cloxacillin Benzathine intended for use in the manufacture of either parenteral preparations or intramammary infusions without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture. If it is intended for use in the manufacture of parenteral preparations or intramammary infusions, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Cloxacillin Benzathine Intramammary Infusion (Dry Cow/ Buffalo)

Cloxacillin Benzathine Intramammary Injection; Cloxacillin Intramammary Infusion (Dry Cow/Buffalo); Cloxacillin Intramammary Infusion (DC/B)

Cloxacillin Benzathine Intramammary Infusion (Dry Cow/ Buffalo) is a sterile suspension of Cloxacillin Benzathine in a suitable non-aqueous vehicle containing suitable suspending agents.

Cloxacillin Benzathine Intramammary Infusion (Dry Cow/ Buffalo) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strength. The equivalent of 500 mg of cloxacillin.

Identification

Extract a quantity containing 75 mg of cloxacillin with three quantities, each of 15 ml, of *light petroleum (120° to 160°)*. Discard the extracts, wash the residue with 10 ml of *ether* and dry in a current of air. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin benzathine RS* or with the reference spectrum of cloxacillin benzathine.

B. Shake 50 mg with 1 ml of *1 M sodium hydroxide* for 2 minutes, add 2 ml of *ether*, shake for 1 minute and allow to separate. Evaporate 1 ml of the ether layer to dryness, dissolve the residue in 2 ml of *glacial acetic acid* and add 1 ml of *dilute potassium dichromate solution*; a golden yellow precipitate is produced.

Tests

Water (2.3.43). Not more than 2.0 per cent, determined on 3 g and using a mixture of 70 volumes of *dichloromethane* and 30 volumes of *anhydrous methanol* as the solvent.

Other tests. Complies with the tests stated under Intramammary Infusions.

Assay. Weigh and mix the contents of 10 containers. Weigh accurately a quantity of the mixed contents containing 80 mg of cloxacillin and extract with three quantities, each of 15 ml, of *light petroleum (120° to 160°)* previously saturated with cloxacillin benzathine. Discard the extracts, wash the residue with *ether* previously saturated with cloxacillin benzathine. Dry in a current of air, dissolve in 25 ml of *methanol* and dilute to 50.0 ml with *water*. Dilute 2.0 ml to 100.0 ml with *buffered cupric sulphate solution pH 2.0*, transfer 10.0 ml to a stoppered test-tube and heat in a water-bath at 70° for 20 minutes. Cool to room temperature rapidly, dilute to 20.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 338 nm (2.4.7), using as the blank 10.0 ml of the unheated buffered solution of the substance under examination after dilution to 20.0 ml with *ethanol*.

Calculate the content of $C_{19}H_{18}ClN_3O_5S$ in a container of average weight from the absorbance obtained by carrying out the procedure simultaneously using 2.0 ml of a solution prepared by dissolving 105 mg of *cloxacillin benzathine RS* in 50.0 ml of a mixture of equal volumes of *methanol* and *water*.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin in the sealed container.

Cloxacillin Sodium Intramammary Infusion (Lactating Cow/Buffalo)

Cloxacillin Intramammary Injection; Cloxacillin Intramammary Infusion (Lactating Cow/Buffalo); Cloxacillin Intramammary Infusion (LC/B)

Cloxacillin Sodium Intramammary Infusion (Lactating Cow/Buffalo) is a sterile suspension of Cloxacillin Sodium in a suitable non-aqueous vehicle containing suitable suspending and dispersing agents.

Cloxacillin Sodium Intramammary Infusion (Lactating Cow/Buffalo) contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of cloxacillin, $C_{19}H_{18}ClN_3O_5S$.

Usual strength. Equivalent of 200 mg of cloxacillin.

Identification

Extract a quantity containing 75 mg of cloxacillin with three quantities, each of 15 ml, of *light petroleum (120° to 160°)*. Discard the extracts, wash the residue with 10 ml of *ether* and dry in a current of air. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *cloxacillin sodium RS* or with the reference spectrum of cloxacillin sodium.

B. Gives reaction A of sodium salts (2.3.1).

Tests

Water (2.3.43). Not more than 1.0 per cent, determined on 3 g using a mixture of 70 volumes of *dichloromethane* and 30 volumes of *anhydrous methanol* as the solvent.

Other tests. Complies with the tests stated under Intramammary Infusions.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of mixed contents of 10 containers containing about 50 mg of cloxacillin with 15 ml of *petroleum spirit (boiling range 120° to 160°)*, centrifuge and discard the supernatant liquid. Repeat the extraction with a further two 15 ml quantities of *petroleum spirit (boiling range 120° to 160°)*. Shake the residue with 20 ml of *ether*, centrifuge and dry in a current of air until the solvent get evaporated. Dissolve the final residue in 50 ml of the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

Reference solution (a). A 0.011 per cent w/v each of *cloxacillin sodium RS* in the mobile phase.

Reference solution (b). A solution containing 0.01 per cent w/v each of *cloxacillin sodium RS* and *flucloxacillin sodium RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 25 volumes of *acetonitrile* and 75 volumes of 0.27 per cent w/v solution of *potassium dihydrogen orthophosphate*, adjusted to pH 5.0 with *2 M sodium hydroxide*,

- flow rate. 1 ml per minute,
- spectrophotometer set at 225 nm,
- injection volume.20 µl.

Inject reference solution (b). The test is not valid unless the resolution between the peaks due to cloxacillin and flucoxacillin is not less than 2.5.

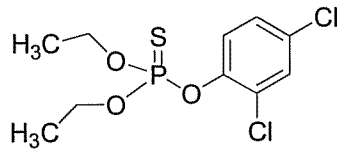
Inject the test solution and reference solution (a).

Calculate the content of $C_{19}H_{18}ClN_3O_5S$.

1 mg of $C_{19}H_{17}ClN_3NaO_5S$ is equivalent to 0.952 mg of $C_{19}H_{18}ClN_3O_5S$.

Labelling. The label states the strength in terms of the equivalent amount of cloxacillin.

Dichlofenthion



$C_{10}H_{13}Cl_2O_3PS$ Mol. Wt. 315.2

Dichlofenthion is *O*-2,4-dichlorophenyl-*O*,*O*-diethyl phosphorothioate.

Dichlofenthion contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{10}H_{13}Cl_2O_3PS$.

Category. Insecticide.

Description. A colourless or pale yellow, oily substance.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dichlofenthion RS* or with the reference spectrum of dichlofenthion.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 95 volumes of *hexane* and 5 volumes of *2-butanone*.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of *methanol*.

Reference solution. A 0.5 per cent w/v solution of *dichlofenthion RS* in *methanol*.

Apply to the plate 2 µl of each solution. After development, dry the plate in air and spray with a 2 per cent w/v solution of *4-(4-nitrobenzyl)pyridine* in *ethyl acetate*. Heat the plate at 130° for 10 minutes, allow to cool and spray with a 2 per cent w/v solution of *lithium hydroxide* in a mixture of 8 volumes of *methanol*, 1 volume of *diethylene glycol* and 1 volume of *water*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

C. Burn 50 mg by the oxygen-flask method (2.3.34), using 20 ml of *1 M sodium hydroxide* as the absorbing liquid. The solution obtained, after acidification with *2 M nitric acid* gives reaction A of chlorides and reaction C of phosphates (2.3.1).

Tests

Refractive index (2.4.27). 1.530 to 1.533.

Weight per ml (2.4.29). 1.296 to 1.316 g.

Assay. Determine by gas chromatography (2.4.13).

Test solution (a). Dissolve 0.3 g of the substance under examination in 100 ml of *dichloromethane*.

Test solution (b). A solution containing 0.3 per cent w/v of the substance under examination and 0.2 per cent w/v of *methyl stearate* (internal standard) in *dichloromethane*.

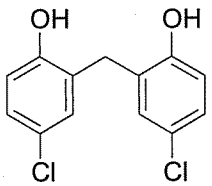
Reference solution. A solution containing 0.3 per cent w/w of *dichlofenthion RS* and 0.2 per cent w/v of *methyl stearate* (internal standard) in *dichloromethane*.

Chromatographic system

- a glass column 1.5 m × 4 mm, packed with 3 per cent w/w of phenyl methyl silicone fluid (50 per cent phenyl) on acid-washed, silanised diatomaceous support (80 to 100 mesh) (such as OV-17),
- temperature:
  column 190°,
  inlet port and detector. 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{10}H_{13}Cl_2O_3PS$.

Dichlorophen



$C_{13}H_{10}Cl_2O_2$ Mol. Wt. 269.1

Dichlorophen is 2,2'-methylenebis(4-chlorophenol).

Dichlorophen contains not less than 97.0 per cent and not more than 101.0 per cent of $C_{13}H_{10}Cl_2O_2$, calculated on the dried basis.

Category. Anthelmintic and fungicide.

Dose. *Dogs and cats*. As anthelmintic, 200 mg per kg of body weight.

Description. A white or almost white powder; odour, slightly phenolic.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 245 nm and 304 nm. The absorbances of the solution after further dilution with an equal volume of *0.1 M sodium hydroxide* at these maxima are about 0.65 and 0.27 respectively.

B. Dissolve 0.2 g in 10 ml of *2.5 M sodium hydroxide*, cool in ice and add a solution prepared by mixing 1 ml of *sodium nitrite solution* with a cold solution containing 0.15 ml of *aniline* in a mixture of 4 ml of *water* and 1 ml of *hydrochloric acid*; a reddish-brown precipitate is produced.

C. Fuse 0.5 g with 2 g of *anhydrous sodium carbonate*, cool, extract the residue with *water* and filter. The filtrate gives reaction A of chlorides (2.3.1).

D. Melting point (2.4.21). about 175°.

Tests

Chlorides (2.3.12). Shake 3.0 g with 6 ml of *ethanol (95 per cent)*, dilute with *water* to 100 ml, allow to stand for 5 minutes and filter. 25 ml of the filtrate complies with the limit test for chlorides (330 ppm).

Sulphates (2.3.17). Shake 1.0 g with 20 ml of *water* for 2 minutes and filter. 5 ml of the filtrate complies with the limit test for sulphates (600 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10 ml of the mobile phase.

Reference solution (a). A 1.0 per cent w/v solution of *dichlorophen impurity standard RS* in the mobile phase.

Reference solution (b). A 0.0010 per cent w/v solution of *4-chlorophenol* in the mobile phase.

Chromatographic system
- a stainless steel column 20 cm × 5 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Spherisorb ODS 1),
- mobile phase: a filtered and degassed mixture of 75 volumes of *methanol*, 25 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of the peak corresponding to 4-dichlorophenol is not more than the area of the principal peak in the chromatogram obtained with reeference solution (b). The content of 4,4′-dichloro-2,2′-(2-hydroxy-4-chloro-*m*-xylene-a,a-diyl)diphenol in the substance under examination does not exceed 8.0 per cent w/w and the sum of the contents of any other impurities, excluding 4-chlorophenol, is not more than 2.0 per cent w/w.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 3 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in 20 ml of *2-propanol*. Titrate with *0.1 M tetrabutylammonium hydroxide*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02691 g of $C_{13}H_{10}Cl_2O_2$.

Labelling. The label states that the substance is intended for animal treatment only.

Dichlorophen Veterinary Aerosol

Dichlorophen Veterinary Aerosol Spray; Dichlorophen Veterinary Spray

Dichlorophen Veterinary Aerosol is a solution of Dichlorophen in a suitable solvent to which suitable propellants have been added. It may contain a suitable dye as a marker.

Dichlorophen Veterinary Aerosol contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of dichlorophen, $C_{13}H_{10}Cl_2O_2$.

Usual strengths. 2 per cent w/w; 7 per cent w/w; 7.5 per cent w/w; 10 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *hexane* and 40 volumes of *acetone*.

Test solution. Solution A obtained in the Assay diluted with *methanol* to contain the equivalent of 1 per cent w/v of Dichlorophen.

Reference solution. A 1.0 per cent w/v solution of *dichlorophen RS* in *methanol*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a freshly prepared solution containing 3.5 per cent w/v of *ferric chloride* and 0.25 per cent w/v of *potassium ferricyanide*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Veterinary Aerosols.

Assay. Weigh the intact container. Place the container in an ice-bath for 15 minutes. Make a small hole about 1 cm from the top of the body of the container and let the propellant escape. When the flow of propellant stops, enlarge the hole. Transfer the contents of the container to a tared vessel capable of being fitted with a reflux condenser. Remove the top of the container carefully retaining all fragments. Wash the container with suitable solvents and add the washings to the tared vessel. Dry the container and reweigh to obtain the net weight of the contents. Heat the contents of the tared vessel under reflux for 30 minutes, cool and weigh (solution A). Dilute an accurately measured volume of the resulting solution containing about 0.25 g of Dichlorophen to 100.0 ml with *acetone*. Dilute 2.0 ml of this solution to 200.0 ml with *ammonia buffer pH 10.9* and mix. To 10.0 ml of the resulting solution add 20 ml of *ammonia buffer pH 10.9* and 2 ml of a freshly prepared 2 per cent w/v solution of *4-aminophenazone*, mix, and add 2 ml of a freshly prepared 8 per cent w/v solution of *potassium ferricyanide*. Dilute to 50.0 ml with *ammonia buffer pH 10.9* and allow to stand for 15 minutes. Measure the absorbance of the resulting solution at the maximum at about 510 nm (2.4.7), using as the blank a solution obtained in a similar manner by carrying out the procedure simultaneously, beginning at the words "To 10.0 ml of the resulting solution....." but omitting the 4-aminophenazone solution. Calculate the weight of $C_{13}H_{10}Cl_2O_2$ in the container from the absorbance obtained by repeating the operation using a 0.25 per cent w/v solution of *dichlorophen* in *acetone* beginning at the words "Dilute 2.0 ml.....".

Labelling. The label states (1) the weight of Dichlorophen present in the container; (2) the total weight of contents; (3) the name and proportion of any added dye.

Dichlorophen Tablets

Dichlorophen Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dichlorophen, $C_{13}H_{10}Cl_2O_2$.

Usual strength. 500 mg.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Dichlorophen with 50 ml of *0.1 M sodium hydroxide* for 15 minutes, add sufficient *0.1 M sodium hydroxide* to produce 100 ml, centrifuge and dilute a suitable volume of the supernatant liquid with *0.1 M sodium hydroxide* to produce a solution containing 0.002 per cent w/v of Dichlorophen.

When examined in the range 220 to 360 nm (2.4.7), the resulting solution shows absorption maxima at about 245 nm and 304 nm; absorbances at about 245 nm and 304 nm, about 1.3 and 0.54 respectively.

B. Shake a quantity of the powdered tablets containing 0.2 g of Dichlorophen with a mixture of 5 ml of *water* and 5 ml of *5 M sodium hydroxide*, filter, cool in ice and add a solution prepared by mixing 1 ml of *sodium nitrite solution* with a cold solution containing 0.15 ml of *aniline* in a mixture of 4 ml of *water* and 1 ml of *hydrochloric acid*; a reddish-brown precipitate is produced.

C. Fuse a quantity of the powdered tablets containing 0.5 g of Dichlorophen with 2 g of *anhydrous sodium carbonate*, cool, extract the residue with *water* and filter. The filtrate gives reaction A of chlorides (2.3.1).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Shake a quantity of the powdered tablets containing 0.50 g of Dichlorophen with 20 ml of *methanol* for 10 minutes, filter, add 7 ml of *water* and dilute to 50 ml with the mobile phase.

Reference solution (a). A 1.0 per cent w/v solution of *dichlorophen impurity standard RS* in the mobile phase.

Reference solution (b). A 0.0010 per cent w/v solution of *4-chlorophenol* in the mobile phase.

Chromatographic system

- a stainless steel column 20 cm × 5 mm, packed with octadecylsilane bonded to porous silica (10 µm) (such as Spherisorb ODS 1),
- mobile phase: a filtered and degassed mixture of 75 volumes of *methanol*, 25 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 1.5 ml per minute,
- spectrophotometer set at 280 nm,
- injection volume. 20 µl.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the area of the peak corresponding to 4-dichlorophenol is not more than the area of the principal peak in the chromatogram obtained with reeference solution (b). The content of 4,4′-dichloro-2,

2′-(2-hydroxy-4-chloro-*m*-xylene-a,a′ -diyl) diphenol in the substance under examination does not exceed 8.0 per cent w/w and the sum of the contents of any other impurities, excluding 4-chlorophenol, is not more than 2.0 per cent w/w.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.1 g of Dichlorophen, shake with 50 ml of *0.1 M sodium hydroxide* for 15 minutes and add sufficient *0.1 M sodium hydroxide* to produce 100.0 ml. Centrifuge and dilute 10.0 ml of the clear supernatant liquid to 100.0 ml with *0.1 M sodium hydroxide*. Dilute 20.0 ml of this solution to 100.0 ml with *0.1 M sodium hydroxide* and measure the absorbance of the resulting solution at the maximum at about 304 nm (2.4.7). Calculate the content of $C_{13}H_{10}Cl_2O_2$ taking 275 as the specific absorbance at 304 nm.

Diethylcarbamazine Injection

Diethylcarbamazine Citrate Injection.

Diethylcarbamazine Injection is a sterile solution of Diethylcarbamazine Citrate in Water for Injections.

Diethylcarbamazine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of diethylcarbamazine citrate, $C_{10}H_{21}N_3O,C_6H_8O_7$.

Usual strength. 400 mg in 1 ml.

Identification

A. To a volume containing 0.5 g of Diethylcarbamazine Citrate add 2 ml of *water* and make alkaline with *5 M sodium hydroxide*. Extract with four quantities, each of 5 ml, of *dichloromethane*, reserve the aqueous solution for test B, wash the combined dichloromethane extracts with *water* and remove the dichloromethane by evaporation. Add 0.5 ml of *iodoethane* to the residue and heat gently under a reflux condenser for 5 minutes. Remove the excess iodoethane with a current of air, dissolve the viscous yellow oil in 2 ml of *ethanol (95 per cent)* and add, with continuous stirring, sufficient *ether* to precipitate the quaternary ammonium salt. Decant off the ether, dissolve the residue in 2 ml of *ethanol (95 per cent)*, reprecipitate with *ether* and dry at 105°; the residue melts at about 152° (2.4.21).

B. Neutralise the aqueous solution obtained in test A with *1 M sulphuric acid*, add an excess of *mercuric sulphate solution*, boil and add a few drops of *potassium permanganate solution*; a white precipitate is produced.

Tests

pH (2.4.24). 6.0 to 7.0.

N,N'-Dimethylpiperazine* and *N-methylpiperazine. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 65 volumes of *methanol*, 30 volumes of *2-butanone* and 5 volumes of *strong ammonia solution*.

Test solution. Dilute a volume of the injection with sufficient *methanol* to produce a solution containing the equivalent of 5.0 per cent w/v of Diethylcarbamazine Citrate.

Reference solution (a). A 5.0 per cent w/v solution of *diethylcarbamazine citrate RS* in *methanol*.

Reference solution (b). A 0.010 per cent w/v solution of *N,N′-dimethylpiperazine* in *methanol*.

Reference solution (c). A 0.010 per cent w/v solution *N-methylpiperazine* in *methanol*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate at 105° and expose it to iodine vapours for 30 minutes. Any spots corresponding to *N,N'-dimethylpiperazine and N-* methylpiperazine in the chromatogram obtained with the test solution are not more intense than the spots in the chromatograms obtained with reference solutions (b) and (c) respectively.

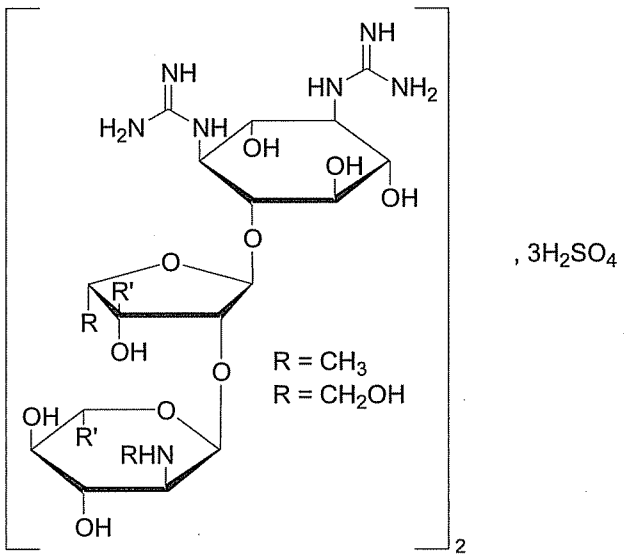
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing 8 g of Diethylcarbamazine Citrate add sufficient *water* to produce 100.0 ml. To 10.0 ml of this solution add 2 ml of *5 M sodium hydroxide* and extract with four quantities, each of 25 ml, of *dichloromethane*. Wash each extract with the same two quantities, each of 20 ml, of *water* and with a third quantity if the second becomes alkaline to *phenolphthalein solution*. Extract the combined dichloromethane extracts in succession with 25.0 ml of *0.05 M sulphuric acid* and 15 ml and 10 ml of *water*. Combine the acid and water extracts, remove the dichloromethane, by warming, cool and titrate the excess of acid with *0.1 M sodium hydroxide* using *bromocresol green solution* as indicator.

1 ml of *0.05 M sulphuric acid* is equivalent to 0.03914 g of $C_{10}H_{21}N_3O,C_6H_8O_7$.

Storage. Store protected from light.

Dihydrostreptomycin Sulphate



, $3H_2SO_4$

$(C_{21}H_{41}N_7O_{12})_2,3H_2SO_4$ Mol. Wt. 1461.4

Dihydrostreptomycin sulphate is *O*-2-deoxy-2-methylamino-*d*-L-lyxofuranosyl-(1→4)-N^1,N^3-diamidino-D-streptamine sulphate.

Dihydrostreptomycin Sulphate contains not less than 95.0 per cent and not more than 102.0 per cent sum of $C_{42}H_{88}N_{14}O_{36}S_3$ and $C_{42}H_{84}N_{14}O_{36}S_3$, calculated on dried basis.

Category. Antibacterial.

Dose. *All species except cats*. By subcutaneous or intramuscular injection, the equivalent of 10 mg of dihydrostreptomycin per kg of body weight twice daily.

(Each 12.8 mg of dihydrostreptomycin sulphate is approximately equivalent to 10 mg of dihydrostreptomycin).

Description. A white or almost white powder; may be hygroscopic.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate in the following manner. Mix 0.3 g of *carbomer* with 240 ml of *water*, allow to stand with moderate stirring for 1 hour, adjust to pH 7.0 by the gradual addition with constant shaking of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. Dissolve 100 mg of the substance under examination in 100 ml of *water*.

Reference solution (a). A 0.10 per cent w/v of *dihydrostreptomycin sulphate RS* in *water*.

Reference solution (b). A solution containing 0.10 per cent w/v of *dihydrostreptomycin sulphate RS*, 0.10 per cent w/v of *neomycin sulphate RS* and 0.10 per cent w/v of *kanamycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray it with a mixture of equal volumes of a 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and a 46 per cent w/v solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.1 g in 2 ml of *water* and add 1 ml of *dilute 1-naphthol solution* and 2 ml of a mixture of equal volumes of *sodium hypochlorite solution (3* per cent *Cl)* and *water*; a red colour is produced.

C. Dissolve 10 mg in 5 ml of *water* and add 1 ml of *1 M hydrochloric acid*. Heat in a water-bath for 2 minutes. Add 2 ml of a 0.5 per cent w/v solution of *1-naphthol* in *1 M sodium hydroxide* and heat in a water-bath for 1 minute; a violet-pink colour is produced (distinction from streptomycin).

D. Gives the reactions of sulphates (2.3.1).

Tests

Appearance of solution. A 25 per cent w/v solution in *carbon dioxide-free water* is not more intensely coloured than degree 4 of the appropriate range of reference solutions (2.4.1). The solution, after standing protected from light at a temperature of about 20° for 24 hours, is not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 5.0 to 7.0, determined in a 25 per cent w/v solution.

Specific optical rotation (2.4.22). –83° to –91°, calculated on the dried basis, determined in a 2 per cent w/v solution in *water*.

Sulphate. 18.0 per cent to 21.5 per cent, calculated on the dried basis.

Dissolve 0.25 g in 100 ml of *water*, adjust the pH to 11 with *strong ammonia solution* and add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate the excess of barium chloride with *0.1 M disodium edetate*, adding 50 ml of *ethanol (95 per cent)* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of sulphate, SO_4.

Streptomycin. Weigh accurately about 0.10 g and dissolve in sufficient *water* to produce 5.0 ml. Add 5.0 ml of *0.2 M sodium hydroxide* and heat for exactly 10 minutes in a water-bath. Cool in ice for exactly 5 minutes, add 3 ml of a 1.5 per cent w/v solution of *ferric ammonium sulphate* in *0.25 M sulphuric acid* and sufficient *water* to produce 25.0 ml, and mix. Exactly 20 minutes after the addition of the ferric ammonium sulphate solution, measure the absorbance of a 2-cm layer at the maximum at about 525 nm (2.4.7), using as the blank a solution prepared in the same manner, omitting the substance under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously using 5.0 ml of a solution prepared by dissolving 10 mg, accurately weighed, of *streptomycin sulphate RS* in sufficient *water* to produce 50 ml and beginning at the words "Add 5.0 ml....", both absorbances being calculated on the dried basis.

Methanol. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 4.0 g of the substance under examination in 100 ml of *water*.

Reference solution. A 0.008 per cent w/v of *methanol*.

Chromatographic system
- a glass column 1.5 to 2.0 m × 2 to 4 mm, packed with ethylvinylbenzenedivinylbenzene copolymer (150 to 180 mm) porous polymer beads (such as Porapak Q),
- temperature:
  column 50°,
  inlet port and detector. 280°,
- flow rate. 30 to 40 ml per minute of the carrier gas.

The area of any peak corresponding to methanol in the chromatogram obtained with test solution is not more than that of the peak in the chromatogram obtained with reference solution (0.2 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 5 per cent, determined on 1 g by drying over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 4 hours.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 50 mg of the substance under examination in 10 ml of *water*.

Reference solution. Dissolve the contents of a vial of *dihydrostreptomycin sulphate RS* (containing impurities A,B,C) in 5.0 ml of *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm),
- coloumn temperature. 45°,
- mobile phase: mix 4.6 g of *anhydrous sodium sulphate*, 1.5 g of *sodium octanesulphonate*, 120 ml of *acetonitrile* and 50 ml of 2.72 per cent w/v solution of *potassium dihydrogen phosphate* and dilute to 1000 ml with *water*, adjusted to pH 3.0 with 2.25 per cent *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 205 nm,
- injection volume. 20 µl.

The relative retention time with reference to dihydrostreptomycin for impurity A is about 0.2, for impurity B is about 0.8, for streptomycin is about 0.9 and for impurity C is about 0.95.

Inject the reference solution. Run the chromatogram 1.5 times the retention time of dihydrostgreptomycin.

Inject the test solution and the reference solution.

Calculate the content of $C_{42}H_{88}N_{14}O_{36}S_3$ and of $C_{42}H_{84}N_{14}O_{36}S_3$. Calculate the sum of these contents.

Dihydrostreptomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg of dihydrostreptomycin sulphate.

Dihydrostreptomycin Sulphate intended for use in the manufacture of parenteral preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light, at a temperature not exceeding 30°. If it is intended for use in the manufacture of parenteral preparations or intramammary infusions, the container should be sterile and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) the name and quantity of any added stabiliser; (3) whether or not the contents are intended for use in the manufacture of Parenteral Preparations or intramammary infusions; (4) that the substance is meant for veterinary use only; (5) the storage conditions; (6) the date after which the contents are not intended to be used.

Dihydrostreptomycin Injection

Dihydrostreptomycin Sulphate Injection.

Dihydrostreptomycin Injection is a sterile solution of Dihydrostreptomycin Sulphate in Water for Injections. It is prepared by dissolving the contents of a sealed container in the requisite amount of Water for Injections immediately before use.

Dihydrostreptomycin Injection contains not less than 90.0 per cent and not more than 115.0 per cent of the stated amount of dihydrostreptomycin, $C_{21}H_{41}N_7O_{12}$, calculated on the dried basis.

Usual strength. The equivalent of 250 mg of dihydrostreptomycin.

Description. A white or almost white powder which yields a clear, colourless or faintly yellow solution when dissolved in *water*.

The injection complies with the tests stated under Parenteral Preparations (Powders for Injection).

The contents of the sealed container comply with the following requirements.

Identification

A. Determine by thin-layer chromatography (2.4.17), prepared by mixing 0.3 g of *carbomer* with 240 ml of *water*, allow to stand with moderate stirring for 1 hour, adjust to pH 7.0 by the gradual addition with constant shaking of *2 M sodium hydroxide* and add 30 g of *silica gel H*. Spread a uniform layer of the resulting suspension 0.75 mm thick. Heat the plate at 110° for 1 hour, allow to cool and use immediately.

Mobile phase. A 7 per cent w/v solution of *potassium dihydrogen phosphate*.

Test solution. Dissolve 100 mg of the sunbstance under examination in 100 ml of *water*.

Reference solution (a). A 0.10 per cent w/v of *dihydrostreptomycin sulphate RS* in *water*.

Reference solution (b). A solution containing 0.10 per cent w/v of *dihydrostreptomycin sulphate RS*, 0.10 per cent w/v of *neomycin sulphate RS* and 0.10 per cent w/v of *kanamycin sulphate RS* in *water*.

Apply to the plate 10 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, spray it with a mixture of equal volumes of a 0.2 per cent w/v solution of *naphthalene-1,3-diol* in *ethanol (95 per cent)* and 46 per cent w/v solution of *sulphuric acid* and heat at 150° for 5 to 10 minutes. The principal spot in the chromatogram obtained with test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. Dissolve 0.1 g in 2 ml of *water* and add 1 ml of *dilute 1-naphthol solution* and 2 ml of a mixture of equal volumes of *sodium hypochlorite solution (3* per cent *Cl)* and *water*; a red colour is produced.

C. Dissolve 10 mg in 5 ml of *water* and add 1 ml of *1 M hydrochloric acid*. Heat in a water-bath for 2 minutes. Add 2 ml of a 0.5 per cent w/v solution of *1-naphthol* in *1 M sodium hydroxide* and heat in a water-bath for 1 minute; a violet-pink colour is produced (distinction from streptomycin).

D. Gives the reactions of sulphates (2.3.1).

Tests

Appearance of solution. A 25 per cent w/v solution in *carbon dioxide-free water* is not more intensely coloured than degree 4 of the appropriate range of reference solutions (2.4.1). The solution, after standing protected from light at a temperature of about 20° for 24 hours, is not more opalescent than opalescence standard OS2 (2.4.1).

pH (2.4.24). 5.0 to 7.0, determined in a 25 per cent w/v solution.

Specific optical rotation (2.4.22). –83° to –91°, calculated on the dried basis, determined in a 2 per cent w/v solution in *water*.

Sulphate. 18.0 per cent to 21.5 per cent, calculated on the dried basis.

Dissolve 0.25 g in 100 ml of *water*, adjust the pH to 11 with *strong ammonia solution* and add 10.0 ml of *0.1 M barium chloride* and 0.5 mg of *metalphthalein*. Titrate the excess of barium chloride with *0.1 M disodium edetate*, adding 50 ml of *ethanol (95 per cent)* when the colour of the solution begins to change and continuing the titration until the violet-blue colour disappears.

1 ml of *0.1 M barium chloride* is equivalent to 0.009606 g of sulphate, SO_4.

Streptomycin. Weigh accurately about 0.10 g and dissolve in sufficient *water* to produce 5.0 ml. Add 5.0 ml of *0.2 M sodium hydroxide* and heat for exactly 10 minutes in a water-bath. Cool in ice for exactly 5 minutes, add 3 ml of a 1.5 per cent w/v solution of *ferric ammonium sulphate* in *0.25 M sulphuric acid* and sufficient *water* to produce 25.0 ml, and mix. Exactly 20 minutes after the addition of the ferric ammonium sulphate solution, measure the absorbance of a 2-cm layer at the maximum at about 525 nm (2.4.7), using as the blank a solution prepared in the same manner, omitting the substance under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously using 5.0 ml of a solution prepared by dissolving 10 mg, accurately weighed, of *streptomycin sulphate RS* in sufficient *water* to produce 50 ml and beginning at the words "Add 5.0 ml....", both absorbances being calculated on the dried basis.

Methanol. Determine by gas chromatography (2.4.13).

Test solution. Dissolve 4.0 g of the substance under examination in 100 ml of *water*.

Reference solution. A 0.008 per cent w/v of *methanol*.

Chromatographic system
- a glass column 1.5 to 2.0 m × 2 to 4 mm, packed with ethylvinylbenzene-divinylbenzene copolymer (150 to 180 mm) porous polymer beads (such as Porapak Q),
- temperature:
  column 50°,
  inlet port and detector. 280°,
- flow rate. 30 to 40 ml per minute of the carrier gas.

The area of any peak corresponding to methanol in the chromatogram obtained with test solution is not more than that of the peak in the chromatogram obtained with reference solution (0.2 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent.

Loss on drying (2.4.19). Not more than 5 per cent, determined on 1 g by drying over *phosphorus pentoxide* at 60° at a pressure not exceeding 0.1 kPa for 4 hours.

Assay. On the mixed contents of ten containers carry out the microbiological assay, Method A or B (2.2.10), and express the result in Units of dihydrostreptomycin per mg.

Dihydrostreptomycin Sulphate intended for use in the manufacture of Parenteral Preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per mg of dihydrostreptomycin sulphate.

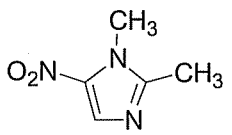
Dihydrostreptomycin Sulphate intended for use in the manufacture of Parenteral Preparations without a further appropriate sterilization procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light. Use the injection within 7 days of the preparation of the solution when stored in a cool place or within 1 month when stored in a cold place.

Labelling. The label states (1) the strength in terms of the equivalent amount of dihydrostreptomycin in a suitable dose-volume; (2) that the contents are meant for veterinary use only; (3) the storage conditions; (4) the date after which the contents are not intended to be used.

Dimetridazole



$C_5H_7N_3O_2$ Mol. Wt. 141.1

Dimetridazole is 1,2-dimethyl-5-nitro-1*H*-imidazole.

Dimetridazole contains not less than 98.0 per cent and not more than 101.0 per cent of the stated amount of dimetridazole, $C_5H_7N_3O_2$, calculated on the anhydrous basis.

Category. Antiprotozoal.

Dose. In drinking water. Pigs. 250 mg per litre. Poultry. 75 to 250 mg per litre. In feed. Pigs. Therapeutic dose, upto 500 g per tonne; prophylactic dose, upto 200 g per tonne. Poultry. 75 to 500 g per tonne.

Description. An almost white to brownish yellow powder; darkens on exposure to light, odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dimetridazole RS* or with the reference spectrum of dimetridazole.

B. When examined in the range of 230 to 360 nm (2.4.7), a 0.002 per cent w/v solution in *methanol* shows a well-defined absorption maximum only at about 309 nm; absorbance at about 309 nm, about 1.3.

C. Dissolve 0.1 g in 20 ml of *ether*, add 10 ml of a 1 per cent w/v solution of *picric acid* in *ether*, induce crystallisation by scratching the sides of the vessel and allow to stand. Wash the precipitate obtained with *ether* and dry at 105°; the residue melts at about 160° (2.4.21).

Tests

2-Methyl-5-nitroimidazole. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 90 volumes of *dichloromethane* and 10 volumes of *2-propanol*.

Test solution. Dissolve 2 g of the substance under examination in 100 ml of *dichloromethane*.

Reference solution. A 0.010 per cent w/v of *2-methyl-5-nitroimidazole RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to 2-methyl-5-nitroimidazole in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1 g.

Assay. Weigh accurately about 0.3 g, dissolve in 30 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01411 g of $C_5H_7N_3O_2$.

Storage. Store protected from light.

Dimetridazole Premix

Dimetridazole Premix contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dimetridazole, $C_5H_7N_3O_2$.

Usual strength. 22.5 per cent w/w.

Identification

Mix a quantity containing 0.1 g of Dimetridazole with 20 ml of *ether*, shake and filter. To the filtrate add 10 ml of a 1 per cent w/v solution of *picric acid* in *ether*, stir to induce crystallisation and allow to stand. Wash the precipitate obtained with *ether* and dry at 105°; the residue melts at about 160° (2.4.21).

Tests

Other tests. Complies with the tests stated under Premixes.

Assay. Weigh accurately a quantity containing 0.45 g of Dimetridazole, transfer to a sintered glass funnel (porosity No. 4), add 10 ml of *dichloromethane*, stir for 1 minute, and apply gentle suction. Repeat the extraction with four further quantities, each of 10 ml, of *dichloromethane*. To the combined dichloromethane extracts add 50 ml of *anhydrous glacial acetic acid* previously neutralised to *crystal violet solution* by the dropwise addition of *0.1 M perchloric acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01411 g of $C_5H_7N_3O_2$.

Storage. Store protected from light.

Dimetridazole Veterinary Oral Powder

Dimetridazole Veterinary Oral Powder is a mixture of Dimetridazole and a suitable water-soluble diluent.

Dimetridazole Veterinary Oral Powder contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of dimetridazole, $C_5H_7N_3O_2$.

Usual strength. 40 per cent w/w.

Identification

Mix a quantity containing 0.1 g of Dimetridazole with 20 ml of *ether*, shake and filter. To the filtrate add 10 ml of a 1 per cent w/v solution of *picric acid* in *ether*, stir to induce crystallisation and allow to stand. Wash the precipitate obtained with *ether* and dry at 105°; the residue melts at about 160° (2.4.21).

Tests

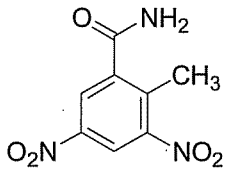
Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. Weigh accurately a quantity containing 0.4 g of Dimetridazole, transfer to a sintered glass funnel (porosity No. 4), add 10 ml of *dichloromethane*, stir for 1 minute, and apply gentle suction. Repeat the extraction with four further quantities, each of 10 ml, of *dichloromethane*. To the combined dichloromethane extracts add 50 ml of *anhydrous glacial acetic acid* previously neutralised to *crystal violet solution* by the dropwise addition of *0.1 M perchloric acid*. Titrate with *0.1 M perchloric acid*, using *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.01411 g of $C_5H_7N_3O_2$.

Storage. Store protected from light.

Dinitolmide



$C_8H_7N_3O_5$ Mol. Wt. 225.2

Dinitolmide is 3,5-dinitro-2-methylbenzamide.

Dinitolmide contains not less than 98.0 per cent and not more than 100.5 per cent of $C_8H_7N_3O_5$, calculated on the dried basis.

Category. Coccidiostat.

Dose. *Poultry*. Upto 200 g per tonne of feed.

Description. A cream to light tan powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *dinitolmide RS* or with the reference spectrum of dinitolmide.

B. Heat 1 g with 20 ml of *9 M sulphuric acid* under a reflux condenser for 1 hour, cool, add 50 ml of *water* and filter. The residue after washing with *water* and drying at 105° melts at about 205° (2.4.21).

Tests

Acid value (2.3.23). Not more than 5.0, determined on 0.5 g and using 50 ml of *ethanol (95 per cent)* as the solvent.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 85 volumes of *dichloromethane,* 10 volumes of *methanol* and 5 volumes of *glacial acetic acid.*

Test solution. Dissolve 2.5 g of the substance under examination in 100 ml of *acetone.*

Reference solution (a). A 0.0125 per cent w/v of the substance under examination in *acetone.*

Reference solution (b). A 0.0125 per cent w/v of o-*toluic acid* in *acetone.*

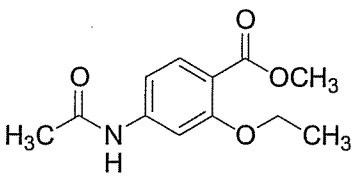
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Spray with *titanium trichloride solution,* diluted 5 times with *water,* heat at 100° for 5 minutes and spray with *ethanolic dimethylaminobenzaldehyde solution.* When viewed under ultraviolet light at 354 nm the spot in the chromatogram obtained with reference solution (b) is more intense than any corresponding spot in the chromatogram obtained with the test solution. By both methods of visualisation any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.15 g, dissolve in *acetone* and dilute to 50.0 ml. To 10.0 ml of the solution add 10 ml of *glacial acetic acid* and 15 ml of a 40 per cent w/v solution of *sodium acetate.* Maintain a stream of carbon dioxide through the flask throughout the determination. Add 25.0 ml of *0.1 M titanium trichloride* and allow to stand for 5 minutes. Add 10 ml of *hydrochloric acid,* 10 ml of *water* and 1 ml of *potassium thiocyanate solution.* Titrate with *0.1 M ferric ammonium sulphate* until the solution becomes first colourless and then orange. Repeat the operation without the substance under examination. The difference between the titrations represents the amount of titanium trichloride required.

1 ml of *0.1 M titanium trichloride* is equivalent to 0.001876 g of $C_8H_7N_3O_5$.

Ethopabate



$C_{12}H_{15}NO_4$ Mol. Wt. 237.3

Ethopabate is methyl 4-acetamido-2-ethoxybenzoate.

Ethopabate contains not less than 96.0 per cent and not more than 104.0 per cent of ethopabate, $C_{12}H_{15}NO_4$, calculated on the dried basis.

Category. Coccidiostat.

Dose. *Poultry.* 4 to 8 g per tonne of feed.

Description. A white or pinkish white powder, odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ethopabate RS* or with the reference spectrum of ethopabate.

B. When examined in the range 230 to 360 nm (2.4.7), a 0.0016 per cent w/v solution in *methanol* shows absorption maxima at about 268 nm and at about 299 nm; absorbance at about 268 nm, about 1.3 and at about 299 nm, about 0.58.

C. Melts at about 148° (2.4.21).

Tests

Diazotisable substances. Dissolve 0.2 g in 10 ml of *dichloromethane* and extract in succession with 100 ml and 90 ml of *0.1 M hydrochloric acid,* combine the acid extracts, wash with 5 ml of *dichloromethane,* dilute to 200 ml with *0.1 M hydrochloric acid* and filter. To 5 ml, add 6 ml of *1 M hydrochloric acid* and 1 ml of a 0.1 per cent w/v solution of *sodium nitrite,* mix, and allow to stand for 4 minutes. Add 1 ml of a 0.5 per cent w/v solution of *ammonium sulphamate,* mix and allow to stand for 3 minutes. Add 1.0 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride,* mix, and allow to stand for 30 minutes. Absorbance of the resulting solution at about 545 nm (2.4.7), not more than 0.70.

Phenolic substances. Dissolve 0.25 g in 15 ml of *methanol* and add sufficient *methanol* to produce 25 ml. To 5 ml add 5 ml of a 3 per cent w/v solution of *anhydrous ferric chloride,* mix and allow to stand for 10 minutes. Absorbance of the resulting solution at about 525 nm (2.4.7), not more than 0.70, using as the blank a solution prepared by adding 5 ml of a 3 per cent w/v solution of *anhydrous ferric chloride* to 5 ml of *methanol.*

Sulphated ash (2.3.18). Not more than 0.5 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g, by drying in an oven at 105° at a pressure not exceeding 0.7 kPa.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water.*

Test solution. Dissolve 20 mg of the substance under examination in 100 ml of the solvent mixture. Dilute 1.0 ml of this solution to 10.0 ml with the solvent mixture.

Reference solution (a). A 0.002 per cent w/v solution of *ethopabate RS* in the solvent mixture.

Reference solution (b). A solution containing 0.002 per cent w/v of *ethopabate RS* and 0.01 per cent w/v of *methyl-4-acetamido- 2-hydroxybenzoate RS* in the solvent mixture.

Chromatographic system
- a stainless steel column 30 cm x 3.9 mm, packed with silica particles the surface of which has been modified with chemically bonded phenyl groups (10 µm),
- column temperature. 45°,
- mobile phase: a mixture of 3 volumes of *acetonitrile*, 15 volumes of *methanol* and 45 volumes of *0.15 M sodium hexanesulphonate*, adjusted to pH 2.5 with *orthophosphoric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 268 nm,
- injection volume. 20 µl.

Inject reference solution (b). The test is not valid unless the resolution factor between the peaks due to ethopabate and methyl-4-acetamido-2 hydroxybenzoate is not less than 1.2.

Inject the test solution and reference solution (a).

Calculate the content of $C_{12}H_{15}NO_4$.

Furazolidone Veterinary Oral Suspension

Furazolidone Veterinary Mixture; Furazolidone Mixture; Furazolidone Drench

Furazolidone Veterinary Oral Suspension is an aqueous suspension of Furazolidone.

Furazolidone Veterinary Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.

Usual strengths. 5 per cent w/v; 7.5 per cent w/v.

Identification

A. Add 0.2 ml to a mixture of 15 ml of *dimethylformamide* and 1 ml of *0.5 M ethanolic potassium hydroxide*; a blue colour is produced.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *dichloromethane* and 40 volumes of *nitromethane* and 10 volumes of *methanol*.

Test solution. Shake a quantity of the suspension containing 5 mg of Furazolidone with 1 ml of *acetone*, allow to stand, and use the supernatant liquid.

Reference solution. A 0.5 per cent w/v solution of *furazolidone RS* in *acetone*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. *Protect the solutions from light throughout the assay.*

Weigh accurately a quantity of the well-shaken suspension containing 35 mg of Furazolidone, add slowly and with stirring, 50 ml of *dimethylformamide*. Warm on a water-bath, with occasional stirring, until most of the solid is dissolved. Decant the supernatant liquid and extract the residue further with two quantities, each of 50 ml, of *dimethylformamide*, decanting the supernatant solution. No yellow colour should be visible in the third extract. Cool the combined dimethylformamide extracts, add sufficient *water* to produce 500.0 ml and filter. To 10.0 ml of the filtrate add sufficient *water* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7). Calculate the content of $C_8H_7N_3O_5$ taking 754 as the specific absorbance at 367 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of furazolidone, weight in volume.

Labelling. The label states that the oral suspension should be administered undiluted.

Furazolidone Premix

Furazolidone Premix contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of furazolidone, $C_8H_7N_3O_5$.

Usual strengths. 4.4 per cent w/v; 22.4 per cent w/v.

Identification

A. To a mixture of 15 ml of *dimethylformamide* and 1 ml of *0.5 M ethanolic potassium hydroxide* add 5 mg of the premix; a blue colour is produced.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *dichloromethane* and 40 volumes of *nitromethane* and 10 volumes of *methanol*.

Test solution. The supernatant liquid obtained by shaking a quantity of the premix containing 5 mg of furazolidone with 1 ml of *acetone.*

Reference solution. A 0.5 per cent w/v solution of *furazolidone RS* in *acetone.*

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Premixes.

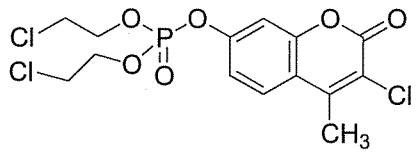
Assay. *Protect the solutions from light throughout the assay.*

Weigh accurately a quantity of the premix containing 35 mg of Furazolidone, add 50 ml of *dimethylformamide* and shake for 20 minutes. Add sufficient *water* to produce 500.0 ml and filter. To 10.0 ml of the filtrate add sufficient *water* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 367 nm (2.4.7). Calculate the content of $C_8H_7N_3O_5$ taking 754 as the specific absorbance at 367 nm.

Determine the weight per ml, (2.4.29), and calculate the content of furazolidone, weight in volume.

Storage. Store protected from light and moisture.

Haloxon



$C_{14}H_{14}Cl_3O_6P$ Mol. Wt. 415.6

Haloxone is phosphoric acid bis(2-chloroethyl) 3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl ester.

Haloxon contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{14}H_{14}Cl_3O_6P$, calculated on the dried basis

Category. Anthelmintic.

Dose. *All species except poultry.* 50 mg per kg of body weight. *Poultry.* 50 to 75 mg per kg of body weight.

Description. A white or almost white powder.

Identification

A. Dissolve about 20 mg in 10 ml of *dioxan*, add 0.5 ml of *0.1 M hydrochloric acid* and dilute to 25 ml with *methanol.* Dilute 1 ml to 25 ml with *methanol.*

When examined in the range 230 to 360 nm (2.4.7), the resulting solution exhibits a maximum at about 290 nm and a less well defined maximum at about 312 nm. Ratio of the absorbance at about 312 nm to that at about 290 nm, about 1.08.

B. Dissolve 0.1 g in 5 ml of *5 M sodium hydroxide* with the aid of warming, cool, acidify 1 ml of the solution by the addition of *2 M nitiric acid* and add 1 ml of *silver nitrate solution*, a white precipitate is formed. The precipitate is soluble in *5 M ammonia* giving a brown solution which exhibits a green fluorescence when viewed under screened ultraviolet light.

C. Melting range (2.4.21). 88° to 93°.

Tests

Acidity. Dissolve 0.1 g in 10 ml of *ethanol (95 per cent)* previously neutralised to *methyl red solution*; the solution requires for neutralisation not more than 0.1 ml of *0.1 M sodium hydroxide.*

3-Chloro-4-methylumbelliferone. Not more than 2.0 per cent.

NOTE — Prepare the solutions immediately before use and protected from light.

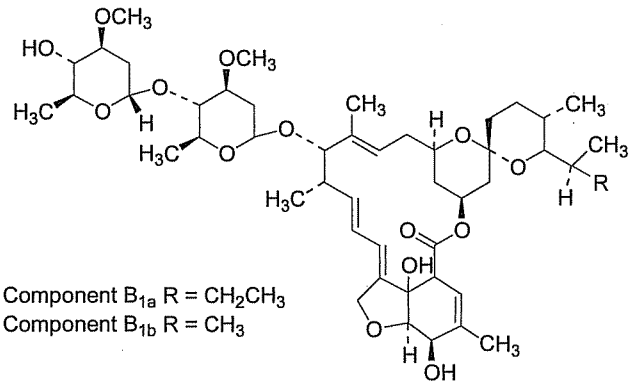
Dissolve 0.20 g in 50 ml of *0.01 M methanolic hydrochloric acid* and dilute 5 ml to 100 ml with *0.01 M methanolic hydrochloric acid.* Measure the fluorescence of the resulting solution (2.4.5), using an excitation wavelength of about 345 nm and an emission wavelength of about 400 nm and setting the spectrofluorimeter to zero with *0.01 M methanolic hydrochloric acid* and to 100 with a standard solution prepared by dissolving 25 mg of *3-chloro-4-methyl- umbelliferone RS* in sufficient *0.01 M methanolic hydrochloric acid* to produce 250 ml (solution A) and diluting 5 ml to 100 ml with *0.01 M methanolic hydrochloric acid.* Calculate the content of 3-chloro-4-methylumbelliferone from a calibration curve prepared by measuring the fluorescence of suitable dilutions of solution A.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 80° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.25 g and dissolve in sufficient *acetonitrile* to produce 10 ml and record the infrared absorption of a 0.2 mm layer of the solution at the maximum at about 1155 cm^{-1} (2.4.6). Construct a base line between the minima at about 1125 cm^{-1} and 1180 cm^{-1}. Calculate the content of $C_{14}H_{14}Cl_3O_6P$ from the absorption obtained by repeating the procedure using *haloxon RS* in place of the substance under examination.

Storage. Avoid contact with metals.

Ivermectin



Component B_{1a} R = CH_2CH_3
Component B_{1b} R = CH_3

$C_{48}H_{74}O_{14}$, H_2B_{1a} Mol.Wt. 875.1

$C_{47}H_{72}O_{14}$, H_2B_{1b} Mol.Wt. 861.1

Ivermectin contains not less than 95.0 per cent and not more than 102.0 per cent of H_2B_{1a} + H_2B_{1b}, calculated on the anhydrous and solvent free basis.

The ratio $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$, determined by liquid chromatography is not less than 90.0 per cent.

Category. Anthelmintic.

Description. A white crystalline powder, slightly hygroscopic.

Identification

A. Determine by infrared absorption spectrophotometery (2.4.6). Compare the spectrum with that obtained with *ivermectin RS*.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Appearance of solution. A 2.0 per cent w/v solution in *toluene* is clear (2.4.1) and not more intensely colored than reference solution BY57 (2.4.1).

Specific optical rotation (2.4.22) -17.0 to -20.0, determined on a 2.5 per cent w/v solution in *methanol*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 40 mg of the substance under examination in 50 ml of *methanol*.

Reference solution (a). A 0.08 per cent w/v solution of *ivermectin RS* in *methanol*.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100 ml with *methanol*.

Reference solution (c). Dilute 5 ml of reference solution (b) to 100 ml with *methanol*.

Chromatographic system
- a stainless steel column 25 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 15 volumes of *water*, 34 volumes of *methanol* and 51 volumes of *acetonitrile*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). This test is not valid unless resolution between the component H_2B_{1b} (first peak) and component H_2B_{1a} (second peak) is not less than 3.0.

Inject the test solution and reference solution (b). In the chromatogram obtained with the test solution the impurity with a relative retention of 1.3 to 1.5 with reference to the principal peak is not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (2.5 per cent). The area of any other peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent) and the sum of all the secondary peaks is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (5.0 per cent).

Ethanol and formamide. Ethanol. Not more than 5.0 per cent and formamide. Not more than 3.0 per cent, determined by gas chromatography (2.4.13).

Internal standard solution. Dilute 0.5 ml of *propanol* to 100 ml with *water*.

Test solution. Dissolve 0.120 g of the substance under examination in 2.0 ml of *m-xylene* by heating on a water-bath at 40 to 50°, add 2.0 ml of *water*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene*.

Reference solution (a). Dilute 3.0 g of *ethanol* to 100 ml with *water*.

Reference solution (b). Dilute 1.0 g of *formamide* to 100 ml with *water*.

Reference solution (c). Dilute 5.0 ml of reference solution (a) and 5 ml of reference solution (b) to 50.0 ml with *water*. Transfer 2.0 ml of this solution to a centrifuge tube, add 2 ml of *m-xylene*, mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene*.

Reference solution (d). Dilute 10.0 ml of reference solution (a) and 10.0 ml of reference solution (b) to 50.0 ml with *water*.

Transfer 2.0 ml of this solution to a centrifuge tube, add 2 ml of *m-xylene,* mix thoroughly and centrifuge. Remove the upper layer and extract it with 2.0 ml of *water*. Discard the upper layer and combine the aqueous layers. Add 1.0 ml of the internal standard solution. Centrifuge and discard any remaining *m-xylene.*

Chromatographic system
- a glass column 30 m × 0.53 mm, packed with fused silica with macrogol 20,000 with film thickness 1 mm,
- temperature
  column 80° increase @ 60° per minute to 240°,
  injection port 220° and detector 280°,
- flow rate. 7.5 ml per minute of helium as carrier gas.

Inject 1 µl of the test solution and reference solutions (c) and (d).

Calculate the content of *ethanol* is not more than 5.0 per cent and *formamide* not more than 3.0 per cent.

Heavy metals (2.3.13). 1 g complies with the limit test for heavy metals, Method C (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 0.5 gm.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject the test solution and reference solution (a).

Calculate the percentage contents of ivermectin ($H_2B_{1a} + H_2B_{1b}$) and the ratio $H_2B_{1a}/(H_2B_{1a} + H_2B_{1b})$.

Storage. Store protected from light.

Ivermectin Injection

Ivermectin Injection is a sterile solution of Ivermectin with or with out one or more anaesthetics, preservatives and solvents.

Ivermectin Injection contains not less than 90 per cent and not more than 110 per cent of H_2B_1a, and not more than 5 per cent of H_2B_1b.

The content of $H_2B_{1a} + H_2B_{1b}$ is not less than 95 per cent and not more than 110 per cent of the stated amount of Ivermectin.

Usual strengths. *for dogs*. 0.2 ml per 33 kg of body weight; *for other animals*. 1 ml per 50 kg of body weight.

Description. A clear, colourless to yellow colour solution.

Identification

When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum at about 245 nm.

Tests

Pyrogens (2.2.8). Complies with the test for pyrogens, by injecting 0.2 mg of Ivermectin per kg body weight of rabbit.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dilute accurately a volume of the injection containing 5 mg of Ivermectin to 100 ml with *water*.

Reference solution. A 0.005 per cent w/v solution of *ivermectin RS* in *water*.

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 9 volumes of *methanol* and 1 volume of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 245 nm,
- injection volume. 20 µl.

Inject the reference solution. The test is not valid unless the tailing factor is not less than 2.0

Inject alternatively the test solution and the reference solution.

Calculate the content of ivermectin in the injection.

Storage. Store protected from light.

Labelling. The label states (1) the strength in mg of Ivermectin per ml; (2) that the contents are to be used for subcutaneous use only; (3) the names of any preservatives used.

Kaolin Veterinary Oral Suspension

Kaolin Veterinary Mixture; Kaolin Mixture

Light Kaolin	200 g
Light Magnesium Carbonate	50 g
Sodium Bicarbonate	50 g
Water to produce	1000 ml

Kaolin Veterinary Oral Suspension should be freshly prepared, unless the Light Kaolin has been sterilised.

Kaolin Veterinary Oral Suspension contains not less than 1.04 per cent w/w and not more than 1.25 per cent w/w of the stated amount of magnesium, Mg and not less than 4.05 per cent w/w and not more than 4.65 per cent w/w of the stated amount of sodium bicarbonate, $NaHCO_3$.

Usual strengths. *All species*. 15 to 30 mg per kg of body weight.

Tests

Acid-insoluble matter. 13.8 to 18.4 per cent w/w, determined by the following method. Weigh accurately about 3 g, add 15 ml of *water* and make acid to *litmus paper* by the cautious addition of *2 M hydrochloric acid*; boil for 5 minutes, replacing water lost by evaporation, cool and decant the supernatant layer through a filter. Boil the residue with 20 ml of *water* and 10 ml of *2 M hydrochloric acid*, cool, filter through the same filter, and wash the residue with *water* until the washings are free from chloride, reserving the filtrate and washings for the Assay *for magnesium*. Dry and ignite the residue to constant weight at red heat.

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. *For magnesium* — Dilute the combined filtrate and washings reserved in the determination of acid-insoluble matter to 100.0 ml with *water*. To 20.0 ml add 0.1 g of *ascorbic acid*, make slightly alkaline to *litmus paper* with *5 M ammonia* and add 10 ml of *triethanolamine*, 10 ml of *ammonia buffer pH 10.9* and 1 ml of *potassium cyanide solution*. Titrate with *0.05 M disodium edetate* using *eriochrome black T solution* as indicator.

1 ml of *0.05 M disodium edetate* is equivalent to 0.001215 g of Mg.

For sodium bicarbonate — Weigh accurately about 10 g, boil with 100 ml of *water* for 5 minutes and filter. Boil the residue with 100 ml of *water* for 5 minutes and filter. Cool the combined filtrates and titrate with *0.5 M hydrochloric acid* using *methyl orange-xylene cyanol FF solution* as indicator. Add 10 ml of *ammonia buffer pH 10.9* and titrate with *0.05 M disodium edetate* using *eriochrome black T solution* as indicator.

1 ml of *0.5 M hydrochloric acid* after subtracting one fifth of the volume of *0.05 M disodium edetate* is equivalent to 0.0420 g of $NaHCO_3$.

Levamisole Injection

Levamisole Hydrochloride Injection

Levamisole Injection is a sterile solution of Levamisole Hydrochloride in Water for Injections. It may contain suitable colouring agents.

Levamisole Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of levamisole hydrochloride, $C_{11}H_{12}N_2S,HCl$.

Usual strength. 75 mg in 1 ml.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Dilute a volume of the injection to produce a solution containing 1.0 per cent w/v of Levamisole Hydrochloride in *methanol*.

Reference solution. A 1.0 per cent w/v of *levamisole hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. Dilute a volume of the injection containing 0.75 g of Levamisole Hydrochloride to 20 ml with *water* and add 6 ml of *1 M sodium hydroxide*. Extract with 20 ml of *dichloromethane*, discard the aqueous layer and wash the dichloromethane layer with 10 ml of *water*. Dry by shaking with *anhydrous sodium sulphate*, filter and evaporate the solvent at room temperature. The residue, after drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa at a temperature not exceeding 40°, melts at about 59° (2.4.21).

C. The injection is laevorotatory.

D. Gives reaction B of chlorides (2.3.1).

Tests

pH (2.4.24). 3.3 to 3.7.

2,3-Dihydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 45 volumes of *toluene*, 8 volumes of *methanol* and 4 volumes of *anhydrous glacial acetic acid*.

Test solution. Dilute a volume of the injection with *methanol* to produce a solution containing 5.0 per cent w/v of Levamisole Hydrochloride.

Reference solution. A 0.025 per cent w/v of *2,3-dihydro-6-phenylimidazo[2,1-b]thiazole hydrochloride RS* in *methanol*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with *potassium iodoplatinate solution*. Any spot corresponding to 2,3-dihydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing 0.75 g of Levamisole Hydrochloride add 50 ml of *water* and 15 ml of *2 M sodium hydroxide*, extract with three quantities, each of 25 ml, 20 ml and 15 ml of *dichloromethane*, wash the combined extracts with two quantities, each of 10 ml, of *water* and discard the washings. To the clear dichloromethane solution, after drying with *anhydrous sodium sulphate*, add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02408 g of $C_{11}H_{12}N_2S,HCl$.

Storage. Store protected from light.

Levamisole Hydrochloride Veterinary Oral Solution

Levamisole Hydrochloride Veterinary Mixture; Levamisole Veterinary Oral Solution; Levamisole Veterinary Mixture

Levamisole Hydrochloride Veterinary Oral Solution is an aqueous solution of Levamisole Hydrochloride containing suitable stabilising agents.

Levamisole Hydrochloride Veterinary Oral Solution contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of levamisole hydrochloride, $C_{11}H_{12}N_2S,HCl$.

Usual strength. 0.25 per cent w/w; 1.5 per cent w/w.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Dilute a volume of the preparation under examination with *methanol* to produce a solution containing 1.0 per cent w/v of Levamisole Hydrochloride.

Reference solution. A 1.0 per cent w/v of *levamisole hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air and spray with *potassium iodoplatinate solution*. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

B. To a quantity containing 0.3 g of Levamisole Hydrochloride add 10 ml of *water* and 6 ml of *1 M sodium hydroxide*. Extract with 20 ml of *dichloromethane*, discard the aqueous layer and wash the dichloromethane layer with 10 ml of *water*. Dry by shaking with *anhydrous sodium sulphate*, filter and allow the dichloromethane to evaporate at room temperature. The residue, after drying over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa at a temperature not exceeding 40°, melts at about 59° (2.4.21).

C. The solution is laevorotatory.

Tests

2,3-Dihydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 45 volumes of *toluene*, 8 volumes of *methanol* and 4 volumes of *anhydrous glacial acetic acid*.

Test solution. Dilute a volume of the preparation under examination with *methanol* to produce a solution containing 1.0 per cent w/v of Levamisole Hydrochloride.

Reference solution. A 0.025 per cent w/v of *2,3-dihydro-6-phenylimidazo[2,1-b]thiazole hydrochloride RS* in *methanol*.

Apply to the plate 50 µl of the test solution and 10 µl of the reference solution. After development, dry the plate in air and spray with *potassium iodoplatinate solution*. Any spot corresponding to 2,3-dihydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. Weigh accurately a quantity containing 0.75 g of Levamisole Hydrochloride add 15 ml of *2 M sodium hydroxide*, extract with three quantities each of 25 ml, 20 ml and 15 ml of *dichloromethane*, wash the combined extracts with two quantities, each of 10 ml, of *water* and discard the washings. To the clear dichloromethane solution, after drying with *anhydrous sodium sulphate*, add 50 ml of *anhydrous glacial acetic acid*. Titrate with *0.1 M perchloric acid*, using *1-naphtholbenzein solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02408 g of $C_{11}H_{12}N_2S,HCl$.

Lincomycin Premix

Lincomycin Hydrochloride Premix.

Lincomycin Premix contains Lincomycin Hydrochloride.

Lincomycin Premix contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of lincomycin, $C_{18}H_{34}N_2O_6S$.

Usual strength. 4.4 per cent w/w.

Identification

In the Assay, the chromatogram obtained with the test solution corresponds to the chromatogram obtained with the reference solution.

Tests

Lincomycin B. Examine test solution as described in the Assay but increasing the sensitivity by 8 to 10 times while recording the peak due to the trimethylsilyl derivative of lincomycin B, which is eluted immediately before the trimethylsilyl derivative of lincomycin. The area of the peak due to the trimethylsilyl derivative of lincomycin B, after correction for the sensitivity factor, is not more than 5 per cent of the area of the peak due to the trimethylsilyl derivative of lincomycin.

Other tests. Complies with the tests stated under Premixes.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of premix containing about 12 mg of Lincomycin Hydrochloride in 10 ml of the mobile phase.

Reference solution. A 0.12 per cent w/v solution of *lincomycin hydrochloride RS* in the mobile phase.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octylsilane bonded to porous silica (5 µm),
- column temperature. 45°,
- mobile phase: a mixture of 78 volumes a solution prepared by diluting 13.5 ml of *orthophosphoric acid* to 1000 ml of *water*, adjusted to pH 6.0 with *ammonium hydroxide*, 15 volumes of *acetonitrile* and 15 volumes of *methanol*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume. 20 µl.

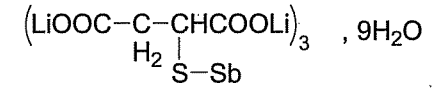
Inject the reference solution. The test is not valid unless the tailing factor is not more than 1.3; the column efficiency is not less than 4000 theoretical plates and relative standard devation for replicate injections is not more than 2.0. The relative rentention time with reference to lincomycin for lincomycin B is about 0.5.

Inject the reference solution and the test solution.

Calculate the content of $C_{18}H_{34}N_2O_6S$ in premix.

Labelling. The label states the strength in terms of the equivalent amount of lincomycin.

Lithium Antimony Thiomalate



$C_{12}H_9Li_6O_{12}S_3Sb,9H_2O$ Mol. Wt. 766.9

Lithium Antimony Thiomalate contains not less than 15.5 per cent and not more than 16.5 per cent of Sb and not less than 5.1 per cent and not more than 5.7 per cent of Li, calculated on the dried, solvent-free basis.

Category. Anthelmintic against trematodes.

Dose. *Cattle.* 1 to 1.2 g.

Description. A pinkish white or creamy powder; hygroscopic.

Identification

A. To 0.2 g dissolved in 5 ml of *water* add 2 ml of *hydrochloric acid* and 5 ml of *sodium sulphide solution*; a yellowish- orange precipitate is produced which does not dissolve on addition of *dilute ammonia solution.*

B. When moistened with *hydrochloric acid* and introduced on a platinum wire it imparts a red colour to a non-luminous flame.

Tests

Appearance of solution. A 6 per cent w/v solution in *carbon dioxide-free water* is clear (2.4.1), and not more intensely coloured than reference solution RS3 (2.4.1).

pH (2.4.24). 9.0 to 10.5, determined in a 6 per cent w/v solution in *carbon dioxide-free water.*

Assay. *For antimony* — Weigh accurately about 0.50 g , add 35 ml of *water* and swirl to dissolve. Add 5 g of *ammonium persulphate*, 10 ml of *sodium hydroxide solution* and 3 or 4 glass beads (approximately 0.5 cm diameter). Place a small funnel in the neck of the flask and boil gently for 20 minutes at such a rate that the volume is not reduced appreciably. Cool, add through the funnel 0.25 ml of *phenolphthalein solution* and sufficient *0.1 M hydrochloric acid* until the last trace of pink colour disappears. Add 25 ml of a 10 per cent w/v solution of *oxalic acid* through the funnel and boil vigorously for 3 minutes. Rinse the funnel, with a small quantity of *water*, remove it and add 5 ml of *hydrochloric acid* and 2 g of *potassium iodide.* Allow to stand for 10 minutes and boil until the solution becomes yellow and shows no further decrease in colour, but taking care to see that the volume is not reduced to less than about 30 ml. Cool and remove a small drop of the solution with a sealed capillary melting point tube and add to *starch iodide paper.* If a bluish colour is produced, add 1 drop of *0.1 M sodium thiosulphate* while swirling and again test with *starch iodide paper.* Repeat if necessary until a bluish colour is no longer produced.

Add 5 g of *sodium potassium tartrate*, cool to about 15° to 20° and cautiously add small portions of sodium bicarbonate until no further effervescence is produced. Add 2 to 4 g more of *sodium bicarbonate* and titrate with *0.1 M iodine* until the first permanent light yellow colour is produced.

1 ml of *0.1 M iodine* is equivalent to 0.006088 g of Sb.

For lithium — Weigh accurately about 0.2 g, dissolve in 50 ml of *glacial acetic acid* . Titrate with *0.1 M perchloric acid*, using 1 ml of *crystal violet solution* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.000694 g of Li.

Storage. Store protected from light and moisture.

Lithium Antimony Thiomalate Injection

Lithium Antimony Thiomalate Injection is a sterile solution of Lithium Antimony Thiomalate in Water for Injection containing a suitable antimicrobial preservative.

Lithium Antimony Thiomalate Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of lithium antimony thiomalate, $C_{12}H_9Li_6O_{12}S_3Sb,9H_2O$.

Usual strength. 6 per cent w/v.

Identification

A. Dilute a volume containing 0.2 g of Lithium Antimony Thiomalate to 5 ml with *water*. Add 2 ml of *hydrochloric acid* and 5 ml of *sodium sulphide solution;* a yellowish orange precipitate is produced which does not dissolve on addition of *dilute ammonia solution.*

B. Dilute 0.2 ml of the injection under examination to 10 ml with a 5 per cent w/v solution of *sodium potassium tartrate*. To 2 ml of the solution add *sodium sulphide solution* dropwise; a reddish orange precipitate is produced. The precipitate dissolves on adding *dilute sodium hydroxide solution.*

Tests

Appearance of solution. The solution is clear (2.4.1), and not more intensely coloured than reference solution RS3 (2.4.1).

pH (2.4.24). 9.0 to 10.5.

Pyrogens. Complies with the test for pyrogens (2.2.8), using per 1.5 kg of the rabbit's weight, a volume containing 0.012 g of Lithium Antimony Thiomalate.

Sterility (2.2.11). Complies with the test for sterility.

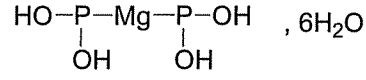
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Dilute 10.0 ml with 25 ml of *water*, add 7.5 g of *ammonium persulphate* and 16 ml of *sodium hydroxide solution*, boil gently for 20 minutes, cool and add 0.5 ml of *phenolphthalein solution*. Neutralise the solution with *dilute hydrochloric acid* and boil for 3 minutes. Add 50 ml of a 10 per cent w/v solution of *oxalic acid*, 7.5 ml of *hydrochloric acid* and sufficient *water* to make up the volume, if necessary. Add 2 g of *potassium iodide* to the hot solution, allow to stand for 10 minutes and boil until it acquires a pale yellow colour (about 10 minutes). Cool and remove the colour by adding *0.1 M sodium thiosulphate* using *starch iodide solution* as an external indicator. Add 7.5 g of *sodium potassium tartrate* and dilute to 200 ml. Add *sodium bicarbonate* carefully (avoiding loss by spurting due to effervescence) till alkaline to *litmus paper* and titrate with *0.05 M iodine* using 1 ml of *starch solution,* added towards the end of the titration, as indicator.

1 ml of *0.05 M iodine* is equivalent to 0.03834 g of $C_{12}H_9Li_6O_{12}S_3Sb,9H_2O$.

Storage. Store protected from light.

Magnesium Hypophosphite



$Mg(H_2PO_2)_2,6H_2O$ Mol. Wt. 262.4

Magnesium Hypophosphite contains not less than 98.5 per cent and not more than 101.0 per cent of $Mg(H_2PO_2)_2,6H_2O$.

Category. Supplement in deficiency conditions; nerve tonic.

Description. Colourless crystals or white crystalline powder.

Identification

A. Gives the reactions of magnesium salts (2.3.1).

B. Dissolve about 50 mg in 5 ml of *water* and add 0.5 ml of *mercuric chloride solution*; a white precipitate is produced.

C. Dissolve about 50 mg in 5 ml of *water* and acidify with *sulphuric acid.* Add 0.5 ml of *cupric sulphate solution* and warm; a red precipitate is produced.

Tests

Appearance of solution. A 5 per cent w/v solution is clear (2.4.1) and colourless (2.4.1).

Heavy metals (2.3.13). Dissolve 1.0 g in 20 ml of *water*, add 2 ml of *dilute hydrochloric acid* and sufficient *water* to produce 25 ml. The resulting solution complies with the limit test for heavy metals, Method A (20 ppm).

Chlorides (2.3.12). To 1 g add 200 ml of *water* and filter. 10 ml of the filtrate complies with the limit test for chlorides (0.1 per cent).

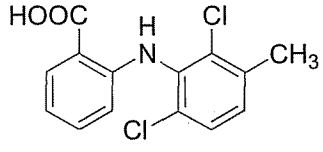
Sulphates (2.3.17). 1 g complies with the limit test for sulphates (0.015 per cent).

Assay. Weigh accurately about 0.2 g, dissolve in 50 ml of *water*, add 5 ml of *strong ammonia-ammonium chloride solution* and titrate with *0.05 M disodium edetate* using 0.1 g of *mordant black II mixture* as indicator, until a blue colour is obtained.

1 ml of *0.05 M disodium edetate* is equivalent to 0.01312 g of $Mg(H_2PO_2)_2,6H_2O$.

Storage. Store protected from moisture.

Meclofenamic Acid



$C_{14}H_{11}Cl_2NO_2$ Mol. Wt. 296.2

Meclofenamic acid is *N*-(2,6-dichloro-3-methylphenyl) anthranilic acid.

Meclofenamic Acid contains not less than 98.5 per cent and not more than 100.5 per cent of the stated amount of $C_{14}H_{11}Cl_2NO_2$, calculated on the dried basis.

Category. Anti-inflammatory; analgesic; antipyretic.

Dose. *Horses*. 2.2 mg per kg of body weight daily, for 5 to 7 days.

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *meclofenamic acid RS* or with the reference spectrum of meclofenamic acid.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M sodium hydroxide* shows absorption maxima at about 279 nm, and 371 nm; absorbance at about 279 nm, about 0.45, and at about 317 nm, about 0.33.

C. Dissolve 25 mg in 15 ml of *dichloromethane*; the solution exhibits a strong blue fluorescene when examined under ultraviolet light.

D. Dissolve 1 mg in 2 ml of *sulphuric acid* and add 0.05 ml of *0.02 M potassium dichromate*; an intense purple colour is produced, which rapidly fades to purple brown.

Tests

Appearance of solution. A 5.0 per cent w/v solution in *1 M sodium hydroxide* is not more opalescent than reference suspension OS2 (2.4.1) and is not more intensely coloured than reference solution BYS5 (2.4.1).

Light absorption (2.4.7). Absorbance of a 0.002 per cent w/v solution in *0.01 M methanolic hydrochloric acid* at the maximum at about 279 nm, not less than 0.400 and not more than 0.445, and at the maximum at about 335 nm, not less than 0.440 and not more than 0.490.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 1.0 g of the substance under examination in 100 ml of *ethanol*.

Reference solution (a). A 0.0035 per cent w/v solution of *ethyl meclofenamate RS* (internal standard). in *ethanol*.

Reference solution (b). A solution containing 1.0 per cent w/v of the substance under examination and 0.0035 per cent w/v of *ethyl meclofenamate RS* (internal standard) in *ethanol*.

Chromatographic system
- a stainless steel column 20 cm × 4 mm, packed with octadecasilane bonded to porous silica (10 µm) (such as Spherisorb ODS),
- mobile phase: a mixture of 75 volumes of *methanol*, 25 volumes of *water* and 1 volume of *glacial acetic acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 4500 theoretical plates.

Inject reference solution (b). The area of the peak immediately preceding the peak due to meclofenamic acid is not more than one-seventh of the area of the peak due to the internal standard. The area of any other peak is not more than the area of the peak due to the internal standard.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method D (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 100 ml of warm *ethanol* previously neutralised to *phenol red solution* and titrate with *0.1 M sodium hydroxide*, using *phenol red solution* as indicator.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.02962 g of $C_{14}H_{11}Cl_2NO_2$.

Storage. Store protected from moisture.

Mepyramine Injection

Mepyramine Maleate Injection; Pyrilamine Maleate Injection; Pyrilamine Injection

Mepyramine Injection is a sterile solution of Mepyramine Maleate in Water for Injections.

Mepyramine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of mepyramine maleate, $C_{17}H_{23}N_3O,C_4H_4O_4$.

Usual strengths. 25 mg in 1 ml; 50 mg in 1 ml.

Description. Colourless or almost colourless solution.

Identification

A. To a volume containing 0.1 g of Mepyramine Maleate add 2 ml of *5 M sodium hydroxide* and shake with three quantities, each of 3 ml, of *ether*. Warm the aqueous layer in a water-bath for 10 minutes with 2 ml of *bromine solution*, heat to boiling, cool, and add 0.2 ml to a solution of 10 mg of *resorcinol* in 3 ml of *sulphuric acid*; a blue-black colour develops on heating for 15 minutes in a water-bath.

B. Dilute a volume containing 20 mg of mepyramine maleate to 2 ml with *water*, add 1 ml of *cyanogen bromide solution* and 5 ml of a 2 per cent w/v solution of *potassium hydrogen phthalate*, mix, allow to stand for 15 minutes and add 1 ml of a 4 per cent solution of *aniline* in *ethanol (95 per cent)*; a yellow colour is produced.

Tests

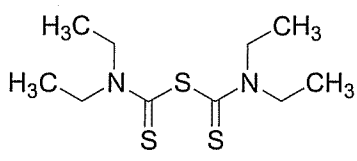
pH (2.4.24). 5.5 to 6.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 25 mg of mepyramine maleate add sufficient *0.01 M hydrochloric acid* to produce 100.0 ml. Dilute 10.0 ml of this solution to 100.0 ml with *0.01 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 316 nm (2.4.7). Calculate the content of $C_{17}H_{23}N_3O,C_4H_4O_4$, taking 206 as the specific absorbance at 316 nm.

Monosulfiram

Sulfiram



$C_{10}H_{20}N_2S_3$ Mol. Wt. 264.5

Monosulfiram is bis(diethylthiocarbamoyl)sulphide.

Monosulfiram contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{10}H_{20}N_2S_3$, calculated on the anhydrous basis.

Category. Insecticide.

Description. A yellow or yellowish-brown soft solid; odour, sulphurous.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), a 2-cm layer of a 0.001 per cent w/v solution in *methanol* shows a well-defined absorption maximum only at about 281 nm; absorbance at about 281 nm, about 1.3.

B. Dissolve 0.1 g in a mixture of 0.15 ml of a 1 per cent w/v solution of *cupric sulphate* and 5 ml of *ethanol (95 per cent)*, evaporate on a water-bath and dissolve the residue in *dichloromethane*; a deep yellowish brown colour is produced.

C. Boil 0.1 g with *2 M hydrochloric acid*; hydrogen sulphide is evolved which has a characteristic odour and turns filter paper treated with *lead acetate solution*, black.

Tests

Freezing point (2.4.11). 28.5° to 32.0°.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

NOTE — Carry out the test in subdued light.

Mobile phase. A mixture of 70 volumes of n-*hexane* and 30 volumes of *butyl acetate*.

Test solution. Dissolve 2.5 g of the substance under examination in 100 ml of *ethyl acetate*.

Reference solution (a). A 0.125 per cent w/v solution of *disulfiram RS* in *ethyl acetate*.

Reference solution (b). A 0.050 per cent w/v solution of the substance under examination in *ethyl acetate*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any secondary spot is not more intense than the spot in the chromatogram obtained with reference solution (a) and any other spot, apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b).

Water (2.3.43). Not more than 1.0 per cent, determined on 1 g.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Assay. Weigh accurately about 0.35 g, dissolve in 8 ml of *nitrogen-free sulphuric acid* and carry out the method for the determination of nitrogen (2.3.30).

1 ml of *0.05 M sulphuric acid* is equivalent to 0.01322 g of $C_{10}H_{20}N_2S_3$.

Storage. Store protected from light.

Monosulfiram Soap

Monosulfiram Soap contains not less than 5 per cent w/w of monosulfiram in a toilet soap basis which may be perfumed.

Monosulfiram Soap contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of monosulfiram, $C_{10}H_{20}N_2S_3$.

Usual strength. 5 per cent w/w.

Identification

In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

NOTE — Carry out the test in subdued light.

Mobile phase. A mixture of 70 volumes of n-*hexane* and 30 volumes of *butyl acetate.*

Test solution (a). Shake a quantity of the finely shredded soap containing 20 mg of Monosulfiram with 10 ml of *dichloromethane*, filter and wash the filtrate with *dichloromethane*. Evaporate the combined filtrate and washings just to dryness at room temperature in a current of *nitrogen* and dissolve the residue in 1 ml of *ethanol (95 per cent).*

Test solution (b). Dilute 0.5 ml of test solution (a) to 10 ml with *ethanol (95 per cent).*

Reference solution (a). A 0.10 per cent w/v solution of *disulfiram RS* in *ethanol (95 per cent).*

Reference solution (b). A 0.040 per cent w/v solution of *monosulfiram RS* in *ethanol (95 per cent).*

Reference solution (c). A 0.10 per cent w/v solution of *monosulfiram RS* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with test solution any spot running ahead of the principal spot and corresponding in position to disulfiram is not more intense than the spot in the chromatogram obtained with reference solution (a) and any spot running behind the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b). Ignore any subsidiary spots due to the soap basis which may also be observed ahead of the principal spot in the chromatogram obtained with test solution (a).

Assay. Determine by gas chromatography (2.4.13).

NOTE —*Protect the solutions from light throughout the assay.*

Test solution (a). Weigh accurately a quantity of the finely shredded soap containing about 0.25 g of Monosulfiram, shake for 10 minutes with 50 ml of *dimethylformamide*, centrifuge and use the supernatant liquid.

Test solution (b). Weigh accurately a quantity of the finely shredded soap containing about 0.25 g of Monosulfiram, shake for 10 minutes with 50 ml of *dimethylformamide* containing 0.125 g of N-*phenylcarbazole* (internal standard), centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.5 per cent w/v of *monosulfiram RS* and 0.25 per cent w/v of N-*phenylcarbazole* (internal standard) in *dimethylformamide.*

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with 2 per cent w/w of methyl silicone gum on acid-washed, silanised diatomaceous support (80 to 100 mesh) (such as SE 30),
- temperature:
  column 180°,
  inlet port 180° and detector 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{10}H_{20}N_2S_3$.

Labelling. The label states (1) the proportion of Monosulfiram in the preparation; (2) the method of use of the preparation.

Monosulfiram Solution

Monosulfiram Solution is a solution of Monosulfiram in Ethanol (95 per cent) containing a suitable dispersing agent.

In making Monosulfiram Solution the ethanol (95 per cent) may be replaced by Industrial Methylated Spirit provided that the statutory requirements governing the use of Industrial Methylated Spirit are observed.

Monosulfiram Solution contains not less than 94.0 per cent and not more than 106.0 per cent of the stated amount of monosulfiram, $C_{10}H_{20}N_2S_3$.

Usual strength. 25 per cent w/w.

Description. Clear, bright, deep reddish-brown liquid; crystals from which may deposit slowly at low temperatures but dissolve on warming. Yields a pale yellow dispersion on dilution with *water.*

Identification

In the test for Related substances, the principal spot in the chromatogram obtained with test solution (b) corresponds to that in the chromatogram obtained with reference solution (c).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

NOTE — Carry out the test in subdued light.

Mobile phase. A mixture of 70 volumes of n-*hexane* and 30 volumes of *butyl acetate.*

Test solution (a). Dilute a quantity of the solution under examination with *ethanol (95 per cent)* so as to contain of 2.0 per cent w/v of Monosulfiram.

Test solution (b). Dilute 0.5 ml of test solution (a) to 10 ml with *ethanol (95 per cent).*

Reference solution (a). A 0.10 per cent w/v solution of *disulfiram RS* in *ethanol (95 per cent).*

Reference solution (b). A 0.040 per cent w/v solution of *monosulfiram RS* in *ethanol (95 per cent).*

Reference solution (c). A 0.10 per cent w/v solution of *monosulfiram RS* in *ethanol (95 per cent).*

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution any spot running ahead of the principal spot and corresponding in position to disulfiram is not more intense than the spot in the chromatogram obtained with reference solution (a) and any spot running behind the principal spot is not more intense than the spot in the chromatogram obtained with reference solution (b).

Assay. *Protect the solutions from light throughout the assay.*

Determine by gas chromatography (2.4.13).

Test solution (a). Dilute the solution under examination in *dimethylformamide* containing the equivalent of 0.5 per cent w/v of Monosulfiram.

Test solution (b). Weigh accurately a quantity of the finely shredded soap containing about 0.25 g of Monosulfiram, shake for 10 minutes with 50 ml of *dimethylformamide* containing 0.125 g of N-*phenylcarbazole* (internal standard), centrifuge and use the supernatant liquid.

Reference solution. A solution containing 0.5 per cent w/v of *monosulfiram RS* and 0.25 per cent w/v of N-*phenylcarbazole* (internal standard) in *dimethylformamide.*

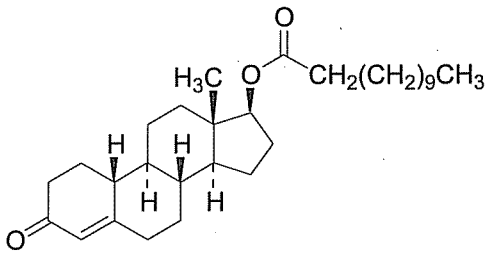
Chromatographic system

- a glass column 1.5 m × 4 mm, packed with 2 per cent w/w of methyl silicone gum on acid-washed, silanised diatomaceous support (80 to 100 mesh) (such as SE 30),
- temperature:
  column 180°,
  inlet port 180° and detector 280°,
- flow rate. 30 ml per minute of the carrier gas.

Calculate the content of $C_{10}H_{20}N_2S_3$.

Labelling. The label states (1) the percentage w/w of monosulfiram; (2) the method of use of the preparation.

Nandrolone Laurate



$C_{30}H_{48}O_3$ Mol. Wt. 456.7

Nandrolone Laurate is 3-oxoestr-4-en-17β-yl-dodecanoate.

Nandrolone Laurate contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{30}H_{48}O_3$, calculated on the dried basis.

Category. Anabolic steroid; androgen.

Dose. *All species.* By subcutaneous or intramuscular injection, 0.2 to 1 mg per kg of body weight once every two weeks.

Description. A white to creamy white, crystalline powder; odour, faint and characteristic.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nandrolone laurate RS* or with the reference spectrum of nandrolone laurate.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254,* surface of which has been modified by chemically bonded octadecylsilyl groups (such as Whatman KC 18F plates).

Mobile phase. A mixture of 60 volumes of *2-propanol,* 40 volumes of *acetonitrile* and 20 volumes of *water.*

Test solution. Dissolve 0.5 g of the substance under examination in *dichloromethane.*

Reference solution (a). A 0.5 per cent w/v of *nandrolone laurate RS* in *dichloromethane.*

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a).

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and heat at 100° for 10 minutes. Allow to cool and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference

solution (a). The test is not valid unless the principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

C. Melts at about 47° (2.4.21).

Tests

Specific optical rotation (2.4.22). +31.0° to +35.0°, determined in a freshly prepared 2 per cent w/v solution in *dioxan*.

Nandrolone. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 70 volumes of n-*heptane* and 30 volumes of *acetone*.

Test solution. Dissolve 1.5 g of the substance under examination in *dichloromethane*.

Reference solution. A 0.030 per cent w/v of *nandrolone RS* in *dichloromethane*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable, spray with a 10 per cent v/v solution of *sulphuric acid* in *ethanol (95 per cent)*, heat at 105° for 30 minutes and examine in ultraviolet light at 365 nm. Any spot in the chromatogram obtained with the test solution corresponding to nandrolone is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 24 hours.

Assay. Weigh accurately about 0.1 g, dissolve in sufficient *ethanol* to produce 100.0 ml and dilute 10.0 ml to 100.0 ml with *ethanol*. Dilute 10.0 ml of this solution to 100.0 ml with *ethanol* and measure the absorbance of the resulting solution at the maximum at about 240 nm (2.4.7). Calculate the content of $C_{30}H_{48}O_3$ taking 380 as the specific absorbance at 240 nm.

Storage. Store protected from light.

Nandrolone Laurate Injection

Nandrolone Laurate Injection is a sterile solution of Nandrolone Laurate in Ethyl Oleate or other suitable ester, in a suitable fixed oil, or in any mixture of these.

Nandrolone Laurate Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of nandrolone laurate, $C_{30}H_{48}O_3$.

Usual strengths. 25 mg in 1 ml; 50 mg in 1 ml.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*, surface of which has been modified by chemically bonded octadecylsilyl groups (such as Whatman KC 18F plates).

Mobile phase. A mixture of 60 volumes of *2-propanol*, 40 volumes of *acetonitrile* and 20 volumes of *water*.

Test solution. Dilute a suitable volume with *dichloromethane* to produce a solution containing 0.5 per cent w/v of Nandrolone Laurate .

Reference solution (a). A 0.5 per cent w/v of *nandrolone laurate RS* in *dichloromethane*.

Reference solution (b). A mixture of equal volumes of the test solution and reference solution (a)

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and heat at 100° for 10 minutes. Allow to cool and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution (a). The test is not valid unless the principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 0.1 g of Nandrolone Laurate add sufficient *dichloromethane* to produce 100.0 ml. Dilute 3.0 ml of the resulting solution to 50.0 ml with *dichloromethane*. To 5.0 ml of this solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of *dichloromethane* treated in a similar manner. Calculate the content of $C_{30}H_{48}O_3$ from the absorbance obtained by repeating the procedure using a suitable quantity of *nandrolone RS*.

1 mg of $C_{18}H_{26}O_2$ is equivalent to 0.001664 g of $C_{30}H_{48}O_3$.

Storage. Store protected from light.

Niclosamide Veterinary Oral Powder

Niclosamide Dispersible Powder for Veterinary Use

Niclosamide Veterinary Oral Powder contains Niclosamide with suitable auxiliary substances.

Niclosamide Veterinary Oral Powder contains not less than 97.0 per cent and not more than 103.0 per cent of the stated amount of niclosamide, $C_{13}H_8Cl_2N_2O_4$.

Usual strength. 75 per cent w/w.

Identification

Heat 50 mg with 5 ml of *1 M hydrochloric acid* and 0.1 g of *zinc powder* in a water-bath for 10 minutes, cool and filter. To the filtrate add 0.5 ml of a 1 per cent w/v solution of *sodium nitrite* and allow to stand for 10 minutes. Add 2 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 10 minutes and add 2 ml of a 0.5 per cent w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*; a deep red colour is produced.

Tests

2-Chloro-4-nitroaniline. Boil a quantity containing 0.10 g of Niclosamide with 20 ml of *methanol* for 2 minutes, cool, add sufficient *1 M hydrochloric acid* to produce 50 ml and filter. To 10 ml of the filtrate add 0.5 ml of a 0.5 per cent w/v solution of *sodium nitrite* and allow to stand for 10 minutes. Add 1 ml of a 2 per cent w/v solution of *ammonium sulphamate*, shake, allow to stand for 10 minutes and add 1 ml of a 0.5 per cent w/v solution of N-*(1-naphthyl)ethylenediamine dihydrochloride*. The colour produced is not more than that produced by simultaneously treating 10 mg of *2-chloro-4-nitroaniline* in the same manner.

5-Chlorosalicylic acid. Boil a quantity containing 0.50 g of Niclosamide with 10 ml of *water* for 2 minutes, cool and filter. To the filtrate add a few drops of *ferric chloride solution*; no red or violet colour is produced.

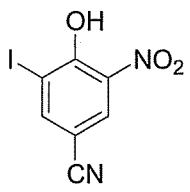
Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 105° for 4 hours.

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. Weigh accurately a quantity containing about 0.3 g of Niclosamide, dissolve in 60 ml of *dimethylformamide* with the aid of gentle heat, cool. Titrate with *0.1 M tetrabutylammonium hydroxide*, maintaining a stream of *nitrogen* through the solution throughout the titration, and determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.03271 g of $C_{13}H_8Cl_2N_2O_4$.

Nitroxynil



$C_7H_3IN_2O_3$ Mol. Wt. 290.0

Nitroxynil is 4-hydroxy-3-iodo-5-nitrobenzonitrile.

Nitroxynil contains not less than 98.0 per cent and not more than 101.0 per cent of $C_7H_3IN_2O_3$, calculated on the dried basis.

Category. Anthelmintic.

Dose. *Cows, buffaloes and sheep*. By subcutaneous injection, 10 mg per kg of body weight, repeated if necessary at intervals of not less than 28 days.

Description. A yellow to yellowish brown powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *nitroxynil RS* or with the reference spectrum of nandrolone nitroxynil.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.01 M sodium hydroxide* exhibits maxima at about 225 nm and at about 271 nm; absorbance at about 271 nm, about 1.3.

C. When heated with *sulphuric acid*, iodine vapours are evolved.

D. Melting range (2.4.21). 136° to 139°.

Tests

Inorganic iodide. To 0.40 g add 0.35 g of N-*methylglucamine* and 10 ml of *water*. Shake to dissolve and add sufficient *water* to produce 50 ml. To 10 ml of the resulting solution add 4 ml of *1 M sulphuric acid* and extract with three quantities, each of 10 ml, of *dichloromethane*. Add to the aqueous extract 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *dichloromethane*, shake for 2 minutes and allow to separate. Any purple colour in the dichloromethane layer is not more intense than that obtained by adding 2 ml of a 0.0026 per cent w/v solution of *potassium iodide* to a mixture of 4 ml of *1 M sulphuric acid* and 8 ml of *water*, adding 10 ml of *dichloromethane*, shaking for 2 minutes, adding to the aqueous layer 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *dichloromethane*, shaking for 2 minutes and allowing to separate (500 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 105° for 4 hours.

Assay. Carry out the oxygen flask method for iodine (2.3.34), using 25 mg.

1 ml of *0.02 M sodium thiosulphate* is equivalent to 0.0009667 g of $C_7H_3IN_2O_3$.

Storage. Store protected from light.

Nitroxynil Injection

Nitroxynil Injection is a sterile solution of the *N*-ethylglucamine salt of Nitroxynil in Water for Injections.

Nitroxynil Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of nitroxynil, $C_7H_3IN_2O_3$.

Usual strengths. 200 mg in 1 ml; 340 mg in 1 ml.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), of the final solution obtained in the Assay exhibits a maximum at about 271 nm.

B. Heat 0.5 ml with 3 ml of *sulphuric acid*; iodine vapours are evolved.

Tests

pH (2.4.24). 5.0 to 7.0, determined by using a 20 per cent w/v solution of *N-ethylglucamine hydrochloride* instead of a saturated solution of *potassium chloride* as the liquid junction solution.

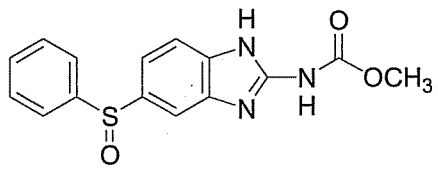
Inorganic iodide. To a volume containing 0.4 g of Nitroxynil add 0.35 g of N-*methylglucamine* and dilute to 100 ml with *water*. To 10 ml of the diluted solution add 4 ml of *1 M sulphuric acid* and extract with three quantities, each of 10 ml, of *dichloromethane*. Add to the aqueous extract 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *dichloromethane*, shake for 2 minutes and allow to separate. Any purple colour in the dichloromethane layer is not more intense than that obtained by adding 2 ml of a 0.0026 per cent w/v solution of *potassium iodide* to a mixture of 4 ml of *1 M sulphuric acid* and 8 ml of *water*, adding 10 ml of *dichloromethane*, shaking for 2 minutes, adding to the aqueous layer 1 ml of *hydrogen peroxide solution (100 vol)* and 1 ml of *dichloromethane*, shaking for 2 minutes and allowing to separate (500 ppm) (0.1 per cent w/v of iodide).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 1.7 g of Nitroxynil add sufficient *0.01 M sodium hydroxide* to produce 500.0 ml. Dilute 20.0 ml of this solution to 500.0 ml with *0.01 M sodium hydroxide*. To 5.0 ml of this solution add sufficient *0.01 M sodium hydroxide* to produce 100.0 ml and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_7H_3IN_2O_3$ taking 660 as the specific absorbance at 271 nm.

Storage. Store protected from light.

Oxfendazole



$C_{15}H_{13}N_3O_3S$ Mol. Wt. 315.4

Oxfendazole is methyl 5-(phenylsulphinyl)-2-benzimidazolecarbamate.

Oxfendazole contains not less than 97.0 per cent and not more than 100.5 per cent of $C_{15}H_{13}N_3O_3S$, calculated on the dried basis.

Category. Anthelmintic.

Dose. *Horses*. 10 mg per kg of body weight. *Cows and buffaloes*. 4.5 mg per kg of body weight. *Sheep*. 5 mg per kg of body weight.

Description. A white or almost white powder; odour, slight and characteristic.

Identification

A. Dissolve 0.1 g in 50 ml of *methanol*, evaporate to a volume of about 2 ml, cool, filter, wash the residue with 2 ml of *water* and dry at 105° at a pressure not exceeding 2.7 kPa. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxfendazole RS* or with the reference spectrum of oxfendazole.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* exhibits two maxima at about 228 nm and about 297 nm; absorbances at about 228 nm, about 1.4 and at about 297 nm, about 0.55.

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 40 volumes of *ethyl acetate* and 10 volumes of *glacial acetic acid*.

Mobile phase. A mixture of 95 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid*.

Test solution. Dissolve 0.5 g of the substance under examination in 100 ml of solvent mixture.

Reference solution (a). A 0.010 per cent w/v solution of the substance under examination in solvent mixture.

Reference solution (b). A 0.0050 per cent w/v solution of *methyl 5-phenylthio-1H-benzimidazol-2-yl carbamate RS* in solvent mixture.)

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to methyl 5-phenylthio-1*H*-benzimidazol-2-yl carbamate in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Any other secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 105° for 2 hours at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.3 g, dissolve in 20 ml of *glacial acetic acid*, add 3 g of *potassium iodide* and 1 ml of *acetyl chloride* and stir for 10 minutes. Add 50 ml of *1 M hydrochloric acid* and 10 ml of *dichloromethane* and titrate immediately with *0.1 M sodium thiosulphate*, shaking after each addition, until the dichloromethane layer is colourless. Repeat the operation omitting the substance under examination; the difference between the titrations represents the amount of sodium thiosulphate required.

1 ml of *0.1 M sodium thiosulphate* is equivalent to 0.01577 g of $C_{15}H_{13}N_3O_3S$.

Storage. Store protected from light.

Oxfendazole Veterinary Oral Suspension

Oxfendazole Veterinary Mixture; Oxfendazole Mixture; Oxfendazole Oral Suspension

Oxfendazole Veterinary Oral Suspension is an aqueous suspension of Oxfendazole containing suitable suspending or dispersing agents.

Oxfendazole Veterinary Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oxfendazole, $C_{15}H_{13}N_3O_3S$.

Usual strengths. 2.265 per cent w/v; 9.06 per cent w/v.

Identification

Shake a quantity containing 0.1 g of Oxfendazole with 50 ml of *methanol* for 15 minutes, centrifuge, evaporate the supernatant liquid to a volume of about 2 ml, cool, filter and wash the residue with 2 ml of *water* and dry at 105° for 1 hour at a pressure not exceeding 2.7 kPa. The residue complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxfendazole RS* or with the reference spectrum of oxfendazole.

B. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *1 M hydrochloric acid* exhibits three maxima, at about 226, 284 and 291 nm.

Tests

pH (2.4.24). 4.3 to 5.3.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. 40 volumes of *ethyl acetate* and 10 volumes of *glacial acetic acid.*

Mobile phase. A mixture of 95 volumes of *ethyl acetate* and 5 volumes of *glacial acetic acid.*

Test solution. Shake a quantity containing 0.1 g of Oxfendazole with 20 ml of solvent mixture and filter.

Reference solution (a). Dilute 1 volume of test solution to 50 volumes with the solvent mixture.

Reference solution (b). A 0.0050 per cent w/v solution of *methyl 5-phenylthio-1H-benzimidazol-2-yl carbamate RS* in solvent mixture.)

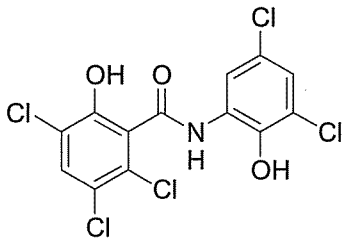
Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any spot corresponding to methyl 5-phenylthio-1*H*-benzimidazol-2-yl carbamate in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (b). Any other secondary spot in the chromatogram obtained with test solution is not more intense than the spot in the chromatogram obtained with reference solution (a).

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. Weigh accurately a quantity of the well-mixed suspension containing about 0.1 g of Oxfendazole and disperse in 15 ml of *water*. Add 200 ml of *methanol* and mix in an ultrasonic bath for 15 minutes, cool, add sufficient *methanol* to produce 500.0 ml and filter. Dilute 2 ml of the filtrate to 50 ml with *methanol* and measure the absorbance of the resulting solution at the maximum at about 296 nm (2.4.7). Calculate the content of $C_{15}H_{13}N_3O_3S$ taking 550 as the specific absorbance at 296 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of oxfendazole, weight in volume.

Oxyclozanide



$C_{13}H_6Cl_5NO_3$ Mol. Wt. 401.5

Oxyclozanide is 2,3,5-trichloro-*N*-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide.

Oxyclozanide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{13}H_6Cl_5NO_3$, calculated on the dried basis.

Category. Anthelmintic.

Dose. *Cows, buffaloes and sheep*. 10 to 15 mg per kg of body weight.

Description. A pale cream to cream-coloured powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *oxyclozanide RS* or with the reference spectrum of oxyclozanide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.003 per cent w/v solution in *1 M methanolic hydrochloric acid* exhibits a maximum only at about 300 nm; absorbance at about 300 nm, about 0.76.

C. Melting range (2.4.21). 208° to 211°.

Tests

Ionisable chlorine. Dissolve 2 g in 100 ml of *methanol*, add 10 ml of *1.5 M nitric acid* and titrate with *0.1 M silver nitrate*, determining the end-point potentiometrically (2.4.25). Not more than 1.4 ml is required (0.25 per cent).

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. A 0.1 per cent w/v solution of the substance under examination prepared by dissolving it in a suitable volume of *methanol* and slowly diluting with *water* containing 0.1 per cent v/v of *phosphoric acid* to give a solution containing about the same proportion of methanol to water as in the mobile phase.

Reference solution. Dilute 1 ml of test solution to 100 ml with the mobile phase.

Chromatographic system

- a stainless steel column 20 cm × 5 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Hypersil ODS),
- mobile phase: a filtered and degassed mixture of 62 volumes of *methanol* and 38 volumes of *water* containing 0.1 per cent v/v of *phosphoric acid*,
- flow rate. 2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume. 20 µl.

Inject alternatively test solution and the reference solution. In the chromatogram obtained with test solution the area of any secondary peak with a retention time less than that of the principal peak is not more than one-third of the area of the principal peak in the chromatogram obtained with reference solution and the area of any secondary peak with a retention time greater than that of the principal peak is not more than the area of the principal peak in the chromatogram obtained with reference solution.

Sulphated ash (2.3.18). Not more than 0.2 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1 g by drying in an oven at 60° at a pressure not exceeding 0.7 kPa.

Assay. Weigh accurately about 0.25 g, dissolve in 75 ml of *anhydrous pyridine* and pass a stream of *nitrogen* through the solution for 5 minutes. Titrate with *0.1 M tetrabutylammonium hydroxide*, maintaining a stream of *nitrogen* through the solution throughout the titration, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M tetrabutylammonium hydroxide* is equivalent to 0.02007 g of $C_{13}H_6Cl_5NO_3$.

Oxyclozanide Veterinary Oral Suspension

Oxyclozanide Oral Suspension; Oxyclozanide Suspension; Oxyclozanide Mixture; Oxyclozanide Drench

Oxyclozanide Veterinary Oral Suspension is an aqueous suspension of Oxyclozanide containing suitable suspending or dispersing agents.

Oxyclozanide Veterinary Oral Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of oxyclozanide, $C_{13}H_6Cl_5NO_3$.

Usual strength. 3.4 per cent w/v.

Identification

In test A for Related substances, the principal spot in the chromatogram obtained with 10 ml of test solution corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *light petroleum (60° to 80°)*, 20 volumes of *acetone* and 5 volumes of *glacial acetic acid.*

Test solution. Dilute a quantity with *acetone* to contain 1.0 per cent w/v of Oxyclozanide, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.050 per cent w/v solution of *3,5,6-trichloro- 2-hydroxybenzoic acid RS* in *acetone.*

Reference solution (b). A 1.0 per cent w/v solution of *oxyclozanide RS* in *acetone.*

Apply to the plate 40 µl and 10 µl of test solution, 4 µl of reference solution (a) and 10 µl of reference solution (b). After development, dry the plate in air and spray with a 3 per cent w/v solution of *ferric chloride* in *methanol*. In the chromatogram obtained with 40 µl of test solution any spot corresponding to 3,5,6-trichloro-2-hydroxybenzoic acid RS is not more intense than that in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution.*

Test solution. Dilute a quantity with *acetone* to contain 1.0 per cent w/v of Oxyclozanide, centrifuge and use the supernatant liquid.

Reference solution. A 0.040 per cent w/v of *2-amino-4,6-dichlorophenol RS* in *acetone.*

Apply to the plate 40 µl of test solution and 4 µl of reference solution. After development, dry the plate in air and spray with *lithium and sodium molybdotungstophosphate solution.* In the chromatogram obtained with test solution any spot corresponding to 2-amino-4,6-dichlorophenol is not more intense than that in the chromatogram obtained with reference solution.

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. Protect the solutions from light throughout the procedure. Weigh accurately a quantity containing about 60 mg of Oxyclozanide, add 60 ml of *acidified methanol* and boil gently on a water-bath. Shake continuously for 20 minutes, cool to 2° and dilute to 100.0 ml with *acidified methanol.* Filter, dilute 5.0 ml of the filtrate to 100.0 ml with *acidified methanol* and measure the absorbance of the resulting solution at the maximum at about 300 nm (2.4.7). Calculate the content of $C_{13}H_6Cl_5NO_3$ taking 254 as the specific absorbance at 300 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of oxyclozanide, weight in volume.

Oxyclozanide Premix

Oxyclozanide Granules

Oxyclozanide Premix contains Oxyclozanide.

Oxyclozanide Premix contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of oxyclozanide, $C_{13}H_6Cl_5NO_3$.

Usual strength. 5 per cent w/w.

Identification

In test A for Related substances, the principal spot in the chromatogram obtained with 10 ml of test solution corresponds to that in the chromatogram obtained with reference solution (b).

Tests

Related substances. A. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 60 volumes of *light petroleum (60° to 80°)*, 20 volumes of *acetone* and 5 volumes of *glacial acetic acid.*

Test solution. Extract the finely powdered preparation under examination with sufficient *acetone* to produce a mixture containing 1.0 per cent w/v of Oxyclozanide, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.050 per cent w/v solution of *3,5,6-trichloro- 2-hydroxybenzoic acid RS* in *acetone.*

Reference solution (b). A 1.0 per cent w/v solution of *oxyclozanide RS* in *acetone.*

Apply to the plate 40 µl and 10 µl of test solution, 4 µl of reference solution (a) and 10 µl of reference solution (b). After development, dry the plate in air and spray with a 3 per cent

w/v solution of *ferric chloride* in *methanol*. In the chromatogram obtained with 40 µl of test solution any spot corresponding to 3,5,6-trichloro-2-hydroxybenzoic acid RS is not more intense than that in the chromatogram obtained with reference solution (a).

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 100 volumes of *ethyl acetate*, 10 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution. Extract the finely powdered preparation under examination with sufficient *acetone* to produce a mixture containing 1.0 per cent w/v of Oxyclozanide, centrifuge and use the supernatant liquid.

Reference solution. A 0.040 per cent w/v of *2-amino-4,6-dichlorophenol RS* in *acetone*.

Apply to the plate 40 µl of test solution and 4 µl of reference solution. After development, dry the plate in air and spray with *lithium and sodium molybdotungstophosphate solution*. In the chromatogram obtained with test solution any spot corresponding to 2-amino-4,6-dichlorophenol is not more intense than that in the chromatogram obtained with reference solution.

Other tests. Complies with the tests stated under Premixes.

Assay. Protect the solutions from light throughout the procedure. Weigh accurately a quantity of the finely powdered preparation under examination containing 60 mg of Oxyclozanide, add 60 ml of *acidified methanol* and boil gently on a water-bath. Shake continuously for 20 minutes, cool to 2° and dilute to 100.0 ml with *acidified methanol*. Filter, dilute 5.0 ml of the filtrate to 100.0 ml with *acidified methanol* and measure the absorbance of the resulting solution at the maximum at about 300 nm (2.4.7). Calculate the content of $C_{13}H_6Cl_5NO_3$ taking 254 as the specific absorbance at 300 nm.

Labelling. The label states (1) the proportion of oxyclozanide in the premix and (2) the method of use of the preparation.

Oxytetracycline Veterinary Oral Powder

Oxytetracycline Hydrochloride Veterinary Oral Powder; Oxytetracycline Hydrochloride Soluble Powder; Oxytetracycline Soluble Powder

Oxytetracycline Veterinary Oral Powder is a mixture of Oxytetracycline Hydrochloride and Lactose or other suitable diluent.

Oxytetracycline Veterinary Oral Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of oxytetracycline hydrochloride, $C_{22}H_{24}N_2O_9,HCl$.

Usual strength. 5.6 per cent w/w of Oxytetracycline Hydrochloride.

Identification

A. Determine by thin-layer chromatography (2.4.17), coating the plate with a substance prepared by mixing 25 g of *silica gel G* with 50 ml of a mixture of 2.5 ml of *glycerin* and 47.5 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*. After spreading the plate, allow it to stand at room temperature till it is dry (70 to 90 minutes).

Mobile phase. The lower layer formed after shaking 200 ml of a mixture of 2 volumes of *ethyl acetate*, 2 volumes of *dichloromethane* and 1 volume of *acetone* with 25 ml of *0.1 M disodium edetate* previously adjusted to pH 7.0 with *dilute ammonia solution*.

Test solution. Extract a quantity of the oral powder containing 10 mg of Oxytetracycline Hydrochloride with 20 ml of *methanol*, centrifuge and use the supernatant liquid.

Reference solution (a). A 0.05 per cent w/v solution of *oxytetracycline hydrochloride RS* in *methanol*.

Reference solution (b). A solution containing 0.05 per cent w/v each of *demethylchlortetracycline hydrochloride RS*, *oxytetracycline hydrochloride RS* and *tetracycline hydrochloride RS* in *methanol*.

Apply to the plate 1 µl of each solution. After development, dry the plate in air, expose to the vapours of ammonia and examine in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The test is not valid unless the chromatogram obtained with reference solution (b) shows three clearly separated spots.

B. To a quantity of the powder containing 0.4 mg of Oxytetracycline Hydrochloride add 5 ml of a 1 per cent w/v solution of *sodium carbonate*, shake and add 2 ml of *diazotised sulphanilic acid solution*; a light brown colour is produced.

C. Shake a quantity of the powder containing 100 mg of Oxytetracyline Hydrochloride with 10 ml of *2 M nitric acid* and filter. To the filtrate add *activated charcoal* to decolorise it and filter again. The filtrate gives the reactions of chlorides (2.3.1).

Tests

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve a quantity of the oral powder containing about 50 mg of Oxytetracycline Hydrochloride in 100.0 ml of

0.01 M hydrochloric acid. Dilute 1.0 ml of this solution to 10.0 ml with *0.01 M hydrochloric acid.*

Reference solution (a). A 0.005 per cent w/v solution of *oxytetracycline RS* in *0.01 M hydrochloric acid.*

Reference solution (b). A 0.1 per cent w/v solution of *4-epioxytetracycline RS* in *0.01 M hydrochloric acid.*

Reference solution (c). A 0.1 per cent w/v solution of *tetracycline hydrochloride RS* in *0.01 M hydrochloric acid.*

Reference solution (d). Dilute 1.5 ml of a 0.1 per cent w/v solution of *oxytetracycline RS* in *0.01 M hydrochloric acid,* 1.0 ml of reference solution (b) and 3.0 ml of reference solution (c) to 25.0 ml with *0.01 M hydrochloric acid.*

Chromatographic system
- a stainless steel column 25 cm x 4.6 mm, packed with styrene divinylbenzene copolymer (8 to 10 µm),
- column temperature. 60°,
- mobile phase: to 50 g of *2 methylpropan-2-ol,* add 200 ml of *water,* 60 ml of *0.33 M phosphate buffer pH 7.5,* 50 ml of 1.0 per cent w/v solution of *tetrabutylammonium hydrogen sulphate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and 10 ml of a 0.04 per cent w/v solution of *disodium edetate* previously adjusted to pH 7.5 with *2 M sodium hydroxide* and dilute to 1000 ml with *water,*
- flow rate. 1 ml per minute,
- spectrophotometer set at 254 nm,
- injection volume. 20 µl.

Inject reference solution (d). The test is not valid unless the resolution between the peaks due to 4- epioxytetracycline and oxytetracycline is not less than 4.0, the resolution between the peak due to oxytetracycline and tetracycline is not less than 5.0 and the tailing factor of the principal peak due to oxytetracycline is not more than 1.25.

Inject the test solution and reference solution (a).

Calculate the content of $C_{22}H_{24}N_2O_9$,HCl in the oral powder.

1 mg of $C_{22}H_{24}N_2O_9$ is equivalent to 1.079 mg of $C_{22}H_{24}N_2O_9$,HCl.

Storage. Store at a temperature not exceeding 15°.

Pentobarbitone Injection

Pentobarbitone Sodium Injection; Pentobarbital Sodium Injection

Pentobarbitone Injection is a sterile solution of Pentobarbitone Sodium in a suitable vehicle.

Solutions containing 20 per cent w/v of Pentobarbitone Sodium in 100-ml and 500-ml containers are also available for use other than for injection. Such solutions may be coloured and need not be sterile but must comply with all other requirements of this monograph.

Pentobarbitone Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of pentobarbitone sodium, $C_{11}H_{17}N_2NaO_3$.

Usual strength. 60 mg in 1 ml.

Description. A clear, colourless or almost colourless solution.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *pentobarbitone RS* or with the reference spectrum of pentobarbitone.

B. The residue obtained in the Assay melts at about 128° (2.4.21).

C. When introduced on a platinum wire into a Bunsen burner flame, a golden yellow colour is imparted to the flame.

Tests

pH (2.4.24). 10.0 to 11.5.

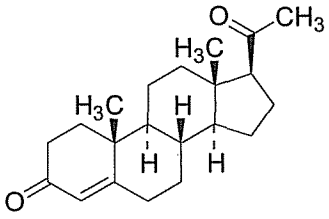
Isomer. To a volume of the injection containing 0.3 g of Pentobarbitone Sodium diluted, if necessary, to 5 ml with *water* add 0.3 g of *4-nitrobenzyl bromide* dissolved in 10 ml of *ethanol (95 per cent).* Heat under a reflux condenser for 30 minutes, cool to 25°, scratch the sides of the vessel with a glass rod if necessary to induce crystallisation, filter and wash the residue with five quantities, each of 5 ml, of *water.* Transfer the residue as completely as possible to a small flask, add 25 ml of *ethanol (95 per cent)* and heat under a reflux condenser for 10 minutes. Filter the hot solution, cool to 25° and scratch the sides of the vessel with a glass rod to induce crystallisation. Filter and wash the residue with two quantities, each of 5 ml, of *water* and dry at 105° for 30 minutes. The dried residue melts completely between 136° and 148° (2.4.21).

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 0.5 g of Pentobarbitone Sodium diluted to 15 ml with *water* add 5 ml of *2 M hydrochloric acid,* extract with 50 ml of *ether* and then with successive quantities, each of 25 ml, of *ether* until complete extraction is effected. Wash the combined extracts with two quantities, each of 5 ml, of *water* and wash the combined aqueous extracts with 10 ml of *ether.* Add the ether washings to the main ethereal extract, filter and wash the filter with *ether.* Evaporate the solvent and dry the residue to constant weight at 105°.

1 g of the residue is equivalent to 1.097 g of $C_{11}H_{17}N_2NaO_3$.

Progesterone



$C_{21}H_{30}O_2$ Mol. Wt. 314.5

Progesterone is pregn-4-en-3,20-dione.

Progesterone contains not less than 97.0 per cent and not more than 103.0 per cent of $C_{21}H_{30}O_2$, calculated on the dried basis.

Category. Progestogen.

Dose. By subcutaneous or intramuscular injection. *Cows, buffaloes and horses*. 200 to 800 mg per kg of body weight every 48 hours. *Dogs*. 3 mg per kg of body weight. By implantation. *Cows, buffaloes and horses*. 200 to 400 mg. *Dogs*. 25 to 100 mg.

Description. Colourless crystals or a white or almost white crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *progesterone RS* or with the reference spectrum of progesterone. If the spectra are not concordant, prepare spectra using 5 per cent w/v solutions in *chloroform IR*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 66 volumes of *dichloromethane* and 33 volumes of *ethyl acetate*.

Solvent mixture. 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 100 ml of the solvent mixture.

Reference solution. A 0.1 per cent w/v solution of *progesterone RS* in the solvent mixture.

Apply to the plate 5 µl of each solution. After removal of the plate, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution. Spray the plate with *ethanolic sulphuric acid* (20 per cent), heat at 120° for 15 minutes, allow to cool and examine in day light and under ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds in position, colour in day light, fluorescence under ultraviolet light and size to that in the chromatogram obtained with the reference solution.

Tests

Specific optical rotation (2.4.22). +186° to +194°, determined in a 1.0 per cent w/v solution in *ethanol (95 per cent)*.

Related substances. Determine by thin layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 66 volumes of *dichloromethane* and 33 volumes of *ethyl acetate*.

Solvent mixture. 90 volumes of *dichloromethane* and 10 volumes of *methanol*.

Test solution. Dissolve 0.1 g of the substance under examination in 10.0 ml of the solvent mixture.

Reference solution. Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Apply the plate 5 µl of each solution. After development, dry the plate in air and spray with a saturated solution of *potassium dichromate* in *sulphuric acid* (70 per cent), heat at 130° for 30 minutes and allow to cool. Any secondary spot in the chromatogram obtain with test solution is not more intense than the spot in the chromatogram obtain with reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 0.5 g by drying in an oven at 105° for 2 hours.

Assay. Weigh accurately about 10 mg and dissolve in 100.0 ml of *ethanol (95 per cent)*. Dilute 5.0 ml of the solution to 50.0 ml with *ethanol (95 per cent)*. Measure the absorbance of the resulting solution at the maximum at about 241 nm (2.4.7). Calculate the content of $C_{21}H_{30}O_2$ taking 535 as the specific absorbance at 241 nm.

Storage. Store protected from light.

Progesterone Injection

Progesterone Injection is a sterile solution of Progesterone in Ethyl Oleate or other suitable ester, in a suitable fixed oil or in any mixture of these. It may contain suitable alcohols.

Progesterone Injection contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of progesterone, $C_{21}H_{30}O_2$.

Usual strengths. 250 mg per ml.

Identification

Dissolve a volume containing 50 mg of Progesterone in 8 ml of *light petroleum (40° to 60°)* and extract with three quantities, each of 8 ml, of a mixture of 7 volumes of *glacial acetic acid* and 3 volumes of *water* until the solution becomes turbid, allow to stand in ice for 2 hours and filter. The precipitate, after washing with *water* and drying at 105°, complies with the following tests.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *progesterone RS* or with the reference spectrum of progesterone. If the spectra are not concordant, prepare spectra using 5 per cent w/v solutions in *chloroform IR*.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Solvent mixture. A mixture of 90 volumes of *acetone* and 10 volumes of *1,2-propanediol*.

Mobile phase. A mixture of equal volumes of *cyclohexane* and *light petroleum (40° to 60 °)*.

Test solution. Dissolve 25 mg of the substance under examination in 10 ml of the solvent mixture.

Reference solution (a). A 0.25 per cent w/v solution of *progesterone RS* in the solvent mixture.

Reference solution (b). Mix equal volumes of the test solution and reference solution (a).

Place the dry plate in a tank containing a shallow layer of the solvent mixture, allow the solvent mixture to ascend to the top, remove the plate from the tank and allow the solvent to evaporate. Use within 2 hours, with the flow of the mobile phase in the direction in which the aforementioned treatment was done.

Apply to the plate 2 µl of each solution. Allow the mobile phase to rise 12 cm. Dry the plate in a current of warm air, allow the solvent to evaporate, heat at 120° for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v)*. Heat at 120° for a further 10 minutes, allow to cool and examine in daylight and in ultraviolet light at 365 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution (a). The principal spot in the chromatogram obtained with reference solution (b) appears as a single, compact spot.

Tests

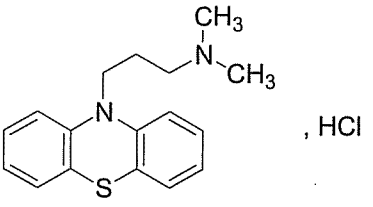
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. To an accurately measured volume containing about 50 mg of Progesterone add sufficient *dichloromethane* to produce 100.0 ml. Dilute 3.0 ml to 50.0 ml with *dichloromethane*. To 5.0 ml of the solution add 10 ml of *isoniazid solution* and sufficient *methanol* to produce 20.0 ml. Allow to stand for 45 minutes and measure the absorbance of the resulting solution at the maximum at about 380 nm (2.4.7), using as the blank 5 ml of *dichloromethane* treated in the same manner. Calculate the content of $C_{21}H_{30}O_2$ from the absorbance obtained by repeating the procedure using a 0.003 per cent w/v solution of *progesterone RS* in *dichloromethane* and beginning at the words "To 5.0 ml of the solution.....".

Storage. Store protected from light. If solid matter separates on standing, it should be redissolved by heating before use.

Labelling. The label states (1) the composition of the solvent; (2) that the preparation is intended for veterinary use by subcutaneous or intramuscular injection only.

Promazine Hydrochloride



$C_{17}H_{20}N_2S,HCl$ Mol. Wt. 320.9

Promazine Hydrochloride is 10-(3-dimethylaminopropyl) phenothiazine hydrochloride.

Promazine Hydrochloride contains not less than 99.0 per cent and not more than 101.0 per cent of $C_{17}H_{20}N_2S,HCl$, calculated on the dried basis.

Category. Sedative.

Dose. *All species*. By intramuscular injection, upto 1 mg per kg of body weight.

Description. A white or almost white, crystalline powder; slightly hygroscopic.

Identification

Test A may be omitted if tests B, C, D and E are carried out. Tests B, C and D may be omitted if tests A and E are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promazine hydrochloride RS* or with the reference spectrum of promazine hydrochloride.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M hydrochloric acid* shows

an absorption maximum at about 252 nm and a less well-defined maximum at about 302 nm; absorbance at about 252 nm, about 0.93.

C. Dissolve 5 mg in 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced.

D. Melting range (2.4.21). 177° to 181°.

E. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.2 to 5.4, determined in a 5 per cent w/v solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.6 g, dissolve in 100 ml of *acetone*. Titrate with *0.1 M perchloric acid*, using 3 ml of a saturated solution of *methyl orange* in *acetone* as indicator. Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.03209 g of $C_{17}H_{20}N_2S,HCl$.

Storage. Store protected from light.

Promazine Injection

Promazine Hydrochloride Injection

Promazine Injection is a sterile solution of Promazine Hydrochloride in Water for Injections free from dissolved air and containing suitable buffering and stabilising agents. The solution is distributed in containers, the air in which is replaced by nitrogen or other suitable gas.

Promazine Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of promazine hydrochloride, $C_{17}H_{20}N_2S,HCl$.

Usual strengths. 50 mg in 1 ml; 100 mg in 2 ml.

Description. A colourless or almost colourless liquid.

Identification

A. To a volume containing 0.1 g of Promazine Hydrochloride add 20 ml of *water* and 2 ml of *10 M sodium hydroxide*. Shake and extract the mixture with 25 ml of *ether*. Wash the ether extract with two quantities, each of 5 ml, of *water*, dry with *anhydrous sodium sulphate* and evaporate the ether. A 10 per cent w/v solution of the oily residue in *chloroform* complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *promazine hydrochloride RS*, treated in the same manner.

B. To a volume containing 5 mg of Promazine Hydrochloride add carefully 2 ml of *sulphuric acid* and allow to stand for 5 minutes; an orange colour is produced.

C. To a volume containing 0.2 g of Promazine Hydrochloride add 1 ml of *1 M sodium hydroxide* and extract with four quantities, each of 10 ml, of *ether*. Wash the combined extracts with 10 ml of *water*, remove the ether and dissolve the residue in 4 ml of *methanol*. Heat on a water-bath almost to boiling, immediately add 2 ml of a boiling 3.5 per cent w/v solution of *picric acid* in *methanol* and boil for 2 minutes. Cool in ice, filter, wash the crystals thrice with *methanol*, dissolve in 10 ml of hot *methanol* and repeat the crystallisation and washing. The rust-red crystals so obtained, after drying at 105° for 1 hour, melt at about 144° (2.4.21).

Tests

pH (2.4.24). 4.4 to 5.2.

Related substances. Carry out the test for identification of related substances in phenothiazines (2.3.5), using *mobile phase A* and applying separately to the plate 10 µl of each of the following freshly-prepared solutions.

Test solution. Dilute a volume of the injection with sufficient *methanol* to produce a solution containing the equivalent of 1.0 per cent w/v of Promazine Hydrochloride.

Reference solution (a). Dilute 1 volume of the test solution to 40 volumes with *methanol*.

Reference solution (b). Dilute 1 volume of the test solution to 200 volumes with *methanol*.

Any secondary spot in the chromatogram obtained with the test solution is more intense than the spot in the chromatogram obtained with reference solution (a) and not more than one such spot is more intense than the spot in the chromatogram obtained with reference solution (b).

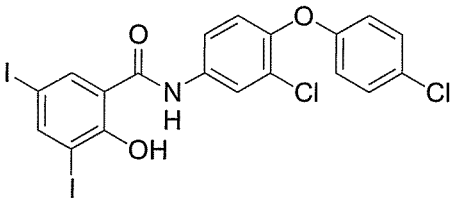
Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *Protect the solutions from light throughout the procedure.*

To an accurately measured volume containing about 50 mg of Promazine Hydrochloride, add 5 ml of *2 M hydrochloric acid* and sufficient *water* to produce 1000.0 ml. To 10.0 ml add 10 ml of *0.1 M hydrochloric acid*, dilute to 100.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 251 nm (2.4.7). Calculate the content of $C_{17}H_{20}N_2S,HCl$ taking 935 as the specific absorbance at 251 nm.

Storage. Store protected from light.

Rafoxanide



$C_{19}H_{11}Cl_2I_2NO_3$ Mol. Wt. 626.0

Rafoxanide is *N*-[3-chloro-4-(4-chlorophenoxy)phenyl]-2-hydroxy-3,5-diiodobenzamide.

Rafoxanide contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{19}H_{11}Cl_2I_2NO_3$, calculated on the dried basis.

Category. Anthelmintic.

Dose. *Cows, buffaloes, sheep and goats*. 7 to 12 mg per kg of body weight.

Description. A greyish-white to brown powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *rafoxanide RS* or with the reference spectrum of rafoxanide.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M methanolic hydrochloric acid* shows absorption maxima at about 280 nm and at 335 nm; absorbance at about 280 nm, about 0.97 and at about 335 nm, about 0.59.

C. Burn 20 mg by the oxygen-flask method (2.3.34), using 5 ml of *2 M sodium hydroxide* as the absorbing liquid, and dilute to 25 ml with *water*. To 5 ml add 1 ml of *silver nitrate solution*; a yellow precipitate is produced; add 5 ml of *5 M ammonia*, shake, filter, and acidify the filtrate with *nitric acid*; a white precipitate is produced.

D. Shake 10 mg with 10 ml of *ethanol (80 per cent)* and add 0.1 ml of *ferric chloride test solution*; a violet colour is produced.

E. Melts at about 175° (2.4.21).

Tests

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

NOTE — Carry out the test in subdued light and use freshly prepared solutions.

Mobile phase. A mixture of 170 volumes of *dichloromethane*, 30 volumes of *methanol* and 2 volumes of *strong ammonia solution*.

Test solution. Dissolve 2 g of the substance under examination in 100 ml in *dichloromethane*.

Reference solution. A 0.010 per cent w/v of *rafoxanide RS* in *dichloromethane*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.5 per cent, determined on 1.0 g by drying in an oven at 90° at a pressure not exceeding 0.7 kPa for 2 hours.

Assay. To 50 ml of *dioxan* add 1 ml of *phenolphthalein solution*, replace the air in the flask with *nitrogen* and titrate with *0.1 M sodium hydroxide*. Weigh accurately about 1.25 g, dissolve it in the mixture and again titrate with *0.1 M sodium hydroxide*. The difference between the titrations represents the amount of *0.1 M sodium hydroxide* required.

1 ml of *0.1 M sodium hydroxide* is equivalent to 0.06260 g of $C_{19}H_{11}Cl_2I_2NO_3$.

Storage. Store protected from light.

Rafoxanide Veterinary Oral Suspension

Rafoxanide Suspension; Rafoxanide Veterinary Mixture; Rafoxanide Mixture

Rafoxanide Veterinary Oral Suspension is an aqueous suspension of Rafoxanide containing suitable suspending and dispersing agents and antimicrobial preservatives.

Rafoxanide Veterinary Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of rafoxanide, $C_{19}H_{11}Cl_2I_2NO_3$.

Usual strength. 3 per cent w/v.

Identification

A. Evaporate a volume containing 0.2 g of Rafoxanide to dryness on a water-bath and heat the residue over a Bunsen burner flame; the vapours turn moistened *starch-iodide paper* blue.

B. In addition to the absorbance at about 335 nm, measure the absorbance at about 280 nm (2.4.7), of the final solution

obtained in the Assay. The ratio of the absorbance at about 280 nm to that at about 335 nm is 1.59 to 1.69.

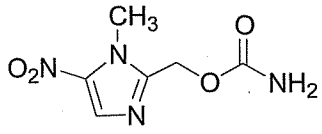
Tests

Other tests. Complies with requirements stated under Veterinary Oral Liquids.

Assay. Weigh accurately a quantity of the well-mixed suspension containing about 0.12 g of Rafoxanide in a stoppered 50-ml test tube and add 15 ml of *0.1 M sodium hydroxide* and 15 ml of *ether*. Shake for 5 minutes and centrifuge. Remove the ether layer and repeat the extraction with three further quantities, each of 15 ml, of *ether*. Dilute the combined ether solutions to 250.0 ml with *ether* and mix. Dilute 5.0 ml of this solution to 100.0 ml with *0.1 M methanolic hydrochloric acid*, mix and measure the absorbance of the resulting solution at about 335 nm (2.4.7). Calculate the content of $C_{19}H_{11}Cl_2I_2NO_3$ taking 149 as the specific absorbance at 335 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of rafoxanide, weight in volume.

Ronidazole



$C_6H_8N_4O_4$ Mol. Wt. 200.2

Ronidazole is 1-methyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole.

Ronidazole contains not less than 98.5 per cent and not more than 101.0 per cent of $C_6H_8N_4O_4$, calculated on the anhydrous basis.

Category. Antiprotozoan.

Dose. *Pigs*. In drinking water, 60 mg per litre of water, offered continuously for 3 days; in feed, 120 mg per kg of feed, offered continuously for 5 days.

Description. A white to yellowish-brown powder; odourless or almost odourless.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ronidazole RS* or with the reference spectrum of ronidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.002 per cent w/v solution in *0.1 M methanolic hydrochloric acid* shows an absorption maximum only at about 270 nm; absorbance at about 270 nm, about 0.64.

C. Melts at about 167° (2.4.21).

Tests

Appearance of solution. A 0.5 per cent w/v solution in *methanol* is not more intensely coloured than reference solution YS6 (2.4.1).

(1-Methyl-5-nitroimidazol-2-yl)methano .Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *toluene*, 5 volumes of *methanol* and 5 volumes of *glacial acetic acid.*

Test solution. Dissolve 1 g of the substance under examination in 100 ml in *acetone.*

Reference solution. A 0.0050 per cent w/v of *(1-methyl-5-nitro-imidazol-2-yl)methanol RS* in *acetone.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to (1-methyl-5-nitroimidazol-2-yl)methanol RS is not more intense than the spot in the chromatogram obtained with the reference solution.

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 0.5 per cent, determined on 5 g.

Assay. Weigh accurately about 0.3 g, dissolve in 50 ml of *anhydrous glacial acetic acid.* Titrate with *0.1 M perchloric acid,* determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02002 g of $C_6H_8N_4O_4$.

Storage. Store proted from light.

Ronidazole Veterinary Oral Powder

Ronidazole Veterinary Oral Powder is a mixture of Ronidazole with suitable diluents.

Ronidazole Veterinary Oral Powder contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of ronidazole, $C_6H_8N_4O_4$.

Usual strength. 10 per cent w/w.

Identification

A. Shake a quantity of the powder containing 0.1 g of Ronidazole with 10 ml of *acetone* for 15 minutes, filter and evaporate the filtrate to dryness. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *ronidazole RS* or with the reference spectrum of ronidazole.

B. When examined in the range 230 nm to 360 nm (2.4.7), the final solution obtained in the Assay shows an absorption maximum only at about 281 nm.

Tests

(1-Methyl-5-nitroimidazol-2-yl)methanol. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 80 volumes of *toluene,* 5 volumes of *methanol* and 5 volumes of *glacial acetic acid.*

Test solution. Shake a quantity of the powder containing 0.1 g of Ronidazole with 10 ml of *acetone* for 15 minutes and filter.

Reference solution. A 0.0050 per cent w/v of *(1-methyl-5-nitro-imidazol-2-yl)methanol RS* in *acetone.*

Apply to the plate 20 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. Any secondary spot in the chromatogram obtained with the test solution corresponding to (1-methyl-5-nitro-imidazol-2-yl)methanol RS is not more intense than the spot in the chromatogram obtained with the reference solution.

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. Weigh accurately a quantity of powder containing 2 g of Ronidazole, dissolve in 450 ml of *water* and add sufficient *water* to produce 500.0 ml. Dilute 5.0 ml of this solution to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 281 nm (2.4.7). Calculate the content of $C_6H_8N_4O_4$ taking 279 as the specific absorbance at 281 nm.

Storage. Store proted from light.

Serum Gonadotrophin for Veterinary Use

Equine Serum Gonadotrophin for Veterinary Use

Serum Gonadotrophin for Veterinary Use is a dry preparation of a glycoprotein fraction, obtained from the serum or plasma of pregnant mares in their 60th to 75th day of pregnancy, which stimulates the formation of follicles and induces leutinising activity.

Serum Gonadotrophin for Veterinary Use contains not less than 1000 Units per mg, calculated on the anhydrous basis.

Category. Gonadotrophic hormone.

Dose. By subcutaneous or intramuscular injection. *Cows and she buffaloes.* 2 to 4 Units per kg of body weight. *Pigs.* 5 to 15 Units per kg of body weight. *Sheep.* 15 Units per kg of body weight. *Dogs.* 10 to 20 Units per kg of body weight.

Description. A white or pale grey, amorphous powder.

Identification

Causes enlargement of the ovaries of immature female rats when administered as directed in the Assay.

Tests

Water (2.3.43). Not more than 10.0 per cent, determined on 80 mg.

Assay. Carry out the biological assay of serum gonadotrophin described below.

The potency of serum gonadotrophin for veterinary use is determined by comparing its effect in increasing the weight of the ovaries of immature rats with that of the Standard Preparation of serum gonadotrophin under the conditions of the following method of assay.

Standard Preparation

The Standard Preparation is the 2nd International Standard for serum gonadotrophin, equine, for bioassay, established in 1966, consisting of the freeze-dried active principle from the serum of pregnant mares, with lactose (supplied in ampoules containing 1600 Units), or other suitable preparation the potency of which has been determined in relation to the International Standard.

Method

Test animals. Use immature female rats of the same strain, 21 to 28 days old, differing in age by not more than 3 days and of approximately equal weights such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of not less than five animals. If sets of six litter-mates are available, allot one litter-mate from each set at random to each group and mark according to the litter.

Procedure. Choose three doses of the Standard Preparation and three doses of the preparation under examination such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximal response in all of the rats. Use doses in geometric progression. As an initial approximation total doses of 8, 12 and 18 Units may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely. Dissolve separately the total quantities of the preparation under examination and of the Standard Preparation

corresponding to the doses to be used in sufficient of a sterile *saline solution* containing 1 mg of *bovine albumin* per ml such that each single dose may be administered by the injection of 6 equally-divided portions, in the same volume of about 0.2 ml. Store the solutions at a temperature 2° to 8°. Inject subcutaneously into each rat the dose allocated to its group. Repeat the injections 18, 21, 24, 42 and 48 hours after the first injection. Kill the rats between 40 hours and 72 hours after the last injection and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the two ovaries from each rat.

Calculate the result of the assay by standard statistical methods using the combined weight of the two ovaries of each animal as the response.

Limits of error - The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

Serum Gonadotrophin for Veterinary Use intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of pyrogens complies with the following additional requirement.

Pyrogens. Complies with the test for pyrogens (2.2.8), using per kg of the rabbit's weight 1 ml of a solution in *sodium chloride injection* containing 500 Units per ml.

Serum Gonadotrophin for Veterinary Use intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture and light in a refrigerator (2 to 8). If the contents are sterile, the containers should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) the total number of Units in the container; (3) the date after which the material is not intended to be used; (4) the storage conditions; (5) whether or not it is intended for use in the manufacture of parenteral preparations.

Serum Gonadotrophin Injection for Veterinary Use

Serum Gonadotrophin Injection for Veterinary Use is a sterile material consisting of Serum Gonadotrophin for Veterinary Use with or without buffering agents and other excipients. It is filled in a sealed container.

The injection is constituted by dissolving the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

The constituted solution complies with the requirements for Clarity of solution and Particulate matter stated under Parenteral Preparations (Injections).

Storage. The constituted solution should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Serum Gonadotrophin Injection for veterinary Use contains not less than 80.0 per cent and not more than 125.0 per cent of the stated potency.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strength. 1000 Units.

Identification

Causes enlargement of the ovaries of immature female rats when administered as directed in the Assay.

Tests

Appearance of solution. A solution containing 5000 Units per ml (solution A) is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 6.0 to 8.0, determined on solution A.

Water (2.3.43). Not more than 10.0 per cent, determined on 80 mg.

Assay. Carry out the biological assay of serum gonadotrophin described below.

The potency of serum gonadotrophin for veterinary use is determined by comparing its effect in increasing the weight of the ovaries of immature rats with that of the Standard Preparation of serum gonadotrophin under the conditions of the following method of assay.

Standard Preparation

The Standard Preparation is the 2nd International Standard for serum gonadotrophin, equine, for bioassay, established in 1966, consisting of the freeze-dried active principle from the serum of pregnant mares, with lactose (supplied in ampoules containing 1600 Units), or other suitable preparation the potency of which has been determined in relation to the International Standard.

Test animals. Use immature female rats of the same strain, 21 to 28 days old, differing in age by not more than 3 days and of approximately equal weights such that the difference between the heaviest and the lightest rat is not more than 10 g. Assign the rats at random to six equal groups of not less than five animals. If sets of six litter-mates are available, allot one

litter-mate from each set at random to each group and mark according to the litter.

Procedure. Choose three doses of the Standard Preparation and three doses of the preparation under examination such that the smallest dose is sufficient to produce a positive response in some of the rats and the largest dose does not produce a maximal response in all of the rats. Use doses in geometric progression. As an initial approximation total doses of 8, 12 and 18 Units may be tried although the dose will depend on the sensitivity of the animals used, which may vary widely. Dissolve separately the total quantities of the preparation under examination and of the Standard Preparation corresponding to the doses to be used in sufficient of a sterile *saline solution* containing 1 mg of *bovine albumin* per ml such that each single dose may be administered by the injection of 6 equally-divided portions, in the same volume of about 0.2 ml. Store the solutions at a temperature 2° to 8°. Inject subcutaneously into each rat the dose allocated to its group. Repeat the injections 18, 21, 24, 42 and 48 hours after the first injection. Kill the rats between 40 hours and 72 hours after the last injection and remove the ovaries. Remove any extraneous fluid and tissue and immediately weigh the two ovaries from each rat.

Calculate the result of the assay by standard statistical methods using the combined weight of the two ovaries of each animal as the response.

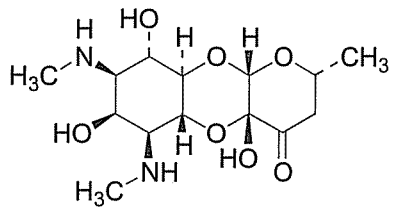
Limits of error - The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The fiducial limits of error ($P = 0.95$) of the estimated potency are not less than 64 per cent and not more than 156 per cent of the stated potency.

Pyrogens. Complies with the test for pyrogens (2.2.8), using per kg of the rabbit's weight 1 ml of a solution in *sodium chloride injection* containing 500 Units per ml.

Storage. Store protected from light in a refrigerator (2° to 8°).

Labelling. The label states the number of Units contained in the sealed container.

Spectinomycin Hydrochloride



, 2HCl, $5H_2O$

$C_{14}H_{24}N_2O_7,2HCl,5H_2O$ Mol. Wt. 495.4

Spectinomycin Hydrochloride is [2*R*-(2α,4aβ,5aβ,6β,7β,8β,9α,9aα,10aβ)]-decahydro-4a,7,9-trihydroxy-2-methyl-6,8-bis(methylamino)-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride pentahydrate.

Spectinomycin Hydrochloride contains not less than 95.0 per cent and not more than 100.5 per cent of $C_{14}H_{24}N_2O_7,2HCl$, calculated on the anhydrous basis.

Category. Antibacterial.

Dose. *Large farm animals and poultry*. By intramuscular or intravenous injection, the equivalent of 10 to 20 mg of spectinomycin per kg of body weight daily.

(Each 30 mg of spectinomycin hydrochloride is approximately equivalent to 20 mg of spectinomycin).

Description. A white or almost white, crystalline powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *spectinomycin hydrochloride RS* or with the reference spectrum of spectinomycin hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

Appearance of solution. A 10 per cent w/v solution is clear (2.4.1), and colourless (2.4.1).

pH (2.4.24). 3.8 to 5.6, determined in a 10 per cent w/v solution.

Specific optical rotation (2.4.22). +15.0° to +21.0°, determined in a 10 per cent w/v solution within 20 minutes of preparation, on the anhydrous basis.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G*.

Mobile phase. A mixture of 50 volumes of *1-propanol*, 40 volumes of *water*, 5 volumes of *glacial acetic acid* and 5 volumes of *pyridine*.

Test solution. Dissolve 2 g of the substance under examination in 100 ml *water*.

Reference solution. A 0.020 per cent w/v solution of the substance under examination in *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *potassium permanganate*. Allow the plate to stand for 2 to 3 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with the reference solution (1 per cent).

Sulphated ash (2.3.18). Not more than 1.0 per cent w/v.

Water (2.3.43). 16.0 to 20.0 per cent, determined on 0.2 g.

Assay. Determine by gas chromatography (2.4.13).

NOTE — Use the solutions within 1 hour after preparation.

Test solution (a). Take 60 mg of the substance under examination in a glass-stoppered conical flask, add 10.0 ml of *dimethylformamide* and 2.0 ml of *hexamethyl- disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide.*

Test solution (b). Take 60 mg of the substance under examination in a glass-stoppered conical flask, add 10.0 ml of a solution containing 0.15 per cent w/v of *phenazone* (internal standard) in *dimethylformamide* and 2.0 ml of *hexamethyl-disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide.*

Reference solution. Take 60 mg of the *spectinomycin hydrochloride RS* in a glass-stoppered conical flask, add 10.0 ml of a solution containing 0.15 per cent w/v of *phenazone* (internal standard) in *dimethylformamide* and 2.0 ml of *hexamethyl- disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide.*

Chromatographic system
- a glass column 1.5 m × 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of *phenylmethylsilicone fluid (50 per cent phenyl)*,
- temperature:
  column 200°,
  inlet port 200° and detector 230°,
- flow rate. 45 ml per minute of the carrier gas.

Inject the chosen volumes of test solutions (a) and (b). The test is not valid unless the resolution factor between the peak due to the internal standard and the principal peak in the chromatogram obtained with test solution (a) is not less than 8.0.

Inject alternately test solution (b) and the reference solution.

Calculate the content of $C_{14}H_{24}N_2O_7$,2HCl.

Spectinomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.09 Endotoxin Unit per mg determined in a 0.42 per cent w/v solution of *sodium bicarbonate.*

Spectinomycin Hydrochloride intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from moisture, at a temperature not exceeding 30°. If the substance is sterile, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the date after which the material is not intended to be used; (2) the storage conditions; (3) whether or not it is intended to be used for manufacture of parenteral preparations.

Spectinomycin Injection

Spectinomycin Hydrochloride Injection

Spectinomycin Injection is a sterile material consisting of Spectinomycin Hydrochloride with or without auxiliary substances. It is filled in a sealed container.

The injection is constituted by suspending the contents of the sealed container in the requisite amount of sterile Water for Injections, immediately before use.

Storage. The constituted suspension should be used immediately after preparation but, in any case, within the period recommended by the manufacturer.

Spectinomycin Injection contains not less than 90.0 per cent and not more than 110.0 per cent the stated amount of spectinomycin, $C_{14}H_{24}N_2O_7$.

The contents of the sealed container comply with the requirements stated under Parenteral Preparations (Powders for Injection) and with the following requirements.

Usual strength. Equivalent of 2 g of spectinomycin.

Dose. *All species except adult ruminants*. 50 to 100 mg per kg of body weight.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *spectinomycin hydrochloride RS* or with the reference spectrum of spectinomycin hydrochloride.

B. Gives reaction A of chlorides (2.3.1).

Tests

pH (2.4.24). 4.0 to 7.0, determined in a suspension of the contents of a sealed container in the volume of the liquid stated on the label.

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel G.*

Mobile phase. A mixture of 50 volumes of *1-propanol*, 40 volumes of *water*, 5 volumes of *glacial acetic acid* and 5 volumes of *pyridine*.

Test solution. Prepare a solution containing the equivalent of 1.4 per cent w/v of spectinomycin in *water*.

Reference solution. Prepare a solution containing the equivalent of 0.014 per cent w/v of spectinomycin in *water*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and spray with a 5 per cent w/v solution of *potassium permanganate*. Allow the plate to stand for 2 to 3 minutes. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution.

Water (2.3.43). Not more than 20.0 per cent, determined on 0.2 g.

Bacterial endotoxins (2.2.3). Not more than 0.09 Endotoxin Unit per ml, determined on a solution prepared by dissolving the contents in a solution containing *0.05 M sodium bicarbonate* in *water BET* to give a solution containing the equivalent of 1 mg of spectinomycin per ml (solution A), and using the maximum valid dilution of solution A calculated from the declared sensitivity of the lysate used in the test.

Assay. Determine by gas chromatography (2.4.13).

NOTE — Use the solutions within 1 hour after preparation.

Test solution (a). Weigh and mix the contents of the 10 containers. To an accurately weighed quantity containing about 60 mg of Spectinomycin Hydrochloride in a glass-stoppered conical flask, add 10.0 ml of *dimethylformamide* and 2.0 ml of *hexamethyl- disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide*.

Test solution (b). To an accurately weighed quantity containing about 60 mg of Spectinomycin Hydrochloride in a glass-stoppered conical flask, add 10.0 ml of a solution containing 0.15 per cent w/v of *phenazone* (internal standard) in *dimethylformamide* and 2.0 ml of *hexamethyl- disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide*.

Reference solution. TO about 60 mg, accurately weighed, of *spectinomycin hydrochloride RS* in a glass-stoppered conical flask, add 10.0 ml of a solution containing 0.15 per cent w/v of *phenazone* (internal standard) in *dimethylformamide* and 2.0 ml of *hexamethyl- disilazane*, shake intermittently for 1 hour and dilute to 20.0 ml with *dimethylformamide*.

Chromatographic system
- a glass column 1.5 m x 4 mm, packed with acid-washed, silanised diatomaceous support (100 to 120 mesh) coated with 3 per cent w/w of *phenylmethylsilicone fluid (50 per cent phenyl)*,
- temperature:
  column 200°,
  inlet port 200° and detector 230°,
- flow rate. 45 ml per minute of the carrier gas.

Inject the chosen volumes of test solutions (a) and (b). The test is not valid unless the resolution factor between the peak due to the internal standard and the principal peak in the chromatogram obtained with test solution (a) is not less than 8.0.

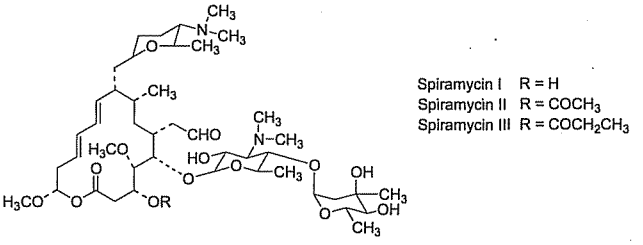
Inject alternately test solution (b) and the reference solution.

Calculate the content of $C_{14}H_{24}N_2O_7,2HCl$.

Storage. Use the injection immediately after preparation but, in any case, within the period recommended by the manufacturer provided it is prepared and stored in accordance with the manufacturer's instructions.

Labelling. The label states the strength in terms of the equivalent amount of spectinomycin.

Spiramycin



$C_{43}H_{74}N_2O_{14}$ Mol. Wt. 843.1

Spiramycin is a mixture comprised primarily of spiramycin I produced by *Streptomyces ambofacien* from soil of northern France.

Spiramycin contains not less than 3900 Units per mg, calculated on the dried basis.

Category. Antibacterial.

Description. A white or slightly yellowish powder; slightly hygroscopic.

Identification

A. When examined in the range 220 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *methanol* shows an absorption maximum only at about 232 nm; absorbance at about 232 nm, about 0.34.

B. Dissolve 0.5 g in a mixture of 10 ml of *0.05 M sulphuric acid* and 25 ml of *water*. Adjust the pH to about 8 by addition of *0.1 M sodium hydroxide* and dilute to 50 ml with *water*. To 5 ml of the resulting solution add 2 ml of a mixture of 1 volume of *water* and 2 volumes of *sulphuric acid*; a brown colour is produced.

Tests

pH (2.4.24). 8.5 to 10.5, determined in a solution prepared by dissolving 0.5 g in 5 ml of *methanol* and diluting to 100 ml with *carbon dioxide-free water*.

Specific optical rotation (2.4.22). –80.0° to –85.0°, determined in a 2 per cent w/v solution in *0.2 M acetic acid*.

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method A (20 ppm).

Related substances. Determine by liquid chromatography (2.4.14).

NOTE–Prepare the solutions immediately before use.

Solvent mixtue. 30 volumes of *methanol* and 70 volumes of *water*.

Test solution. Dissolve 25 mg of the substance under examination in 25.0 ml of the solvent mixture.

Reference solution (a). A 0.1 per cent w/v solution of *spiramycin RS* in the solvent mixture.

Reference solution (b). Dilute 2.0 ml of reference solution (a) to 100 ml with the solvent mixture.

Reference solution (c). Dissolve 5.0 mg *spiramycin RS* in 15.0 ml of *buffer solution pH 2.2* and dilute to 25.0 ml with *water*, heat on water-bath at 60° for 5 minutes and cool.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, endcapped polar embedded octadecylsilane amorphous organosilica polymer (5 µm) (polar embedded octadecylsilane methylsilica (12.5 µm),
- mobile phase: a mixture of 5.0 volumes of 3.48 per cent solution of *dipotassium hydrogen phosphate,* adjusted to pH 6.5 with 2.72 per cent w/v solution of *potassium dihydrogen phosphate*, 40 volumes of *acetonitrile* and 55 volumes of *water*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 232 nm,
- injection volume. 20 µl.

Inject reference solution (a) and (c). Run the chromatogram three times the retention times of spiromycin peak. The relative rentention time with reference to spiramycin I for impurity F is about 0.41, for impurity A is about 0.45, for impurity D is about 0.5, for impurity G is about 0.66, for impurity B is about 0.73, for impurity H is about 0.87, for spiramycin II is about 1.4, for spiramycin III is about 2.0 and for impurity E is about 2.5. The test is not valid unless in the chromatogram obtained with reference solution (c), the resolution between the peaks due to impurity A and spiramycin I is not less than 10.0.

Inject reference solution (b) and the test solution. The area of secondary peak due to impurity A, B, C, D, E, F, G and H is not more than the area of principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent). The area of any other secondary peak is not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2.0 per cent) and the sum of areas of all the secondary peaks is not more than 5 times the area of principal peak in the chromatogram obtained with reference solution (b) (10.0 per cent). Ignore any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the peak due to blank, Spiramycin I, II, III.

Sulphated ash (2.3.18). Not more than 0.1 per cent w/v.

Loss on drying (2.4.19). Not more than 3.5 per cent, determined on 0.5 g by drying over *phosphorus pentoxide* at 80° at a pressure not exceeding 0.7 kPa for 6 hours.

Assay. Carry out the microbiological assay of antibiotics (2.2.10), Method A.

Storage. Store protected from moisture.

Sulphadiazine and Trimethoprim Injection

Trimethoprim and Sulphadiazine Injection; Co-trimazine Injection

Sulphadiazine and Trimethoprim Injection is a sterile suspension in Water for Injections containing Sulphadiazine and Trimethoprim in the proportion of five parts to one part respectively.

Sulphadiazine and Trimethoprim Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sulphadiazine, $C_{10}H_{10}N_4O_2S$ and of trimethoprim, $C_{14}H_{18}N_4O_3$.

Usual strengths. 400 mg of Sulphadiazine and 80 mg of Trimethoprim in 1 ml; 200 mg of Sulphadiazine and 40 mg of Trimethoprim in 1 ml.

Identification

A. When examined in the range 230 nm to 360 nm (2.4.7), the solution obtained in the Assay *for trimethoprim* shows an absorption maximum only at about 271 nm.

B. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 75 volumes of *ethyl acetate,* 15 volumes of *dimethylformamide* and 5 volumes of *water.*

Test solution. Add 4 ml of *hydrochloric acid* to 2.5 ml of the well-mixed contents of the container and dilute to 50 ml with *1.4 M methanolic ammonia.*

Reference solution (a). A 2.0 per cent w/v of *sulphadiazine RS* in *1.4 M methanolic ammonia.*

Reference solution (b). A 0.4 per cent w/v of *trimethoprim RS* in *1.4 M methanolic ammonia.*

Apply to the plate 1 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. One of the principal spots in the chromatogram obtained with the test solution corresponds to the principal spot in the chromatogram obtained with reference solution (a) and the other corresponds to the principal spot in the chromatogram obtained with reference solution (b).

C. To 5 ml of the filtrate obtained in the Assay *for sulphadiazine* add 10 ml of *water* and 5 ml of *thiobarbituric acid-citrate buffer.* Mix and heat on a water-bath for 30 minutes; a pink colour is produced.

Tests

pH (2.4.24). 10.0 to 10.5.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. *For sulphadiazine* — Disperse the trimethoprim evenly throughout the injection solution by gently inverting the container several times without foam formation. Transfer an accurately measured quantity of the injection containing 2 g of Sulphadiazine to a separating funnel containing 50 ml of *0.1 M sodium hydroxide* and extract with two quantities, each of 100 ml and 50 ml of *dichloromethane,* washing the extract with the same 25-ml quantity of *0.1 M sodium hydroxide.* Reserve the combined dichloromethane extracts for the assay for trimethoprim.

Dilute the combined aqueous solutions and washings to 250.0 ml with *water* and filter, and dilute 5.0 ml of the filtrate to 200.0 ml with *water.* Dilute 10.0 ml of this solution to 100.0 ml with *water.* To 3.0 ml of the resulting solution add 1 ml of *2 M hydrochloric acid* and 1 ml of a 0.1 per cent w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 ml of a 0.5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 3 minutes. Add 1 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride,* allow to stand for 10 minutes, add sufficient *water* to produce 25.0 ml and measure the absorbance of the resulting solution at the maximum at about 538 nm (2.4.7). Calculate the content of $C_{10}H_{10}N_4O_2S$ from the absorbance obtained by carrying out the procedure simultaneously, using 3.0 ml of a solution prepared by dissolving 200 mg of *sulphadiazine RS* in 50 ml of *0.1 M sodium hydroxide,* adding sufficient *water* to produce 200.0 ml, diluting 5.0 ml to 250.0 ml with *water* and beginning at the words "add 1 ml of *2 M hydrochloric acid.....*".

For trimethoprim — Extract the dichloromethane solution reserved in the Assay *for sulphadiazine* with three quantities, each of 100 ml, 50 ml and 50 ml, of *1 M acetic acid* and dilute the combined extracts to 500.0 ml with *1 M acetic acid.* To 5.0 ml add 35 ml of *1 M acetic acid* and sufficient *water* to produce 200.0 ml and measure the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm.

Labelling. The label states the content of Sulphadiazine and Trimethoprim in a suitable dose-volume.

Sulphadiazine and Trimethoprim Veterinary Oral Powder

Trimethoprim and Sulphadiazine Veterinary Oral Powder; Sulphadiazine and Trimethoprim Dispersible Powder; Co-trimazine Veterinary Oral Powder

Sulphadiazine and Trimethoprim Veterinary Oral Powder consists of Sulphadiazine and Trimethoprim in the proportion of five parts to one part respectively, mixed with suitable wetting, dispersing and suspending agents.

Sulphadiazine and Trimethoprim Veterinary Oral Powder contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amounts of sulphadiazine, $C_{10}H_{10}N_4O_2S$, and of trimethoprim, $C_{14}H_{18}N_4O_3$.

Usual strength. 10 per cent w/w of Sulphadiazine and 2 per cent w/w of Trimethoprim.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 75 volumes of *ethyl acetate,* 15 volumes of *dimethylformamide* and 5 volumes of *water.*

Test solution (a). The supernatant liquid obtained by shaking a quantity of the powder containing 0.2 g of Sulphadiazine with sufficient *1.4 M methanolic ammonia* to produce 100 ml and centrifuging.

Test solution (b). The supernatant liquid obtained by shaking a quantity of the powder containing 0.2 g of Trimethoprim with sufficient *1.4 M methanolic ammonia* to produce 100 ml and centrifuging.

Reference solution (a). A 0.2 per cent w/v solution of *sulphadiazine RS* in *1.4 M methanolic ammonia*.

Reference solution (b). A 0.2 per cent w/v solution of *trimethoprim RS* in *1.4 M methanolic ammonia*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 0.1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 1 ml of *hydrochloric acid* and 100 ml of *ethanol (95 per cent)*, allow to dry and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with test solution (a) having R_f value of about 0.7 corresponds to the principal spot in the chromatogram obtained with reference solution (a). The spot in the chromatogram obtained with test solution (b) having R_f value of about 0.3 corresponds to the principal spot in the chromatogram obtained with reference solution (b).

Tests

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. *For sulphadiazine* — Weigh accurately a quantity of the powder containing about 0.125 g of Sulphadiazine, transfer into a separator containing 20 ml of *0.1 M sodium hydroxide* and extract with four quantities, each of 50 ml, of *dichloromethane*. Wash each dichloromethane extract with the same two quantities, each of 10 ml, of *0.1 M sodium hydroxide*. Combine the aqueous washings and the aqueous layer from the separator and reserve the combined dichloromethane extracts for the Assay *for trimethoprim*.

Dilute the combined aqueous solutions to 250.0 ml with *water*, filter and dilute 10.0 ml of the filtrate to 200.0 ml with *water*. To 2.0 ml of the resulting solution add 0.5 ml of *4 M hydrochloric acid* and 1 ml of a 0.1 per cent w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 ml of a 0.5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 3 minutes. Add 1 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, allow to stand for 10 minutes. Dilute the solution to 25.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 538 nm (2.4.7), using as the blank a solution prepared in the same manner using 2 ml of *water* and beginning at the words "add 0.5 ml of *4 M hydrochloric acid*.....". Calculate the content of $C_{10}H_{10}N_4O_2S$ from the absorbance obtained by carrying out the procedure simultaneously, with 2.0 ml of a 0.0025 per cent w/v solution of *sulphadiazine RS* in *0.0005 M sodium hydroxide* and beginning at the words "add 0.5 ml of *4 M hydrochloric acid*.....".

For trimethoprim — Extract the combined dichloromethane extracts from the Assay *for sulphadiazine* with four quantities, each of 50 ml, of a 5 per cent v/v solution of *6 M acetic acid*; wash the combined aqueous extracts with 5 ml of *dichloromethane*, discard the dichloromethane layer and dilute to 250.0 ml with a 5 per cent v/v solution of *6 M acetic acid*. Dilute 20.0 ml to 100.0 ml with *water* and determine the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm.

Sulphadiazine and Trimethoprim Veterinary Oral Suspension

Sulphadiazine and Trimethoprim Mixture; Trimethoprim and Sulphadiazine Veterinary Oral Suspension; Co-trimazine Oral Suspension; Co-trimazine Mixture

Sulphadiazine and Trimethoprim Veterinary Oral Suspension is a suspension of Sulphadiazine and Trimethoprim in the proportion of five parts to one part respectively, containing suitable suspending and dispersing agents. It may contain suitable antimicrobial preservatives.

Sulphadiazine and Trimethoprim Veterinary Oral Suspension contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sulphadiazine, $C_{10}H_{10}N_4O_2S$, and of trimethoprim, $C_{14}H_{18}N_4O_3$.

Usual strengths. 40 per cent w/v of Sulphadiazine and 8 per cent w/v of Trimethoprim; 4.55 per cent w/v of Sulphadiazine and 0.91 per cent w/v of Trimethoprim.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 75 volumes of *ethyl acetate*, 15 volumes of *dimethylformamide* and 5 volumes of *water*.

Test solution (a). A dilution of the oral suspension in *1.4 M methanolic ammonia* containing the equivalent of 0.2 per cent w/v of Sulphadiazine.

Test solution (b). A dilution of the oral suspension in *1.4 M methanolic ammonia* containing the equivalent of 0.2 per cent w/v of Trimethoprim.

Reference solution (a). A 0.2 per cent w/v solution of *sulphadiazine RS* in *1.4 M methanolic ammonia*.

Reference solution (b). A 0.2 per cent w/v solution of *trimethoprim RS* in *1.4 M methanolic ammonia*.

Apply to the plate 5 µl of each solution. After development, dry the plate in air and spray with a 0.1 per cent w/v solution

of *4-dimethylaminobenzaldehyde* in a mixture of 1 ml of *hydrochloric acid* and 100 ml of *ethanol (95 per cent)*, allow to dry and spray with *dilute potassium iodobismuthate solution*. The spot in the chromatogram obtained with test solution (a) having R_f value of about 0.7 corresponds to the principal spot in the chromatogram obtained with reference solution (a). The spot in the chromatogram obtained with test solution (b) having R_f value of about 0.3 corresponds to the principal spot in the chromatogram obtained with reference solution (b).

Tests

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. *For sulphadiazine* — Transfer an accurately weighed quantity of the oral suspension containing about 0.125 g of Sulphadiazine, into a separator containing 20 ml of *0.1 M sodium hydroxide* and extract with four quantities, each of 50 ml, of *dichloromethane*. Wash each dichloromethane extract with the same two quantities, each of 10 ml, of *0.1 M sodium hydroxide*. Combine the aqueous washings and the aqueous layer from the separator and reserve the combined dichloromethane extracts for the Assay *for trimethoprim.*

Dilute the combined aqueous solutions to 250.0 ml with *water*, filter and dilute 10.0 ml of the filtrate to 200.0 ml with *water*. To 2.0 ml of the resulting solution add 0.5 ml of *4 M hydrochloric acid* and 1 ml of a 0.1 per cent w/v solution of *sodium nitrite* and allow to stand for 2 minutes. Add 1 ml of a 0.5 per cent w/v solution of *ammonium sulphamate* and allow to stand for 3 minutes. Add 1 ml of a 0.1 per cent w/v solution of *N-(1-naphthyl)ethylenediamine dihydrochloride*, allow to stand for 10 minutes. Dilute the solution to 25.0 ml with *water* and measure the absorbance of the resulting solution at the maximum at about 538 nm (2.4.7), using as the blank a solution prepared in the same manner using 2 ml of *water* and beginning at the words "add 0.5 ml of *4 M hydrochloric acid*.....".

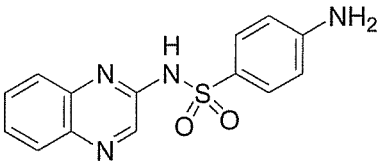
Calculate the content of $C_{10}H_{10}N_4O_2S$ from the absorbance obtained by carrying out the procedure simultaneously, with 2.0 ml of a 0.0025 per cent w/v solution of *sulphadiazine RS* in *0.0005 M sodium hydroxide* and beginning at the words "add 0.5 ml of *4 M hydrochloric acid*.....".

For trimethoprim — Extract the combined dichloromethane extracts from the Assay *for sulphadiazine* with four quantities, each of 50 ml, of a 5 per cent v/v solution of *6 M acetic acid*; wash the combined aqueous extracts with 5 ml of *dichloromethane*, discard the dichloromethane layer and dilute to 250.0 ml with a 5 per cent v/v solution of *6 M acetic acid*. Dilute 20.0 ml to 100.0 ml with *water* and determine the absorbance of the resulting solution at the maximum at about 271 nm (2.4.7). Calculate the content of $C_{14}H_{18}N_4O_3$ taking 204 as the specific absorbance at 271 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the contents of sulphadiazine and trimethoprim, weight in volume.

Labelling. The label states the strength in terms of the amounts of Sulphadiazine and Trimethoprim.

Sulphaquinoxaline



$C_{14}H_{12}N_4O_2S$ Mol. Wt. 300.3

Sulphaquinoxaline is 4-amino-*N*-2-quinoxalinylbenzene-sulphonamide.

Sulphaquinoxaline contains not less than 98.0 per cent and not more than 101.0 per cent of $C_{14}H_{12}N_4O_2S$, calculated on the dried basis.

Category. Antibacterial.

Dose. *Poultry*. Prophylactic, upto 125 g per tonne of feed for 8 weeks or 300 to 500 mg per litre of drinking water.

Description. A yellow colour powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphaquinoxaline RS* or with the reference spectrum of sulphaquinoxaline.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.001 per cent w/v solution in *0.01 M sodium hydroxide* shows an absorption maximum only at about 252 nm; about 1.1.

C. Dissolve 4 mg in 2 ml of warm *2 M hydrochloric acid*. The solution gives the reaction of primary aromatic amines (2.3.1).

Tests

Acidity. To 2 g add 100 ml of *water*, heat at 70° for 5 minutes, cool to 20°, and filter. Titrate 50 ml of the filtrate to pH 7.0 with *0.1 M sodium hydroxide*; not more than 0.2 ml of *0.1 M sodium hydroxide* is required.

Heavy metals. Dissolve the residue obtained in the test for Sulphated ash in 1 ml of *2 M hydrochloric acid* and dilute to 14 ml with *water*. 12 ml of the solution complies with limit test for heavy metals, Method D (2.3.13) (20 ppm).

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 60 volumes of *dichloromethane*, 40 volumes of *methanol* and 20 volumes of *strong ammonia solution.*

Test solution. Dissolve 0.20 g of the substance under examination in 2 ml of *1 M sodium hydroxide* and add sufficient *methanol* to produce 50 ml.

Reference solution (a). A 0.012 per cent w/v solution of *N^1,N^2-diquinoxalin- 2-ylsulphanilamide RS* in *methanol.*

Reference solution (b). A 0.0040 per cent w/v solution of *sulphanilamide RS* in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and examine in ultraviolet light at 254 nm. Any spot corresponding to N^1, N^2-diquinoxalin-2-ylsulphanilamide in the chromatogram obtained with the test solution not more intense than that of the spot in the chromatogram obtained with reference solution (a). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than that in the chromatogram obtained by reference solution (b).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 1.0 per cent, determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.65 g and dissolve in 10 ml of a mixture of equal volumes of *1 M sodium hydroxide* and *water*. Add 20 ml of *glycerin*, 20 ml of *9 M sulphuric acid* and 5 g of *potassium bromide,* cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.03003 g of $C_{14}H_{12}N_4O_2S$.

Storage. Store protected from light.

Sulphaquinoxaline Sodium Solution

Sulphaquinoxaline Sodium Solution is an aqueous solution of sulphaquinoxaline sodium prepared by the interaction of Sulphaquinoxaline and Sodium Hydroxide.

Sulphaquinoxaline Sodium Solution contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sulphaquinoxaline, $C_{14}H_{12}N_4O_2S$.

Usual strength. The equivalent of 96 mg of Sulphaquinoxaline in 1 ml.

Description. A clear, yellow to brown solution.

Identification

A. To a volume containing 1 g of Sulphaquinoxaline add 10 ml of *water* and 3 ml of *2 M hydrochloric acid*, filter, wash the precipitate with *water* and dry for 2 hours at 105°. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphaquinoxaline RS* or with the reference spectrum of sulphaquinoxaline.

B. Dissolve 4 mg of the residue obtained in test A in 2 ml of warm *2 M hydrochloric acid.* The solution gives the reaction of primary aromatic amines (2.3.1).

C. Acidify with *6 M acetic acid*, filter and evaporate the filtrate to dryness. The incinerated residue, when moistened with *hydrochloric acid* and introduced on a platinum wire into a Bunsen burner flame, gives a yellow colour to the flame.

Tests

pH (2.4.24). 12.2 to 12.8, determined in a 9.6 per cent w/v solution in *carbon dioxide-free water.*

Related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254.*

Mobile phase. A mixture of 60 volumes of *dichloromethane*, 40 volumes of *methanol* and 20 volumes of *strong ammonia solution.*

Test solution. Dilute a solution containing 0.20 g of Sulphaquinoxaline to 50 ml with *methanol.*

Reference solution (a). A 0.012 per cent w/v solution of *N^1, N^2-diquinoxalin- 2-ylsulphanilamide RS* in *methanol.*

Reference solution (b). A 0.0040 per cent w/v solution of *sulphanilamide RS* in *methanol.*

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of the solvent is no longer detectable and examine in ultraviolet light at 254 nm. Any spot corresponding to N^1,N^2-diquinoxalin-2-ylsulphanilamide in the chromatogram obtained with the test solution not more intense than that in the chromatogram obtained with reference solution (a). Any other secondary spot in the chromatogram obtained with the test solution is not more intense than that of the spot in the chromatogram obtained with reference solution (b).

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

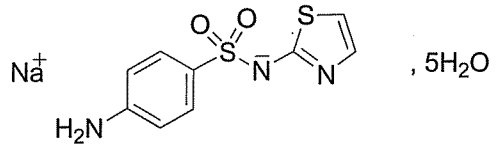
Assay. To an accurately measured volume containing about 0.48 g of Sulphaquinoxaline add 30 ml *water*, 20 ml of *glycerin*, 20 ml of *9 M sulphuric acid* and 5 g of *potassium bromide,* cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.03003 g of $C_{14}H_{12}N_4O_2S$.

Storage. Store protected from light.

Labelling. The label states the strength in terms of the equivalent amount of Sulphaquinoxaline in a suitable dose-volume.

Sulphathiazole Sodium



$C_9H_8N_3NaO_2S_2,1\frac{1}{2}H_2O$ Mol. Wt. 304.3

$C_9H_8N_3NaO_2S_2,5H_2O$ Mol. Wt. 367.4

Sulphathiazole Sodium is sodium salt of 4-amino-*N*-2-thiazolylbenzenesulphonamide with five or one and half molecules of water.

Sulphathiazole Sodium contains not less than 99.0 per cent and not more than 101.0 per cent of $C_9H_8N_3NaO_2S_2$, calculated on the dried basis.

Category. Antibacterial.

Dose. *Pigs*. 2.5 g of the powder per gallon (4.5 litres) of drinking water offered continuously for 3 days.

Description. A white or yellowish white, crystalline powder or granules.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphathiazole sodium RS* or with the reference spectrum of sulphathiazole sodium.

B. Dissolve 1 g in 25 ml of *water* and add 2 ml of *6 M acetic acid*. Wash the precipitate formed with *water* and dry for 4 hours at 105°. The residue melts at about 201° (2.4.21).

C. The precipitate obtained in test B gives the reaction of primary aromatic amines (2.3.1).

Tests

pH (2.4.24). 9.0 to 10.0, determined in a 1 per cent w/v solution.

Heavy metals. Dissolve 2.5 g of the substance under examination in 10 ml of *water*, add 15 ml of *2 M acetic acid*, shake for 30 minutes and filter.

12 ml of this solution complies with the limit test for heavy metals, Method D (2.3.13) (20 ppm).

Related substances. Complies with test A for related substances in sulphonamides (2.3.7).

Loss on drying (2.4.19). Not less than 6.0 per cent and not more than 10.0 per cent (sesquihydrate) or not less than 22.0 per cent and not more than 27.0 per cent (pentahydrate), determined on 1.0 g by drying in an oven at 105°.

Assay. Weigh accurately about 0.5 g, dissolve in a mixture of 75 ml of *water* and 10 ml of *hydrochloric acid*, add 3 g of *potassium bromide*, cool in ice and carry out the nitrite titration (2.3.31).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.02773 g of $C_9H_8N_3NaO_2S_2$.

Storage. Store protected from light.

Labelling. The label states whether the substance is the sesquihydrate or the pentahydrate.

Thiabendazole Veterinary Oral Suspension

Thiabendazole Oral Suspension; Thiabendazole Mixture; Thiabendazole Drench

Thiabendazole Veterinary Oral Suspension is an aqueous suspension of Thiabendazole containing suitable suspending agents and antimicrobial preservatives.

Thiabendazole Veterinary Oral Suspension contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of thiabendazole, $C_{10}H_7N_3S$.

Usual strength. 13.3 per cent w/v.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *toluene*, 20 volumes of *glacial acetic acid*, 8 volumes of *acetone* and 2 volumes of *water*.

Test solution. Add 50 ml of *ethyl acetate* and 2 ml of *glacial acetic acid* to a volume of the well-mixed oral suspension containing about 0.25 g of Thiabendazole. Shake for 5 minutes, heat to boiling, cool, shake for a further 15 minutes and filter.

Reference solution. Dissolve 0.25 g of *thiabendazole RS* in 50 ml of *ethyl acetate* and add 2 ml of *glacial acetic acid*.

Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with reference solution.

Tests

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. Weigh accurately a quantity of the well-mixed oral suspension containing about 1 g of Thiabendazole, add to 700 ml of *0.1 M hydrochloric acid*, shake for 30 minutes, add sufficient *0.1 M hydrochloric acid* to produce 1000.0 ml, mix and filter. Dilute 10.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid*. Dilute 5.0 ml to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7). Calculate the content of $C_{10}H_7N_3S$ taking 1230 as the specific absorbance at 302 nm.

Determine the weight per ml of the suspension (2.4.29), and calculate the content of thiabendazole, weight in volume.

Labelling. The label states that the suspension should be administered undiluted.

Thiabendazole and Rafoxanide Veterinary Oral Suspension

Thiabendazole and Rafoxanide Suspension; Thiabendazole and Rafoxanide Mixture

Thiabendazole and Rafoxanide Veterinary Oral Suspension is an aqueous suspension of Thiabendazole and Rafoxanide containing suitable suspending and dispersing agents.

Thiabendazole and Rafoxanide Veterinary Oral Suspension contains not less than 92.5 per cent and not more than 107.5 per cent of the stated amount of thiabendazole, $C_{10}H_7N_3S$, and not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of rafoxanide, $C_{19}H_{11}Cl_2I_2NO_3$.

Usual strength. 13.3 per cent w/v of Thiabendazole and 2.27 per cent w/v of Rafoxanide.

Identification

A. Mix a volume containing 20 mg of Thiabendazole with 5 ml of *0.1 M hydrochloric acid*, add 3 mg of *4-phenylenediamine dihydrochloride*, mix, add 0.1 g of *zinc powder* and allow to stand for 2 minutes. Add 10 ml of *ferric ammonium sulphate solution*; a deep blue or blue violet colour is produced.

B. In addition to the absorbance at about 335 nm, measure the absorbance at about 280 nm (2.4.7), of the final solution obtained in the Assay. The ratio of the absorbance at about 280 nm to that at about 335 nm is 1.59 to 1.69.

Tests

Other tests. Complies with the tests stated under Veterinary Oral Liquids.

Assay. *For thiabendazole* — Weigh accurately a volume of the well-mixed suspension containing about 85 mg of Thiabendazole, add 20 ml of *water* and 9 ml of *0.1 M hydrochloric acid* and warm on a water-bath for 30 minutes with occasional stirring. Transfer the suspension to a flask, rinse the vessel with *water* and add the washings to the flask. Cool, add sufficient *water* to produce 1000.0 ml and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7). Calculate the content of $C_{10}H_7N_3S$ taking 1230 as the specific absorbance at 302 nm.

For rafoxanide — Protect the solutions from light throughout the determination.

Weigh accurately a volume of the well-mixed suspension containing about 0.1 g of Rafoxanide in a 500-ml stoppered flask and add sufficient *water* to produce 100 ml. Swirl to disperse, add 20 ml of *1 M hydrochloric acid*, mix well and add 300 ml of *ethyl acetate*. Shake the mixture for 1 hour, set aside for separation of the immiscible layers and centrifuge a portion of the ethyl acetate layer. Transfer 15.0 ml of the clear solution to a 50-ml centrifuge tube, add 20 ml of *0.1 M hydrochloric acid*, stopper the tube, shake for 15 minutes, and centrifuge. Remove and discard the aqueous layer. Repeat the washing with two quantities, each of 20 ml, of *0.1 M hydrochloric acid*. Evaporate the ethyl acetate solution almost to dryness in a warm water-bath, passing a stream of *nitrogen* over the surface of the liquid. Add 10 ml of *water*, warm on a water-bath for 10 minutes, add 5 ml of *1 M sodium hydroxide* and mix. Add 15 ml of *ether*, shake for 15 minutes, centrifuge, and remove the ether layer. Repeat the extraction with two quantities, each of 15 ml, of *ether*. Evaporate the combined ether extracts almost to dryness on a warm water-bath, passing a stream of *nitrogen* over the surface of the liquid. Dissolve the residue in sufficient *0.1 M methanolic hydrochloric acid* to produce 200.0 ml and measure the absorbance of the resulting solution at the maximum at about 335 nm (2.4.7).

Calculate the content of $C_{19}H_{11}Cl_2I_2NO_3$ from the absorbance obtained by carrying out the procedure simultaneously, using 0.1 g of *rafoxanide RS* and beginning at the words, "add sufficient *water* to produce 100 ml.....".

Determine the weight per ml of the suspension (2.4.29), and calculate the content of thiabendazole and rafoxanide, weight in volume.

Thiabendazole Premix

Thiabendazole Premix contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of thiabendazole, $C_{10}H_7N_3S$.

Usual strengths. 22.5 per cent w/w; 33.3 per cent w/w.

Identification

Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel GF254*.

Mobile phase. A mixture of 50 volumes of *toluene*, 20 volumes of *glacial acetic acid*, 8 volumes of *acetone* and 2 volumes of *water*.

Test solution. To a quantity of the premix containing 0.25 g of Thiabendazole, finely powdered if necessary, add 50 ml of *ethyl acetate* and 2 ml of *glacial acetic acid*, shake for 5 minutes, heat to boiling, cool, shake for a further 15 minutes and filter.

Reference solution. Dissolve 0.25 g of *thiabendazole RS* in 50 ml of *ethyl acetate* and add 2 ml of *glacial acetic acid.*

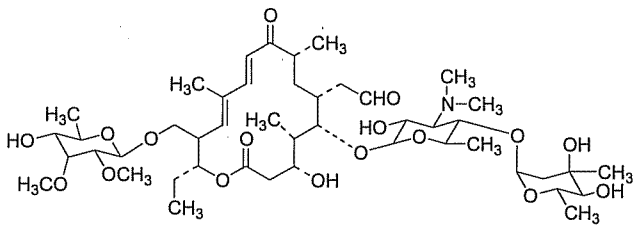
Apply to the plate 10 µl of each solution. After development, dry the plate in air and examine in ultraviolet light at 254 nm. The principal spot in the chromatogram obtained with the test solution corresponds to that in the chromatogram obtained with the reference solution.

Tests

Other tests. Complies with the tests stated under Premixes.

Assay. Weigh accurately a quantity containing about 0.1 g of Thiabendazole, add 700 ml of *0.1 M hydrochloric acid*, shake for 30 minutes, dilute to 1000.0 ml with *0.1 M hydrochloric acid* and filter. Dilute 5.0 ml of the filtrate to 100.0 ml with *0.1 M hydrochloric acid* and measure the absorbance of the resulting solution at the maximum at about 302 nm (2.4.7). Calculate the content of $C_{10}H_7N_3S$, taking 1230 as the specific absorbance at 302 nm.

Tylosin



$C_{46}H_{77}NO_{17}$ Mol. Wt. 916.1

Tylosin is a macrolide antibiotic isolatede from a strain of Stryptomycetes fradiae found in soil from Thailand.

Tylosin has a potency of not less than 900 Units per mg, calculated on the dried basis. The content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 95.0 per cent.

Category. Antibacterial.

Dose. *All species except adult ruminants.* Orally, 20 to 45 mg per kg of body weight daily in divided doses. By intramuscular injection, 2 to 10 mg per kg of body weight.

Description. Almost white or slightly yellow powder.

Identification

Tests B and C may be omitted if tests A, D and E are carried out. Tests D and E may be omitted if tests A, B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tylosin RS* or with the reference spectrum of tylosin.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* (solution A) shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.94.

C. To 10 ml of solution A add 1 ml of *2 M sodium hydroxide*, heat on a water-bath for 20 minutes and cool. When examined in the range 250 nm to 430 nm (2.4.7), of the resulting solution shows an absorption maximum only at about 332 nm.

D. In the test for Tylosin A and other tylosins, the retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with reference solution (a).

E. Dissolve about 30 mg in a mixture of 0.15 ml of *water*, 2.5 ml of *acetic anhydride* and 7.5 ml of *pyridine*. Allow to stand for 10 minutes; no green colour develops.

Tests

pH (2.4.24). 8.5 to 10.5, determined in a 2.5 per cent w/v suspension in *carbon dioxide-free water.*

Heavy metals. To the residue obtained in the test for Sulphated ash add 2 ml of *hydrochloric acid* and evaporate slowly to dryness on a water-bath. Moisten the residue with 0.05 ml of *hydrochloric acid*, add 10 ml of boiling *water* and heat for 10 minutes on a water-bath. Cool and dilute to 25 ml with *water*. 12 ml of the solution complies with the limit test for heavy metals, Method D (2.3.13) (20 ppm).

Tyramine. Dissolve 50 mg in 5 ml of *0.03 M phosphoric acid* in a 25-ml volumetric flask, add 1 ml of *pyridine* and 2 ml of a saturated solution of *ninhydrin* in *water* (approximately 4 per cent w/v). Close the flask by covering with a piece of aluminium foil and heat in a water-bath at 85° for at least 20 minutes. Cool rapidly and add sufficient *water* to produce 25 ml. Mix and measure without delay the absorbance of the solution at about 570 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the substance under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously, using 5 ml of a solution in *0.03 M phosphoric acid* containing 35 mg of *tyramine* per ml and beginning at the words "add 1 ml of *pyridine*....." (0.35 per cent).

Sulphated ash (2.3.18). Not more than 3.0 per cent.

Loss on drying (2.4.19). Not more than 5.0 per cent, determined on 1.0 g by drying at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Tylosin A and other tylosins. Determine by liquid chromatography (2.4.14).

NOTE— Use freshly prepared solutions.

Test solution. Dissolve 20 mg of the substance under examination in 100 ml of a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (a). A 0.02 per cent w/v solution of *tylosin RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (b). A solution containing 0.02 per cent w/v each of *tylosin A RS* and *tylosin D RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 60 volumes of *0.85 M sodium perchlorate* and 40 volumes of *acetonitrile* adjusted to pH 2.5 with *1 M hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject reference solution (b). If necessary, adjust the molarity of the sodium perchlorate or increase the temperature of the column to a maximum of 50° so as to obtain a retention time of about 12 minutes for tylosin A. The test is not valid unless the resolution between the peaks due to tylosin A and tylosin D is at least 2.0.

Inject reference solution (a). The column efficiency, determined using the peak due to tylosin A, should be not less than 22,000 theoretical plates per metre.

Inject alternatively the test solution and reference solution (a). The order of elution of the major components of the substance under examination is desmycinosyltylosin, tylosin C, tylosin B, tylosin D, tylosin A aldol and tylosin A.

Calculate the percentage content of components from the areas of the peaks in the chromatogram obtained with the test solution by normalisation.

Assay. Carry out the microbiological assay of antibiotics (2.2.10).

Tylosin intended for use in the manufacture of Parenteral Preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light. If it is intended for use in the manufacture of Parenteral Preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) the date after which the material is not intended to be used; (3) the storage conditions; (4) where applicable, that it is suitable for use in the manufacture of Parenteral Preparations; (5) that the preparation is intended for veterinary use.

Tylosin Injection

Tylosin Injection is a sterile solution of Tylosin in a mixture of equal volumes of Propylene Glycol and Water for Injections.

Tylosin Injection contains not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tylosin. The content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 90.0 per cent.

Usual strengths. 2.5 g in 50 ml; 20 g in 100 ml.

Description. A pale yellow to amber-coloured solution.

Identification

A. To a volume containing 0.1 g of Tylosin add sufficient *water* to obtain a solution containing 0.02 per cent w/v of Tylosin. To 5 ml of this solution add 10 ml of *0.1 M sodium hydroxide* and extract with 10 ml of *dichloromethane*. Separate the dichloromethane layer and extract it with 25 ml of *0.1 M hydrochloric acid*. Discard the dichloromethane layer, wash the aqueous layer with 3 ml of *dichloromethane*, discard the washings and filter. When examined in the range 230 nm to 360 nm (2.4.7), of the resulting solution exhibits a maximum only at about 290 nm; absorbance at about 290 nm, about 0.94.

B. To 10 ml of the filtrate obtained in test A add 1 ml of *2 M sodium hydroxide*, heat in a water-bath for 20 minutes and cool. When examined in the range 250 nm to 430 nm (2.4.7), exhibits a maximum only at about 332 nm.

Tests

Tyramine. Dilute a volume containing 100 mg of Tylosin with 5 ml of *0.03 M phosphoric acid* in a 25-ml volumetric flask, add 1 ml of *pyridine* and 2 ml of a saturated solution of *ninhydrin* in *water* (approximately 4 per cent w/v). Close the flask by covering with a piece of aluminium foil and heat in a water-bath at 85° for at least 20 minutes. Cool rapidly and add

sufficient *water* to produce 25 ml. Mix and measure without delay the absorbance of the resulting solution at about 570 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the preparation under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously, using 5 ml of a solution in *0.03 M phosphoric acid* containing 30 mg of *tyramine* per ml and beginning at the words "add 1 ml of *pyridine*....." (0.15 per cent).

Tylosin A and other tylosins. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Dilute the injection with sufficient of a mixture of equal volumes of *acetonitrile* and *water* to produce a solution containing 0.02 per cent w/v of Tylosin.

Reference solution (a). A 0.02 per cent w/v solution of *tylosin RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (b). A solution containing 0.02 per cent w/v each of *tylosin A RS* and *tylosin D RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system
- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 60 volumes of *0.85 M sodium perchlorate* and 40 volumes of *acetonitrile* adjusted to pH 2.5 with *1 M hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject reference solution (b). If necessary, adjust the molarity of the sodium perchlorate or increase the temperature of the column to a maximum of 50° so as to obtain a retention time of about 12 minutes for tylosin A. The test is not valid unless the resolution between the peaks due to tylosin A and tylosin D is at least 2.0.

Inject reference solution (a). The column efficiency, determined using the peak due to tylosin A, should be not less than 22,000 theoretical plates per metre.

Inject alternatively test solution and reference solution (a). The order of elution of the major components of the substance under examination is desmycinosyltylosin, tylosin C, tylosin B, tylosin D, tylosin A aldol and tylosin A.

Calculate the percentage content of components from the areas of the peaks in the chromatogram obtained with the test solution.

Other tests. Complies with the tests stated under Parenteral Preparations (Injections).

Assay. Carry out the microbiological assay of antibiotics (2.2.10). Calculate the content of tylosin in the injection, taking each 1000 Units found to be equivalent to 1 mg of tylosin.

Storage. Store protected from moisture.

Labelling. The label states that the preparation is intended for veterinary use by intramuscular injection only.

Tylosin Tablets

Tylosin Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of tylosin. The content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 90.0 per cent.

Usual strength. 200 mg.

Identification

A. Triturate a quantity of the powdered tablets containing 0.2 g of Tylosin with 20 ml of *dichloromethane* and filter. Dry the dichloromethane extract by shaking with *anhydrous sodium sulphate*, filter and evaporate the filtrate to dryness. Dry the residue over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 1 hour.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tylosin RS* or with the reference spectrum of tylosin.

B. Triturate a quantity of the powdered tablets containing 0.2 g of Tylosin with two quantities, each of 10 ml, of *0.1 M hydrochloric acid*, filter and dilute the filtrate to 100 ml with *0.1 M hydrochloric acid*. Dilute 10 ml of the resulting solution to 50 ml with the same solvent. Dilute 5 ml of this solution further to 50 ml with the same solvent.

When examined in the range 230 nm to 360 nm (2.4.7), the resulting solution shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.94.

C. To 10 ml of the final solution obtained in test B add 1 ml of *2 M sodium hydroxide*, heat in a water-bath for 20 minutes and cool. When examined in the range 250 nm to 430 nm (2.4.7), exhibits a maximum only at about 332 nm.

Tests

Tyramine. Shake a quantity of the powdered tablets containing 50 mg of Tylosin with 5 ml of *0.03 M phosphoric acid*. Filter into a 25-ml volumetric flask, add 1 ml of *pyridine* and 2 ml of a saturated solution of *ninhydrin* in *water* (approximately 4 per cent w/v). Close the flask by covering with a piece of

aluminium foil and heat in a water-bath at 85° for at least 20 minutes. Cool rapidly and add sufficient *water* to produce 25 ml. Mix and measure without delay the absorbance of the solution at about 570 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the substance under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously, using 5 ml of a solution in *0.03 M phosphoric acid* containing 35 mg of *tyramine* per ml and beginning at the words "add 1 ml of *pyridine*....." (0.35 per cent).

Tylosin A and other tylosins. Determine by liquid chromatography (2.4.14).

NOTE —Use freshly prepared solutions.

Test solution. Shake a quantity of the powdered tablets containing 0.2 g of Tylosin with 50 ml of *methanol*, filter and dilute 5 ml of the filtrate to 100 ml with a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (a). A 0.02 per cent w/v solution of *tylosin RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (b). A solution containing 0.02 per cent w/v each of *tylosin A RS* and *tylosin D RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system

- a stainless steel column 20 cm × 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 60 volumes of *0.85 M sodium perchlorate* and 40 volumes of *acetonitrile* adjusted to pH 2.5 with *1 M hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject reference solution (b). If necessary, adjust the molarity of the sodium perchlorate or increase the temperature of the column to maximum of 50° so as to obtain a retention time of about 12 minutes for tylosin A. The test is not valid unless the resolution between the peaks due to tylosin A and tylosin D is at least 2.0.

Inject reference solution (a). The column efficiency, determined using the peak due to tylosin A, should be not less than 22,000 theoretical plates per metre.

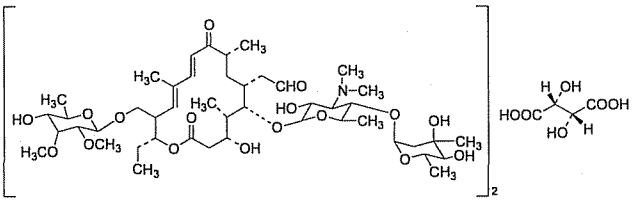
Inject alternatively the test solution and reference solution (a). The order of elution of the major components of the substance under examination is desmycinosyltylosin, tylosin C, tylosin B, tylosin D, tylosin A aldol and tylosin A.

Calculate the percentage content of components from the areas of the peaks in the chromatogram obtained with test solution.

Other tests. Comply with the tests stated under Tablets.

Assay. Carry out the microbiological assay of antibiotics (2.2.10). Calculate the content of tylosin in the tablets, taking each 1000 Units found to be equivalent to 1 mg of tylosin.

Tylosin Tartrate



$(C_{46}H_{77}NO_{17})_2, C_4H_6O_6$ Mol. Wt. 1983.3

Tylosin Tartrate is the tartrate of Tylosin, which is a mixture of antimicrobial macrolides produced by the growth of certain strains of *Streptomyces fradiae* or by any other means. It consists largely of tylosin A tartrate but tartrates of tylosin B (desmycosin), tylosin C (macrocin) and tylosin D (relomycin) may also be present.

Tylosin Tartrate contains not less than 800 Units per mg, calculated on the dried basis. The content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 95.0 per cent.

Category. Antibacterial.

Dose. *Pigs and poultry*. The equivalent of 2.5 g of Tylosin per gallon (4.5 litres) of drinking water offered continuously for 3 days.

Description. An almost white or slightly yellow, hygroscopic powder.

Identification

Tests B and C may be omitted if tests A, D and E are carried out. Tests D and E may be omitted if tests A, B and C are carried out.

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tylosin tartrate RS* or with the reference spectrum of tylosin tartrate.

B. When examined in the range 230 nm to 360 nm (2.4.7), a 0.004 per cent w/v solution in *0.1 M hydrochloric acid* (solution A) shows an absorption maximum only at about 290 nm; absorbance at about 290 nm, about 0.88.

C. To 10 ml of solution A add 1 ml of *2 M sodium hydroxide*, heat on a water-bath for 20 minutes and cool.

When examined in the range 250 nm to 430 nm (2.4.7), the resulting solution shows an absorption maximum only at about 332 nm.

D. In the test for Tylosin A and other tylosins, the retention time and size of the principal peak in the chromatogram obtained with the test solution are approximately the same as those of the principal peak in the chromatogram obtained with the reference solution.

E. Dissolve about 30 mg in a mixture of 0.15 ml of *water*, 2.5 ml of *acetic anhydride* and 7.5 ml of *pyridine*. Allow to stand for 10 minutes; a green colour develops.

Tests

pH (2.4.24). 5.0 to 7.2, determined in a 2.5 per cent w/v solution in *carbon dioxide-free water*.

Tyramine. Dissolve 50 mg in 5 ml of *0.03 M phosphoric acid* in a 25-ml volumetric flask, add 1 ml of *pyridine* and 2 ml of a saturated solution of *ninhydrin* in *water* (approximately 4 per cent w/v). Close the flask by covering with a piece of aluminium foil and heat in a water-bath at 85° for at least 20 minutes. Cool rapidly and add sufficient *water* to produce 25 ml. Mix and measure without delay the absorbance of the solution at about 570 nm (2.4.7), using as the blank a solution prepared in a similar manner but omitting the substance under examination. The absorbance is not more than that obtained by carrying out the procedure simultaneously, using 5 ml of a solution in *0.03 M phosphoric acid* containing 35 mg of *tyramine* per ml and beginning at the words "add 1 ml of *pyridine*....." (0.35 per cent).

Sulphated ash (2.3.18). Not more than 2.5 per cent.

Loss on drying (2.4.19). Not more than 4.5 per cent, determined on 1.0 g by drying at 60° at a pressure not exceeding 0.7 kPa for 3 hours.

Tylosin A and other tylosins. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Dissolve a quantity containing 20 mg of tylosin in 100 ml of a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (a). A 0.02 per cent w/v solution of *tylosin RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (b). A solution containing 0.02 per cent w/v each of *tylosin A RS* and *tylosin D RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system

- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 60 volumes of *0.85 M sodium perchlorate* and 40 volumes of *acetonitrile* adjusted to pH 2.5 with *1 M hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject reference solution (b). If necessary, adjust the molarity of the sodium perchlorate or increase the temperature of the column to a maximum of 50° so as to obtain a retention time of about 12 minutes for tylosin A. The test is not valid unless the resolution between the peaks due to tylosin A and tylosin D is at least 2.0.

Inject reference solution (a). The column efficiency, determined using the peak due to tylosin A, should be not less than 22,000 theoretical plates per metre.

Inject alternatively the test solution and the reference solution (a). The order of elution of the major components of the substance under examination is desmycinosyltylosin, tylosin C, tylosin B, tylosin D, tylosin A aldol and tylosin A.

Calculate the percentage content of components from the areas of the peaks in the chromatogram obtained with test solution.

Assay. Carry out the microbiological assay of antibiotics (2.2.10).

Tylosin Tartrate intended for use in the manufacture of parenteral preparations complies with the above requirements with the following modification.

Tyramine. Carry out the procedure described in the test for Tyramine but using 100 mg in 5 ml of *0.03 M phosphoric acid*. Measure the absorbance of the solution under the conditions described in the test. The absorbance is not more than that obtained by simultaneously carrying out the procedure using 5 ml of a solution in *0.03 M phosphoric acid* containing 30 mg of *tyramine* per ml and beginning at the words "add 1 ml of *pyridine*....." (0.15 per cent).

Tylosin Tartrate intended for use in the manufacture of parenteral preparations without a further appropriate sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light. If it is intended to be used in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units per mg; (2) the quantity of Tylosin Tartrate in terms of equivalent amount of tylosin; (3) the date after which the material is not intended

to be used; (4) the storage conditions; (5) where applicable, that it is suitable for use in the manufacture of parenteral preparations; (6) that the preparation is intended for veterinary use.

Tylosin Tartrate and Sulphathiazole Sodium Veterinary Oral Powder

Tylosin Tartrate and Sulphathiazole Sodium Veterinary Oral Powder is a mixture of Tylosin Tartrate and Sulphathiazole Sodium. It contains 3 parts of Sulphathiazole Sodium for 1 part, by weight, of Tylosin.

Tylosin Tartrate and Sulphathiazole Sodium Veterinary Oral Powder contains not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of tylosin and sulphathiazole sodium sesquihydrate, $C_9H_8NaO_2S_2,1^1/_2H_2O$.

Usual strength. The equivalent of 25 g of tylosin as Tylosin Tartrate and the equivalent of 75 g of sulphathiazole sodium sesquihydrate as Sulphathiazole Sodium.

Identification

A. Triturate a quantity of the powder containing 0.25 g of Tylosin with two quantities, each of 25 ml, of *dichloromethane* and filter. Reserve the dichloromethane-insoluble matter for test B. Wash the combined filtrates by shaking for 1 minute with 20 ml of *0.1 M sodium hydroxide* and dry the dichloromethane layer by the addition of *anhydrous sodium sulphate*. Evaporate the filtrate to dryness and dry the residue over *phosphorus pentoxide* at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *tylosin RS* or with the reference spectrum of tylosin.

B. Dry the dichloromethane-insoluble matter reserved in test A at 105° for 1 hour. The residue complies with the following test.

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sulphathiazole sodium RS* or with the reference spectrum of sulphathiazole sodium.

Tests

Sulphonamide-related substances. Determine by thin-layer chromatography (2.4.17), coating the plate with *silica gel H*.

Solvent mixture. A mixture of 9 volumes of *ethanol (95 per cent)* and 1 volume of *strong ammonia solution*.

Mobile phase. A mixture of 90 volumes of *1-butanol* and 18 volumes of *10 M ammonia*.

Test solution. Shake a quantity of the powder containing 0.1 g of sulphathiazole sodium sesquihydrate with 10 ml of the solvent mixture.

Reference solution. A 0.0050 per cent w/v solution of *sulphanilamide* in the solvent mixture.

Apply to the plate 10 µl of each solution. After development, dry the plate by heating it at 105° for 10 minutes and spray with a 0.1 per cent w/v solution of *4-dimethylaminobenzaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid*. Any secondary spot in the chromatogram obtained with the test solution is not more intense than the spot in the chromatogram obtained with reference solution (0.5 per cent).

Tylosin A and other tylosins. Determine by liquid chromatography (2.4.14).

NOTE — Use freshly prepared solutions.

Test solution. Dissolve a quantity containing 20 mg of tylosin in 100 ml of a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (a). A 0.02 per cent w/v solution of *tylosin RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Reference solution (b). A solution containing 0.02 per cent w/v each of *tylosin A RS* and *tylosin D RS* in a mixture of equal volumes of *acetonitrile* and *water*.

Chromatographic system
- a stainless steel column 20 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature 35°,
- mobile phase: a filtered and degassed mixture of 60 volumes of *0.85 M sodium perchlorate* and 40 volumes of *acetonitrile* adjusted to pH 2.5 with *1 M hydrochloric acid*,
- flow rate. 1 ml per minute,
- spectrophotometer set at 290 nm,
- injection volume. 20 µl.

Inject reference solution (b). If necessary, adjust the molarity of the sodium perchlorate or increase the temperature of the column to a maximum of 50° so as to obtain a retention time of about 12 minutes for tylosin A. The test is not valid unless the resolution between the peaks due to tylosin A and tylosin D is at least 2.0.

Inject reference solution (a). The column efficiency, determined using the peak due to tylosin A, should be not less than 22,000 theoretical plates per metre.

Inject alternatively the test solution and the reference solution (a). The order of elution of the major components of the

substance under examination is desmycinosyltylosin, tylosin C, tylosin B, tylosin D, tylosin A aldol and tylosin A.

Calculate the percentage content of components from the areas of the peaks in the chromatogram obtained with the test solution by normalisation. In the chromatogram obtained with the test solution the content of tylosin A is not less than 80.0 per cent and the sum of the contents of tylosin A, tylosin B, tylosin C and tylosin D is not less than 95.0 per cent.

Other tests. Complies with the tests stated under Veterinary Oral Powders.

Assay. *For tylosin activity* — Weigh accurately a quantity of the powder containing about 0.2 g of Tylosin, transfer to a 100-ml volumetric flask with three quantities, each of 10 ml, of *methanol*, swirl to dissolve and add sufficient sterile *phosphate buffer pH 7.0* to produce 100.0 ml. Filter and dilute 5.0 ml of the filtrate to 100.0 ml with sterile *phosphate buffer pH 7.0*. Carry out the microbiological assay of antibiotics (2.2.10). Calculate the content of tylosin taking each 1000 Units found to be equivalent to 1 mg of tylosin.

For sulphathiazole sodium — Weigh accurately a quantity of the powder containing about 0.4 g of sulphathiazole sodium sesquihydrate, dissolve in a mixture of 75 ml of *water* and 10 ml of *hydrochloric acid,* add 3 g of *potassium bromide*, cool in ice and titrate slowly with *0.1 M sodium nitrite*, stirring constantly and determine the end-point potentiometrically (2.4.25).

1 ml of *0.1 M sodium nitrite* is equivalent to 0.03043 g of $C_9H_8N_3NaO_2S_2,1\frac{1}{2}H_2O$.

Storage. Store protected from moisture.

Labelling. The label states the strength of Tylosin Tartrate in terms of the equivalent amount of tylosin and that of Sulphathiazole Sodium in terms of the equivalent amount of sulphathiazole sodium sesquihydrate.

BIOLOGICAL VETERINARY MONOGRAPHS

Anthrax Spore Vaccine, Live

Anthrax Spore Vaccine, Live consists of a suspension of live spores of an attenuated, non-capsulated strain of *Bacillus anthracis*.

Production

The strain used may either be not lethal to guinea pigs or the mouse or lethal to guinea pigs but not to the rabbit or lethal to some rabbits. At the end of growth the spores are suspended in 50 per cent glycerin saline and counted. The vaccine may contain an adjuvant.

If the immunogenicity tests have been performed with satisfactory results on a representative batch of the vaccine from the seed lot, they may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Identification

B. anthracis present in the vaccine is identified by means of morphological, serological test, culture and biochemical tests.

Tests

Safety. Carry out the test on one of the species for which the vaccine is intended. If the vaccine is intended for several species including the goat, carry out the test on goats. Administer subcutaneously or intramuscularly to each of two animals having no antibodies against *B. anthracis*, twice the dose (example, 2 million spores) stated on the label for the species used and observe the animals for 10 days. No abnormal systemic reaction is produced but a mild local reaction may occur at the site of injection. The severity of the local reaction may vary according to the strain of the *B. anthracis* and the adjuvants used in the preparation, but necrosis does not occur.

Sterility (2.2.11). Complies with the test for sterility.

Spore count. Determine the number of viable spores by plate count. The number of live spores is not less than 80 per cent of that stated on the label. The spore count must be not less than 10 million spores per dose.

Potency. For a strain of *B. anthracis* which is not lethal to the guinea pig or the mouse, the test may be carried out in the guinea pigs. For a strain which is lethal to the guinea pig but not to the rabbit, the test may be carried out in rabbits. For a strain which is lethal to some rabbits, carry out the test in sheep.

If the test is carried out in guinea pigs or rabbits. Inject into animals (each guinea pig weighing not less than 500 g and each rabbit weighing not less than 2.5 kg) subcutaneously or intramuscularly, $1/10^{th}$ of the smallest dose of the vaccine stated on the label for sheep. Observe the animals for 21 days. If more than two animals die from non-specific causes, repeat the test.

If the test is carried out in sheep use 5 healthy animals. Inject subcutaneously or intramuscularly into each sheep weighing not less than 20 kg, $1/10^{th}$ of the smallest dose of the vaccine stated on the label for sheep and observe the animal for 21 days. None of the sheep shows any untoward reaction. If more than 2 animals die from non-specific causes, repeat the test.

Inject subcutaneously into each vaccinated guinea pig or rabbit or sheep at least 100 MLD and to each of these control animals 10 MLD of a strain of *B.anthracis* pathogenic for the species of animal used in the test. Observe all the animals for 10 days, all vaccinated animals should survive and all the controls die from anthrax during the observation period. If a vaccinated animal dies after the challenge, repeat the test. If in the second test, a vaccinated animal dies, the vaccine fails the test.

Labelling. The label states (1) the strain used for the preparation of the vaccine; (2) the number of viable spores per ml.

Avian Infectious Bronchitis Vaccine, Inactivated

Avian Infectious Bronchitis Vaccine, Inactivated consists of an emulsion or a suspension of one or more serotypes of avian infectious bronchitis virus which have been inactivated in such a manner that the immunogenic activity is retained. This monograph applies to vaccines intended to protect birds against drop in egg production or quality; for vaccines also intended for protection against respiratory signs and nephropathic symptoms, a demonstration of efficacy additional to that described under potency is required.

Production

The virus is propagated in embryonated hen's eggs obtained from healthy flocks or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10). The vaccine may contain one or more suitable adjuvant.

Inactivation

An amplification test for residual live avian infectious bronchitis virus is carried out on each batch of antigen immediately after inactivation. The test is carried out in fertilised hen's eggs from flocks free from specified pathogens (SPF) or in suitable cell culture derived from SPF

eggs (2.7.7) and the quantity of inactivated virus used is equivalent to not less than 10 doses of vaccine. No live virus is detected.

A. **In embryonated eggs.** For vaccine prepared with embryo-adapted strains of virus, inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of ten 9 to 11-day-old fertilized hen eggs from an SPF flock and incubate. Observe for 5 to 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those that die within the first 24 hours after injection. Examine for abnormalities in all embryos which die after 24 hours of inoculation or which survive 5 to 6 days. No death or abnormality attributable to the vaccine virus occurs.

Inject into the allantoic cavity of each of ten 9 to 11-day-old fertilized hen eggs from SPF flock, 0.2 ml of the pooled allantoic fluid from the live embryos and into each of 10 similar eggs 0.2 ml of the pooled liquid from the dead embryos and incubate for 5 to 6 days. Examine for abnormalities in all embryos which die after 24 hours of injection or which survive 5 to 6 days. No death or abnormality attributable to the vaccine virus occurs.

If more than 20 per cent of the embryos die at either stage repeat the test from that stage. The vaccine complies with the test if there is no death or abnormality attributable to the vaccine virus. Antibiotics may be used to control extraneous bacterial infection.

B. **In cell culture.** For vaccine prepared with cell-culture-adapted strains of virus, inoculate quantity of inactivated virus equivalent to 10 doses of vaccine into suitable cell culture derived from SPF eggs (2.7.7). Incubate at 36 ± 1° for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) and incubate at 36 ± 1° for 7 days. None of the cultures shows signs of infection.

Identification

In susceptible birds, the vaccine stimulates the production of specific antibodies against each of the virus strain incorporated in the vaccine, detectable by suitable serological method.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Safety. Inject intramuscularly a quantity equivalent to 2 doses into each of ten SPF chickens (2.7.7) or healthy susceptible chickens, 2 to 4 weeks old. Observe the chickens for 14 days. No abnormal systemic or local reaction is seen.

Potency. Inject one dose by the route stated on the label into each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 to 4 weeks old. Use 5 similar chickens as controls and house them together with the vaccinated chickens. After 28 days, collect serum samples from each of the vaccinated and control chickens and perform haemagglutination inhibition (HI) test on each serum using 4 haemagglutinating (HA) units of antigen and chicken erythrocytes, testing all serum samples at the same time. The vaccine passes the test if the mean antibody titre of the vaccinated group is not less than 1:64 and no specific antibody is detected in the control chickens. Alternatively, serum neutralization test may be carried out in SPF eggs (2.7.7). Serum neutralization titre should not be less than 10^2 neutralization units.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label states (1) the strain of virus used in preparing the vaccine; (2) the route of administration.

Avian Infectious Bronchitis Vaccine, Live

Infectious Bronchitis Vaccine, Live, Avian Infectious Bronchitis Vaccine Living

Avian Infectious Bronchitis Vaccine, Live is a preparation of one or more suitable strains of avian infectious bronchitis virus.

Production

The vaccine virus is grown in embryonated hens' eggs or in cell culture derived from SPF eggs (2.7.7).

Substrate for virus propagation

If the vaccine virus is grown in embryonated hen's eggs they are obtained from SPF flock (2.7.7) or in cell culture derived from SPF flocks (2.7.7).

The production is based on an approved seed lot system. Each lot of stock seed virus is tested for immunogenicity in chicken of the same age and source by the method described under immunogenicity test. If the immunogenicity test has been performed with satisfactory results on the representative batch of vaccine from the seed lot, it may be omitted as a routine control of other batches of the vaccine prepared from the same seed lot.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

Carry out either the test A or B.

A. Inoculate 0.2 ml undiluted vaccine in the allantoic sac of SPF embryonated eggs and incubate at 36 ± 1° for 5 to 6 days.

Lesions typical of infectious bronchitis (IB) are observed in the embryos and the allantoic fluid does not agglutinate chicken erythrocytes.

B. Specific antiserum against the strain or each of the strains of the avian infectious bronchitis virus used in the vaccine should neutralise corresponding IB virus. When mixed with specific antiserum, the vaccine no longer infects 9-11 day old embryonated SPF eggs (2.7.7).

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Inject 10 times the dose by the route stated on the label into each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens of 5-10 days old. Observe the birds for 21 days. Not more than one of the vaccinated chickens shows symptoms of or dies from infectious bronchitis. If during the period of observation more than 2 of the vaccinated chickens die from causes not attributable to the vaccine, repeat the test.

Sterility (2.2.11). Complies with the test for sterility.

Virus titre. Titrate the vaccine in cell culture derived from SPF eggs (2.7.7) derived from SPF embryos or by inoculating into the allantoic sac of SPF embryonated eggs, 9 to 11 days old. One dose of the vaccine contains not less than $10^{3.5}$ $TCID_{50}/EID_{50}$.

Immunogenicity. Carry out a test for each route of administration recommended on the label and for each serotype against which protection is claimed and of the minimum age stated for vaccination. Administer to each of 20 SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 to 4 weeks old, for each of the stated routes a volume of reconstituted vaccine containing a quantity of virus equivalent to the minimum titre stated on the label. Ten additional SPF chickens (2.7.7, table 3) or healthy susceptible chickens of same flock for each serotype against which protection is claimed are used as unvaccinated controls. Three to four weeks later, administer by eye drop a virulent strain of bronchitis virus with a titre of at least $10^{3.5}$ EID_{50} per ml to all the vaccinated and control birds. Between the fourth to seventh day after the challenge, take tracheal swabs from each of the vaccinated and control birds. Place each swab in a sterile test tube containing 3 ml of tryptose phosphate broth and antibiotics. Swirl the tubes containing swabs thoroughly and store at -20° pending inoculation into eggs. For each tracheal swab, inoculate at least 5 chicken embryos, 9 to 11 days old, with 0.2 ml of the broth from each tube into the allantoic cavity. All the embryos surviving on the third day after inoculation are used in the evaluation. A tracheal swab is considered positive for recovery of the virus if any of the embryos shows typical infectious bronchitis lesions such as stunting, curling, kidney urates, clubbing down or death between the fourth and seventh day after inoculation. The vaccine complies with the test if not less than 80 per cent of the controls and not more than 20 per cent of the vaccinated chickens are positive for virus recovery. If less than 80 per cent of the vaccinated chickens are negative for virus recovery the stock seed is unsatisfactory. The stock seed virus may be tested for immunogenicity once in 5 years provided it is maintained under standard conditions of storage of the bronchitis virus.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) the minimum virus titre per dose; (2) the dose of vaccine.

Avian Spirochaetosis Vaccine

Avian spirochaetosis Vaccine is a suspension prepared from viscera and membranes of developing chicken embryos of SPF eggs (2.7.7) infected with antigenic strains of *Borrelia anserina,* which has been inactivated in a such a manner that it's immunogenic activity is retained.

Production

Substrate for propagation

The organism is grown in embryonated eggs derived from SPF flocks.

Identification

Protects chickens against infection with *B. anserina.*

Tests

Safety. Inject subcutaneously a quantity equivalent to 2 doses into each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the recommended age at which vaccine is to be used. Observe the chickens for 14 days, no abnormal systemic or local reaction is seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject at least 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 8 to 12 week old, with the minimum dose of vaccine by the route stated on the label. Use 5 chickens of the same stock as controls. Ten days later challenge all the chickens intra peritoneally with an adequate dose of a virulent culture of *B. anserina* used to prepare the vaccine or with a

suspension of liver or kidney tissues obtained from infected chickens. Observe the chickens for 10 days. The vaccinated chickens do not show any symptoms of the disease and presence of *B. anserina* organism in the blood smears of the vaccinated group. The test is not valid unless the control chickens show typical symptoms of spirochaetosis with detection of spirochetes in the blood smears.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) strain of the bacteria used; (2) the route of administration.

Blackquarter Vaccine

Blackquarter Vaccine is a culture of suitable strain or strains of *Clostridium chauvoei* grown in a suitable anaerobic fluid medium and rendered sterile and non-toxic by addition of solution of *formaldehyde* in such a manner that it retains its immunizing properties. The vaccine may contain a suitable adjuvant.

Identification

Protects susceptible animals against infection with *C. chauvoei.*

Tests

Safety. Use two healthy susceptible animals of one of the species for which the vaccine is intended. Inject into each animal by the recommended route twice the maximum dose stated on the label. Observe the animals for 7 days. No significant local or systemic reaction is produced.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject ten healthy adult guinea pigs weighing between 350 and 450 g with a quantity of the product not greater than the minimum dose and route as stated on the label. Repeat the inoculation with the same dose of the vaccine after 28 days. None of the vaccinated guinea pigs shows any systemic reaction. However a minimal local reaction may be observed in the animal.

Fourteen days after the second vaccination, inoculate intramuscularly into each of 10 vaccinated and into each of 5 control guinea pigs with 0.2 ml of virulent culture or 25 viable spore suspension of *C. chauvoei* in 5 per cent of *calcium chloride* solution.

The vaccine complies with the test, if not more than 10 per cent of the vaccinated guinea pigs die from *C. chauvoei* infection within 5 days and all the control animals die from *C. chauvoei* infection within 48 hours of challenge if virulent culture was used or within 72 hours if a spore suspension was used for the challenge. Repeat the test if 20 per cent of the vaccinated animals die.

On repetition, the vaccine complies with the test if not more than 10 per cent of the vaccinated animals die within 5 days and all of the control animals die within 48 hours of challenge if virulent culture was used or within 72 hours if a spore suspension was used for challenge.

Labelling. The label states (a) the method of preparation; (b) the strains of bacteria used to prepare the vaccine.

Canine Contagious Hepatitis Vaccine, Inactivated

Canine Contagious Hepatitis Vaccine, Inactivated is a preparation containing canine contagious hepatitis virus inactivated in such a manner that its immunogenic activity is retained. It may be a freeze-dried preparation or a liquid preparation containing a suitable adjuvant.

Production

The virus is propagated in suitable cell culture systems, the viral suspension is harvested, titrated, inactivated and may contain a suitable stabilizing solution.

Identification

When injected into a susceptible dog, the animal develops specific neutralizing antibodies.

Tests

Water (2.3.43). Not more than 3.0 per cent (for freeze dried vaccine only).

Safety. Inject each of two healthy dogs, between 8 and 14 weeks old, that have been previously tested and shown to be free from canine adenovirus neutralizing antibodies, with twice the dose and by the route stated on the label. Observe the animals for 14 days. No abnormal systemic or local reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of two healthy susceptible dogs, between 8 and 14 weeks old, that have been previously tested and shown to be free from canine adenovirus neutralizing antibodies, with the minimum dose and by the route stated on the label. After 14 days inject a second dose. Between 14 and 21 days after the second injection collect serum from each dog separately and examine each sample as described below.

Inactivate the serum by heating at 56° for 30 min and prepare serial dilutions in a suitable medium. Add to each dilution an equal volume of serum-virus suspension containing approximately 10^2 $TCID_{50}$. Incubate the mixtures for 1 hour at 37°. Add suitable cell culture with minimum of four replicates for each dilution and incubate at 37° for 4 to 8 days. Examine each culture for evidence of specific cytopathic effect. Calculate the antibody titre. The serum from each dog contains not less than 80 SN_{50} per 0.05 ml.

Labelling. The label states (1) the strain used for the preparation; (2) the name of any added adjuvant.

Canine Contagious Hepatitis Vaccine, Live

Canine Contagious Hepatitis/Canine Adenovirus Vaccine is a freeze dried preparation containing one or more attenuated strains of canine adenovirus.

Production

The virus is propagated in a suitable cell cultures, harvested, titrated and may be mixed with a suitable stabilizing solution. The stock seed virus should be tested for immunogenicity at least once in 5 years, if maintained under suitable conditions of storage.

Identification

The vaccine, mixed with one or more specific antisera of canine adenovirus(s), does not produce specific cytopathic effects in susceptible cell cultures.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Virus titre. Not less than 10^3 $TCID_{50}$ virus per dose, determining the titre of the vaccine in a suitable cell culture.

Safety. Inject each of two susceptible dogs, between 8 and 14 weeks old, that have been previously tested and shown to be free from canine adenovirus neutralising antibodies, with 10 times the dose and by the route stated on the label. Observe the animals for 21 days. No abnormal systemic or local reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject five healthy susceptible dogs, between 8 and 14 weeks old, that have been previously tested and shown to be free from canine adenovirus neutralizing antibodies, with a quantity of the vaccine equivalent to the minimum titre and by the route stated on the label. Observe the animals for 21 days. Inject intravenously each of the five vaccinated animals and each of two control animals of the same stock and weight range with a quantity of a virulent strain of canine contagious hepatitis virus sufficient to cause death or typical signs of the disease in a susceptible dog. Observe the animals for a further period of 21 days. The vaccinated animals remain in normal health and the controls die from hepatitis or show typical signs of serious infection. If one of the controls does not show any signs of the disease, repeat the test. The vaccinated animals of the second group remain in normal health and the control animals die from hepatitis or show typical signs of serious infection.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Labelling. The label states (1) the minimum virus titre; (2) the species of the animals in which use of the vaccine is recommended.

Canine Corona Virus Vaccine, Inactivated

Canine Corona Virus Vaccine, Inactivated is a preparation containing canine corona virus inactivated in such a manner that its immunogenic activity is retained. It may be issued as a liquid or as a freeze-dried preparation to be reconstituted with a suitable liquid immediately before use. The liquid vaccine may contain a suitable adjuvant.

Production

The virus is grown in suitable cell culture systems. The vaccine may contain a suitable adjuvant. Only an inactivated viral suspension that complies with the requirements mentioned under final bulk vaccine of each batch is used in the preparation of the final vaccine.

Identification

When inoculated into dogs, the vaccine stimulates the production of specific neutralizing antibodies against canine corona virus as determined by suitable serological tests.

Tests

Water (2.3.43). Not more than 3.0 per cent (for freeze dried vaccine only).

Safety. Inject each of two healthy susceptible dogs, between 8 and 14 weeks old, free from canine corona virus antibodies with a quantity equivalent to 2 doses by the route stated on

the label. Observe the animals for 14 days. No abnormal systemic or local reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either of the test A or B may be carried out.

A. Inject each of five healthy susceptible guinea pigs, each weighing between 350 and 400 g, with half the minimum dose and by the route stated on the label. Repeat the injection after 14 days. Fourteen days after the second injection collect blood samples and obtain the serum from each guinea pig separately. Inactivate each serum by heating at 56° for 30 min. Examine the serum samples for antibodies by the following method.

Prepare 2-fold serial dilutions of serum in a medium suitable for canine cells. Add to each dilution an equal volume of a virus suspension containing approximately 10^2 $TCID_{50}$ and incubate the mixtures at 37° for 1 hour. Inoculate a suitable volume of canine cells into at least 4 replicates of serum virus mixtures and incubate at 37° for 4 days. Examine for evidence of specific cytopathic effects and calculate the antibody titre. The vaccine complies with the test if the mean antibody titre is not less than 45 SN_{50} per 0.05 ml of serum.

B. Inject each of two healthy susceptible dogs, between 8 and 14 weeks old, having antibody titre less than 6 SN_{50} per 0.05 ml of serum, with the dose and by the route recommended on the label. Fourteen days later collect the serum samples from each dog separately. Inactivate each serum by heating at 56° for 30 min. Examine the serum samples individually for neutralizing antibodies by the method described in test A.

If one dog fails to respond, i.e., the antibody titre is less than 6 SN_{50} per 0.05 ml of serum, repeat the test with two more dogs and calculate the mean titres of the three dogs that have responded. The vaccine complies with the test if the median antibody titre of the sera is not less than 45 SN_{50} per 0.05 ml.

Labelling. The label states (1) the recommended routes of administration; (2) that the preparation should be shaken well before use; (3) that the liquid preparation should not be allowed to freeze; (4) that the vaccine should be used immediately after reconstitution.

Canine Distemper Vaccine, Live

Canine Distemper Vaccine, Live is a freeze-dried preparation of a strain of canine distemper virus that has been attenuated for dogs and is grown either in SPF embryonated eggs or in suitable cell cultures.

Production

The virus is propagated in suitable cell culture systems, the viral suspension is harvested, titrated and may contain a suitable stabilizing solution. The stock seed virus should be tested for potency at least once in 5 years, if maintained under suitable conditions of storage.

Identification

A. The vaccine infects the chorioallantoic membranes of SPF embryonated eggs or suitable cell cultures. This effect is neutralized by canine distemper antiserum.

B. An injection into susceptible ferrets or dogs does not cause distemper but immunizes them against the disease. The vaccine strain is satisfactory with respect to absence of increase in virulence.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Virus titre. Not less than 10^3 $EID_{50}/TCID_{50}$ of the virus per dose, determining the titre of the vaccine in a suitable cell culture or SPF eggs (2.7.7).

Extraneous pathogens

A. Mix the vaccine under examination with a mono specific distemper antiserum. It no longer provokes cytopathic effects in susceptible cell cultures and shows no evidence of haemagglutinating or haemadsorbing agents.

B. Use a sufficient number of mice, not less than ten, each weighing between 11 and 15 g, such that a total of three-tenths of the dose of the vaccine is injected. Inject each mouse intracerebrally with 0.03 ml of the vaccine. Observe for 21 days. Not more than two mice die within the first 48 hours. If more than two mice die within the first 48 hours, repeat the test. From the third day to 21 days after the injection, the mice do not show any abnormalities attributable to the vaccine.

Mycoplasmas (2.7.8). Complies with the test for freedom from mycoplasmas.

Safety. Reconstitute the vaccine as recommended on the label and carry out the following tests.

A. *For chicken embryo-adapted vaccine only.* Inject 0.3 ml intracerebrally into each of a group of eight mice, between 3 and 4 weeks old, and 0.5 ml intraperitoneally into each of another eight mice of the same age group.

Observe both the groups for 7 days. Not more than one mouse in either group shows any abnormal local or systemic reaction attributable to the vaccine.

B. Inject each of two susceptible dogs, between 8 and 14 weeks old which do not have antibodies against canine distemper virus with 10 times dose and by the route stated on the label. Observe the animals for 21 days. None of the dogs shows any abnormal local or systemic reaction.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of five susceptible dogs, between 8 and 14 weeks old, that have been previously tested and shown to be free from distemper virus neutralizing antibodies with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titre and by the route stated on the label. Use another two dogs of the same stock and age group as unvaccinated controls. Observe the animals for 21 days. Inject intravenously each of the seven animals with a quantity of virulent strain of canine distemper virus sufficient to cause death or produce typical signs of the disease in a susceptible dog. Observe the animals for a further 21 days. The vaccinated animals survive and show no clinical signs of canine distemper. The test is not valid unless the control dogs die or show symptoms typical of canine distemper. If one of the control animals does not show any sign of distemper, repeat the test. The vaccinated animals of the second group remain in normal health and the control animals die from distemper or show symptoms typical of canine distemper.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Labelling. The label states (1) the strain used for the preparation; (2) virus titre per dose.

Canine Leptospirosis Vaccine, Inactivated

Canine Leptospirosis Vaccine (Inactivated) is a suspension of inactivated whole organisms and/or antigenic extract(s) of one or more suitable strains of one or more of *Leptospira interrogans* serovar canicola, serovar *icterohaemorrhagiae* or any other epidemiologically appropriate serovar, inactivated and prepared in such a way that adequate immunogenicity is maintained.

Production

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial harvests are collected separately and inactivated by a suitable method. The antigen may be concentrated. The vaccine may contain an adjuvant.

Inactivation

Carry out a test for inactivation by inoculation on to a specific medium. Inoculate 1 ml of the vaccine into 100 ml of the medium. Incubate at 30° for 14 days, subculture into a further quantity of the medium and incubate both media at 30° for 14 days: no growth occurs in either medium. At the same time, carry out a control test by inoculating a further quantity of the medium with the vaccine together with a quantity of a culture containing approximately 100 leptospirae and incubating at 30° Growth of leptospirae occurs within 14 days.

Identification

When administered to experimental animals causes the appearance of agglutinating antibodies against the serotype or serotypes used to prepare the vaccine.

Tests

Safety. Use two dogs of the minimum age recommended for vaccination and which do not have antibodies to the leptospira serovar(s) present in the vaccine. Administer 2 doses of the vaccine to each dog by a recommended route. Observe the animals for 14 days. The animals remain in good health and no abnormal local or systemic reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a separate potency test for each serotype if the vaccine is prepared with different serotypes. Inject each of five hamsters not more than 3 months old, the animals being drawn from the same stock, subcutaneously with 1/40 of the dose of the vaccine stated on the label for dogs. Use an equal number of animals of the species used for the test as controls. After 15 to 20 days inject intraperitoneally into each of the vaccinated and control animals an adequate dose of a virulent culture of leptospirae of the serotype used to prepare the vaccine or a suspension of liver or kidney tissue obtained from animals infected with the serotype used to prepare the vaccine. Observe the animals for 14 days after the injection. Not less than four of the control animals die showing typical leptospira infection. Not less than four of the vaccinated animals remain in good health for not less than 14 days after the death of the four control animals.

Labelling. The label states (1) the strain used for the preparation; (2) the name of any added adjuvant.

Canine Parainfluenza Virus Vaccine, Live

Canine Parainfluenza Virus Vaccine, Live is a freeze-dried preparation of tissue culture fluid containing the cell culture-adapted attenuated canine parainfluenza virus of stock seed which has been established as pure, safe and immunogenic.

Production

The virus is propagated in suitable cell culture systems, the viral suspension is harvested, titrated and may contain a suitable stabilizing solution.

Identification

When inoculated into dogs, the vaccine stimulates the production of specific neutralizing antibodies against canine parainfluenza virus as determined by suitable serological tests.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Virus titre. Not less than 10^3 $TCID_{50}$ per dose, determining the titre of the vaccine in a suitable cell culture.

Safety. Inject each of two susceptible dogs, between 8 and 14 weeks old, free from canine parainfluenza virus haemagglutinating antibodies with a dose of the vaccine reconstituted with the sterile diluent equivalent to 10 times the dose and by the route stated on the label. Observe the animals for 14 days. None of the dogs shows any systemic or local reactions.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Use two healthy susceptible dogs, between 8 and 14 weeks old, having haemagglutination inhibition antibody titres less than 1:4 per 0.1 ml of serum. Keep equal number of dogs as unvaccinated controls. Vaccinate each test group dog with the vaccine as per schedule stated on the label. After 21 days, collect the serum from each dog separately and examine each sample as described below. Heat the serum of each animal at 56° for 30 minutes and prepare serial dilutions with saline solution. To 0.025 ml of each dilution add 0.025 ml of a canine parainfluenza virus suspension containing 4 haemagglutinating units. Allow the mixture to stand at room temperature for 30 minutes and add 0.05 ml of a suspension of chicken erythrocytes containing 2 × 107 erythrocytes per ml. Allow to stand for 1 hour and note the last dilution of serum that completely inhibits haemugglutination. Calculate the median antibody litre of the sera which should not be less than 1:40 per 0.025 ml of the serum. If one dog fails to respond, repeat the test using two more dogs and calculate the result as the mean of titres obtained from all of three dogs that have responded which is not less than 1:40 per 0.025 ml of the serum.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine it may be omitted as a routine test during production on the other batches of vaccine prepared from the same seed lot.

Labelling. The label states (1) the strain used for the preparation; (2) the virus titre per dose.

Canine Parvovirus Vaccine, Inactivated

Canine Parvovirus Vaccine, Inactivated is a liquid or freeze dried preparation of canine parvovirus inactivated by a suitable method so that its immunogenic activity is retained.

Production

The virus is grown in suitable cell culture systems. The virus may be cloned, purified and concentrated. The vaccine may contain a suitable adjuvant.

Identification

When inoculated into dogs, the development of specific neutralizing antibodies against canine parvovirus can be demonstrated by suitable serological tests.

Tests

Safety. Inject into each of two healthy susceptible dogs, between 8 and 14 weeks old, preferably sero negative ones with a quantity equivalent to 2 doses by the route stated on the label. Observe the animals for 14 days. No abnormal systemic or local reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either of the test A or B may be carried out.

A. Inject subcutaneously each of five healthy susceptible guinea-pigs, each weighing between 350 and 400g, with half of the dose stated on the label. After 14 days, inject again half of the dose stated on the label. Fourteen days after the second injection collect blood samples and obtain the serum from each guinea pig separately. Inactivate each serum by heating at 56 ° for 30 min. To 1 volume of each serum add 9 volumes of 20 per cent suspension of *light kaolin* in *phosphate buffered saline pH 7.4*. Shake the mixture for 20 minutes and centrifuge at 2,000 rpm for 10 minutes. Collect the supernatant liquid and mix with 1 volume of a *concentrated suspension of Rhesus monkey/pig erythrocytes*. Allow the mixture to stand at 4° for 60 minutes and centrifuge at 2,000 rpm for 10 minutes and collect the supernatant serum obtained in 10-fold dilution. Using each serum, prepare a series of 2-fold dilutions. To 0.025 ml of each of the latter dilutions add 0.025 ml of a suspension of canine parvovirus antigen containing approximately 8 haemagglutinating (HA) units and allow to stand at 37° for 30 minutes. Add 0.05 ml or other suitable quantity of a 1 per cent suspension of Rhesus monkey/pig erythrocytes containing 30×10^6 cells per ml to at least four replicates of each dilution. Allow to stand at 4° for 90 minutes and note the last dilution of the serum that completely inhibits haemagglutination. The vaccine complies with the test if the median antibody titre of the sera is not less than 1:80.

B. Inject each of two healthy susceptible dogs, between 8 and 14 weeks old, having antibody titres less than 4 ND_{50} (50 percent neutralizing dose) per 0.1 ml of serum, with the dose and by the route recommended on the label. Fourteen days later collect the blood/serum samples from each dog separately. Inactivate each serum by heating at 56° for 30 min. Examine the serum samples individually for neutralizing antibodies as follows:

Prepare 2-fold serial dilutions of serum in a medium suitable for canine cells. Add to each dilution an equal volume of virus suspension containing approximately 10^4 $TCID_{50}$ and incubate the mixtures at 37° for one hour. Inoculate a suitable volume of canine cells into at least four replicates of serum virus mixtures and incubate at 37° for 7 days. Examine for evidence of specific cytopathic effects and calculate the antibody titre. The vaccine complies with the test if the mean antibody titre is not less than 32 ND_{50} per 0.1 ml of serum. If one dog fails to respond repeat the test using two more dogs and calculate the mean titres of the three dogs that have responded.

Labelling. The label states (1) the method of preparation; (2) the types and strains of virus used to prepare the vaccine.

Canine Parvovirus Vaccine, Live

Canine Parvovirus Vaccine, Live is a freeze-dried preparation of a strain of canine parvovirus that is attenuated for the target species of dogs.

Production

The attenuated virus is grown in suitable cell culture systems. The viral harvest is titrated and mixed with a suitable stabilizing solution. The stock seed virus should be tested for immunogenicity at least once in 5 years, if maintained under suitable conditions of storage.

Identification

When inoculated into dogs, the development of specific neutralizing antibodies against canine parvovirus can be demonstrated by suitable serological tests.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Virus titre. Not less than 128 HA units per dose when tested using suitable RBC after culturing the virus in a suitable cell culture.

Safety. Inject each of two susceptible dogs, between 8 and 14 weeks old, free from canine parvovirus hemagglutinating antibodies, a quantity of the vaccine reconstituted with the sterile diluent equivalent to 10 times the dose and by the route stated on the label. Observe the animals for 21 days. No abnormal systemic or local reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of seven dogs, between 8 and 14 weeks old, free from canine parvovirus haemagglutinating antibodies, subcutaneously with the minimum dose stated on the label. Use two dogs of the same stock, weight and age range as controls. Not less than 21 days after injection of the vaccine, challenge the vaccinated and control animals through the oronasal route with a virulent strain of infectious canine parvovirus. Observe the animals for 14 days. Not less than five of the vaccinated dogs survive. The test is not valid unless the control dogs die or show clinical signs of canine parvovirus infection.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine it may be omitted as a routine test during production of the other batches of vaccine prepared from the same seed lot.

Labelling. The label states (1) the strain used for the preparation; (2) virus titre per dose.

Multicomponent Clostridium Vaccine, Inactivated

Multicomponent Clostridium Vaccine, Inactivated consists of five highly antigenic components containing the toxoids of *C. perfringens* type 'B' *C. perfringens* type 'C', *C. perfringens* type 'D', *C. oedematiens* and *C. septicum* which are prepared in double strength and then combined in such a proportion that would invoke adequate anti-toxin response in the vaccinated sheep against each antigen incorporated in the vaccine.

Identification

When injected into susceptible animals, it stimulates the production of epsilon and beta antitoxin against *C. perfringens* types 'B', 'C' and 'D' and also antitoxins against *C. septicum* and toxin of *C. oedematiens.*

Tests

Safety. Four sheep each are inoculated with two times the dose of vaccine subcutaneously and are observed for 7 days during which period the animals do not show any local or systemic reaction.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Eight sheep each are inoculated with 2 doses of vaccine subcutaneously at an interval of 21 to 28 days and bled on 10th day after second inoculation for collection of

serum for assessing the antitoxin titre against each antigen incorporated in the vaccine. The post-inoculation serum contains not less than 5 IU of epsilon antitoxin and 10 units of beta antitoxins of *C. perfringens types 'B' and 'C'* and 2.5 IU of *C. septicum* antitoxin and 3.5 IU of *C. oedematiens* antitoxin.

Labelling. The label states (1) the types of Clostridia contained in the vaccine; (2) the preparation should be shaken before use.

Clostridium novyi (Type B) Vaccine Inactivated for Veterinary Use

Clostridium novyi (Type B) Vaccine Inactivated for Veterinary Use is prepared from a liquid culture of a suitable strain of *Clostridium novyi* Type B.

Production

The whole culture or its filtrate or a mixture of the two is inactivated in such a manner that toxicity is eliminated and immunogenic activity is retained. Toxoids and/or inactivated cultures may be treated with a suitable adjuvant, after concentration if necessary.

Choice of vaccine composition. The vaccine is shown to be satisfactory with respect to safety and efficacy (2.7.12). For the latter, it shall be demonstrated that for each target species the vaccine, when administered according to the recommended schedule, stimulates an immune response (for example, induction of antibodies) consistent with the claims made for the product.

Batch testing

Safety. Administer by a recommended route, to each of 2 sheep that have not been vaccinated against *C. novyi* Type B twice the maximum dose of the vaccine stated on the label. Observe the animals for not less than 14 days. No abnormal local or systemic reaction occurs.

Residual toxicity. Inject 0.5 ml of the vaccine subcutaneously into each of 5 mice, each weighing between 17 and 22 g. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

Identification

The vaccine stimulates the formation of *C. novyi* Type B alpha antitoxin when injected into animals.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject subcutaneously into each of not less than 10 healthy rabbits, 3 to 6 months old, a quantity of vaccine not exceeding the minimum dose stated on the label as the first dose. After 21 to 28 days, inject into the same animals a quantity of the vaccine not exceeding the minimum dose stated on the label as the second dose. 10 to 14 days after the second injection, bleed the rabbits and pool the sera.

The alpha antitoxin titre of the pooled sera is not less than 3.5 IU per ml.

The International Unit is the specific neutralising activity for *C. novyi* alpha toxin contained in a stated amount of the International standard, which consists of a quantity of dried immune horse serum. The equivalence in International Units of the International standard is stated by the World Health Organisation.

The potency of the pooled sera obtained from the rabbits is determined by comparing the quantity necessary to protect mice or other suitable animals against the toxic effects of a fixed dose of *C. novyi* alpha toxin with the quantity of a reference preparation of *Clostridium novyi* alpha antitoxin, calibrated in International Units, necessary to give the same protection. For this comparison, a suitable preparation of *C. novyi* alpha toxin for use as a test toxin is required. The dose of the test toxin is determined in relation to the reference preparation; the potency of the serum under examination is determined in relation to the reference preparation using the test toxin.

Preparation of test toxin. Prepare the test toxin from a sterile filtrate of an approximately 3 to 5 day culture in liquid medium of *C. novyi* Type B and dry by a suitable method. Select the test dose of the toxin in mice by determining the L+ 10 dose and the LD_{50} the observation period being 72 hours.

A suitable alpha test toxin contains not less than one L+/10 dose in 0.05 mg and not less than 10 LD_{50} in each L+/10 dose.

Determination of test dose of toxin. Prepare a solution of the reference preparation in a suitable liquid so that it contains 1 IU per ml. Prepare a solution of the test toxin in a suitable liquid so that 1 ml contains a precisely known amount such as 1 mg. Prepare mixtures of the solution of the reference preparation and the solution of the test toxin such that each mixture contains 1.0 ml of the solution of the reference preparation (1 IU), one of a series of graded volumes of the solution of the test toxin and sufficient of a suitable liquid to bring the total volume to 2.0 ml. Allow the mixtures to stand at room temperature for 60 min. Using not less than 2 mice, each weighing between 17 and 22 g, for each mixture, inject a dose of 0.2 ml subcutaneously into each mouse. Observe the mice for 72 hours. If all the mice die, the amount of toxin present in 0.2 ml of the mixture is in excess of the test dose. If none of the mice dies, the amount of toxin present in 0.2 ml of the mixture is less than the test dose. Prepare fresh mixtures such that

2.0 ml of each mixture contains 1.0 ml of the solution of the reference preparation (1 IU) and one of a series of graded volumes of the solution of the test toxin separated from each other by steps of not more than 20 per cent and covering the expected end-point. Allow the mixtures to stand at room temperature for 60 min. Using not less than two mice for each mixture, inject a dose of 0.2 ml subcutaneously into each mouse. Observe the mice for 72 hours. Repeat the determination at least once and combine the results of the separate tests that have been made with mixtures of the same composition so that a series of totals is obtained, each total representing the mortality due to a mixture of a given composition.

The test dose of toxin is the amount present in 0.2 ml of that mixture which causes the death of one half of the total number of mice injected with it.

Determination of the potency of the serum obtained from rabbits

Preliminary test. Dissolve a quantity of the test toxin in a suitable liquid so that 1 ml contains 10 times the test dose (solution of the test toxin). Prepare a series of mixtures of the solution of the test toxin and of the serum under examination such that each mixture contains 1.0 ml of the solution of the test toxin, one of a series of graded volumes of the serum under examination and sufficient of a suitable liquid to bring the final volume to 2.0 ml. Allow the mixtures to stand at room temperature for 60 min. Using not less than 2 mice for each mixture, inject a dose of 0.2 ml subcutaneously into each mouse. Observe the mice for 72 h. If none of the mice dies, 0.2 ml of the mixture contains more than 0.1 IU. If all the mice die, 0.2 ml of the mixture contains less than 0.1 IU.

Final test. Prepare a series of mixtures of the solution of the test toxin and of the serum under examination such that 2.0 ml of each mixture contains 1.0 ml of the solution of the test toxin and one of a series of graded volumes of the serum under examination, separated from each other by steps of not more than 20 per cent and covering the expected end-point as determined by the preliminary test. Prepare further mixtures of the solution of the test toxin and of the solution of the reference preparation such that 2.0 ml of each mixture contains 1.0 ml of the solution of the test toxin and one of a series of graded volumes of the solution of the reference preparation, in order to confirm the test dose of the toxin. Allow the mixtures to stand at room temperature for 60 min. Using not less than 2 mice for each mixture, proceed as described in the preliminary test. The test mixture which contains 0.1 IU in 0.2 ml is that mixture which kills the same or almost the same number of mice as the reference mixture containing 0.1 IU in 0.2 ml. Repeat the determination at least once and calculate the average of all valid estimates. The test is valid only if the reference preparation gives a result within 20 per cent of the expected value.

The confidence limits (P = 0.95) have been estimated to be (a) 85 per cent and 114 per cent when 2 animals per dose are used; (b) 91.5 per cent and 109 per cent when 4 animals per dose are used; (c) 93 per cent and 108 per cent when 6 animals per dose are used.

Labelling. The label states (1) whether the product is a toxoid, a vaccine prepared from a whole inactivated culture or a mixture of the two; (2) that the preparation is to be shaken before use; (3) for each target species, the immunising effect produced (for example, antibody production, protection against signs of disease or infection).

Clostridium Perfringens Vaccine, Inactivated

The vaccines contains inactivated cultures of strains of *C. perfringens* type B (Lamb Dysentery Vaccine), type C (Struck Vaccine) or type D (Enterotoxaemia Vaccine; Pulpy Kidney Vaccine) or any combination of these types.

Production

The organisms grown in an anaerobic medium, the whole culture or their filtrates or a mixture of the two are inactivated in such a manner that toxicity is eliminated and immunogenic activity is retained. Toxoid and or inactivated cultures may contain a suitable adjuvant.

Batch tesing

Safety. Inject subcutaneously two healthy susceptible sheep weighing about 18 kg each or two healthy susceptible rabbits weighing between 1.5 and 2.0 kg each with twice the dose stated on the label and observe the animals for 14 days.

Observe the animals for 14 days. No abnormal local or systemic reaction occurs.

Residual toxicity. Inject 0.5 ml of the vaccine subcutaneously into each of 5 mice, each weighing 17 to 22 g. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency

C. perfringens Type B Vaccine. Inject subcutaneously into each of six healthy susceptible sheep weighing about 18 kg or ten healthy susceptible rabbits weighing between 1.5 and 2.0 kg with the minimum dose of the vaccine stated on the label. Repeat the dose after an interval of 21 to 28 days. Bleed the animals 10 to 14 days after the second dose of the vaccine and determine beta antitoxin titres in the pooled serum sample by

testing in mice as per the method described for *C. perfringens* Type D Vaccine. Product passes the test if the post-inoculation pooled serum contains not less than 10 IU of beta antitoxins, and not less than 5 IU of epsilon antitoxin per milliliter.

C. perfringens Type C Vaccine. Carry out the test for potency as described for *C. perfringens* Type B Vaccine.

1 ml of pooled serum contains not less than 10 IU of beta antitoxin per ml.

C. perfringens Type D (Enterotoxaemia) Vaccine. Carry out the test as described below.

1 ml of pooled serum contains not less than 5 IU of *C. perfringens* epsilon antitoxin per ml.

Identification

A. When injected into susceptible animals, the *C. perfringens* Type B Vaccine stimulates the production of *C. perfringens* beta and epsilon antitoxins.

B. When injected into susceptible animals, the *C. perfringens* Type C Vaccine stimulates the production of *C. perfringens* beta antitoxin.

C. When injected into susceptible animals, the *C. perfringens* Type D Vaccine stimulates the production of *C. perfringens* epsilon antitoxin.

Tests

Potency test. Inject subcutaneously into each of at least six healthy susceptible sheep weighing about 18 kg or ten healthy susceptible rabbits weighing between 2.0 and 2.5 kg with the minimum dose of the vaccine stated on the label. Repeat the dose in each sheep/rabbit after an interval of 21 to 28 days. Bleed the animals 10 to 14 days after the second inoculation. The sera of sheep or rabbits are pooled separately and estimated for the antitoxin levels.

Biological assay of *C. perfringens* antitoxins

The potency of *C. perfringens* beta and epsilon antitoxins is determined by comparing the dose of antitoxin necessary to protect mice or other suitable animals against the toxic effects of *C. perfringens* beta toxin or epsilon toxin with the dose of a standard preparation of the respective antitoxin necessary to give the same protection. For this comparison, the standard preparations of *C. perfringens* beta antitoxin and *C. perfringens* epsilon antitoxin and suitable preparations of *C. perfringens* beta and epsilon toxins are required.

The test dose of each toxin is established in relation to the appropriate Standard preparation of antitoxin and the potency of antitoxin under examination is then determined in relation to the appropriate Standard preparation using the appropriate test toxin.

International standard for the standard preparations

The International units of the antitoxin is the specific neutralizing activity of *C. perfringens* epsilon toxin contained in the stated amount in relation to International standards in the dried Horse serum.

The International units of the antitoxins is the specific neutralizing activity for the *C. perfringens* beta toxin contained in the stated amount in relation to International standards in the dried Horse serum.

Test animals

Use healthy mice having body weights such that the difference between the lightest and heaviest is not more than 5 g.

Suggested method for preparation of test toxin. Prepare *C. perfringens* toxins from sterile supernatants/filtrates of early cultures of *C. perfringens* type B, type C or type D. The supernatants may be purified by precipitation with *ammonium sulphate* and the resulting precipitate collected. This may then be dried over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, powdered and kept dry or re-dissolved and freeze-dried.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities.

L+ and L+/10 doses. These are the smallest quantities of toxin that when mixed respectively with 1 Unit of antitoxin and with 0.1 Unit of antitoxin and injected intravenously into mice cause the death of the animals within 72 hours.

LD_{50}. This is the quantity of toxin that when injected intravenously into mice causes the death of one-half of the mice injected within 72 hours.

A suitable *C. perfringens* beta toxin is one that has an L+ dose in 0.2 mg or less and contains not less than 25 LD_{50} in an L+ dose.

A suitable *Clostridium perfringens* epsilon toxin is one that has an L+/10 dose in 0.005 mg or less and contains not less than 20 LD_{50} in an L+/10 dose.

Determination of test dose of *C. perfringens* beta toxin. Dissolve a quantity of dried toxin in a suitable liquid such that 1.0 ml contains a precise amount such as 10 mg. Reconstitute the standard preparation of *C. perfringens* beta antitoxin with a suitable liquid to give a solution containing 5 Units of *C. perfringens* beta antitoxin in 1 ml.

Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard preparation (10 Units) and one of a series of graded volumes of the solution of the toxin. Dilute each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 30 minutes and then inject a dose of 0.5 ml of each mixture intravenously into each of not less than

two mice. Observe the mice for 72 hours. If all the mice die, the amount of toxin present in 0.5 ml of the mixture is in excess of the test dose. If none of the mice dies, the amount of toxin present in 0.5 ml of the mixture is less than the test dose. Prepare similar fresh mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard preparation (10 Units) and one of a series of graded volumes of the solution of the toxin, separated from each other by steps of not more than 20 per cent and covering the expected end-point.

Allow the mixtures to stand at room temperature, protected from light, for 30 minutes, Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice. Observe the mice for 72 hours. Repeat the determinations at least once and add together the results of the separate tests that have been made with mixtures of the same composition such that a series of totals is obtained, each total representing the mortality due to a mixture of a given composition.

The test dose of toxin is the amount present in 0.5 ml of that mixture that causes the death of one-half of the total number of mice injected with it within 72 hours.

Determination of test dose of *C. perfringens* epsilon toxin. Carry out the method described for the determination of test dose of *C. perfringens* beta toxin with the following modification. Dissolve a quantity of dried toxin in a suitable liquid such that 1.0 ml contains a precise amount such as 1 mg.

Reconstitute the Standard preparation of *C. perfringens* epsilon antitoxin with a suitable liquid to give a solution containing 0.5 Unit in 1 ml (the prepared mixtures will therefore contain 1 Unit of the Standard preparation in 5 ml).

The test dose of toxin is the amount present in 0.5 ml of that mixture that causes the death of one-half of the total number of mice injected with it within 72 hours.

Determination of potency of *C. perfringens* beta antitoxin

Preliminary test. Dilute the test toxin with a suitable liquid such that 2.0 ml contains 10 times the test dose. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the toxin and one of a series of graded volumes of the preparation under examination. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 30 minutes. Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice and observe the mice for 72 hours. If all the mice die, 0.5 ml of the mixture contains less than 1 Unit of antitoxin. If none of the mice dies, 0.5 ml of the mixture contains more than 1 Unit of antitoxin.

Final test. Prepare similar fresh mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the toxin and one of a series of graded volumes of the preparation under examination, separated from each other by steps of not more than 20 per cent and covering the expected end-point. Prepare further mixtures of 5.0 ml containing 2.0 ml of the solution of the toxin and graded volumes of the Standard preparation of *C. perfringens* beta antitoxin to confirm the test dose of the toxin. Allow the mixtures to stand at room temperature, protected from light, for 30 minutes. Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice and observe the mice for 72 hours. The mixture of the antitoxin under examination which contains 1 Unit of *C. perfringens* beta antitoxin in 0.5 ml is that mixture which causes the death of the same or almost the same number of mice as the mixture containing 1 Unit of the Standard preparation of *C. perfringens* beta antitoxin in 0.5 ml. Repeat the determinations at least once and calculate the average of all valid estimates. Estimates are not valid unless the Standard preparation gives a result within 20 per cent of the expected value.

Determination of potency of *C. perfringens* epsilon antitoxin. Carry out the preliminary test and final test as described for the determination of potency of *C. perfringens* beta antitoxin with the following amendments.

Dilute a quantity of the test toxin in a suitable liquid such that 2.0 ml contains 10 times the test dose. The Standard preparation used in these tests is that of *C. perfringens* epsilon antitoxin.

The mixture of the antitoxin under examination which contains 0.1 Unit of *C. perfringens* epsilon antitoxin in 0.5 ml is that mixture which causes the death of the same or almost the same number of mice as the mixture containing 0.1 Unit of the Standard preparation of *C. perfringens* epsilon antitoxin in 0.5 ml.

Limits of error. For the suggested method, the limits of error (P = 0.95) have been estimated to be 85 to 114 per cent when two mice are used per dose, 91.5 to 109 per cent when four mice are used per dose, and 93 to 108 per cent when six mice are used per dose.

Labelling. The label states (a) the type or types of *C. perfringens* from which the vaccine has been prepared; (b) whether the preparation is a toxoid or a vaccine prepared from a whole inactivated culture or a mixture of the two; (c) that the preparation is to be shaken before use; (d) for each target species, the immunising effect produced (for example, antibody production, protection against signs of disease or infection).

Clostridium Septicum Vaccine, Inactivated

Clostridium Septicum Vaccine, Inactivated is a suspension of a culture of a highly toxigenic strain of *C. septicum* grown in an anaerobic medium, or a filtrate from such a culture.

Production

The whole culture or its filtrate or a mixture of the two is inactivated in such a manner that toxicity is eliminated and immunogenic activity is retained. Toxoid and/or inactivated cultures may be treated with a suitable adjuvant.

Batch testing

Residual toxicity. Inject 0.5 ml of the vaccine subcutaneously into each of 5 mice, each weighing between 17 and 22 g. Observe the animals for 7 days. No abnormal local or systemic reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject subcutaneously each of eight healthy susceptible sheep, between 8 and 12 months old, or ten rabbits, between 3 and 6 months old, with the minimum dose of the vaccine stated on the label. Repeat the dose after an interval of 21 to 28 days. 10 to 14 days after the second inoculation, bleed the animals. Pool the sera samples from individual animals and determine the antitoxin titre by the biological assay of *C. septicum* antitoxin described below.

1 ml of serum contains not less than 2.5 Units of *C. septicum* antitoxin by biological assay of *C. septicum* antitoxin.

The potency of *C. septicum* antitoxin is determined by comparing the dose of antitoxin necessary to protect mice or other suitable animals against the lethal effects of *C. septicum* toxin with the dose of a Standard preparation of *C. septicum* antitoxin necessary to give the same protection. For this purpose, the Standard preparation of *C. septicum* antitoxin and a suitable preparation of *C. septicum* toxin for use as a test toxin are required.

Identification

When injected into healthy susceptible animals, it stimulates the production of antitoxins to *C. septicum*.

Tests

The test dose of the toxin is determined in relation to the Standard preparation of antitoxin and the potency of antitoxin under examination is then determined in relation to the Standard preparation using the test toxin.

Assay

Standard preparation

The Standard preparation is the 3rd International standard, established in 1957, consisting of dried hyperimmune horse serum (supplied in ampoules containing 500 Units) or another suitable preparation the potency of which has been determined in relation to the International standard.

Safety. Inject subcutaneously each of two healthy susceptible sheep, between 8 and 12 months old, with twice the dose stated on the label. Observe the animals for 7 days; none of the animals shows any systemic or local reaction. Observe the animals for 14 days.

Test animals. Use healthy mice having body weights such that the difference between the lightest and heaviest is not more than 5 g.

Preparation of test toxin. Prepare *C. septicum* toxin by growing *C. septicum* in a liquid culture medium, filtering the supernatant aseptically and precipitating with *ammonium sulphate*. The resulting precipitate, which contains the toxin, is collected, dried over *phosphorus pentoxide* at a pressure of 1.5 to 2.5 kPa, powdered and kept dry.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities.

L+/5 dose. This is the smallest quantity of the toxin which when mixed with 0.2 Unit of antitoxin and injected intravenously into mice causes the death of the animals within 72 hours.

LD_{50}. This is the quantity of toxin which when injected intravenously into mice causes the death of one-half of the animals within 72 hours.

A suitable *C. septicum* toxin is one that has an L+/5 dose in 1 mg or less and contains not less than 10 LD_{50} in an L+/5 dose.

Determination of test dose of toxin. Weigh accurately a quantity of the dried toxin and dissolve it in a suitable liquid so that 1.0 ml contains a precise known amount, such as 4 mg. Prepare a solution of the Standard preparation in a suitable liquid such that 1.0 ml contains 1 Unit.

Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard preparation (2 Units) and one of a series of graded volumes of the solution of the toxin. Dilute each mixture with a suitable liquid to the same final volume (5.0 ml). Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject a dose of 0.5 ml of each mixture intravenously into each of not less than 2 mice. Observe the mice for 72 hours. If all the mice die the amount of toxin present in 0.5 ml of the mixture is in excess of the test dose. If none of the mice dies, the amount of toxin present in 0.5 ml of the mixture is less than the test dose. Prepare similar fresh mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the Standard preparation (2 Units) and one of a series of graded volumes of the solution of the toxin separated from each other by steps of not more than 20 per cent and covering the expected end-point.

Allow the mixtures to stand at room temperature, protected from light, for 60 minutes. Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice. Observe the mice for 72 hours. Repeat the determinations at least once

and add together the results of the separate tests that have been made with mixtures of the same composition such that a series of totals is obtained, each total representing the mortality due to a mixture of a given composition.

The test dose of toxin is the amount present in 0.5 ml of that mixture that causes the death of one-half of the total number of mice injected within 72 hours.

Determination of potency of the antitoxin

Preliminary test. Dilute the test toxin with a suitable liquid such that 2.0 ml contains 10 times the test dose. Prepare mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the toxin and one of a series of graded volumes of the preparation under examination. Adjust each mixture to the same final volume with a suitable liquid.

Allow the mixtures to stand at room temperature, protected from light, for 60 minutes. Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice and observe the mice for 72 hours. If all the mice die, 0.5 ml of the mixture contains less than 0.2 Unit of antitoxin. If none of the mice dies, 0.5 ml of the mixture contains more than 0.2 Unit of antitoxin.

Final test. Prepare similar fresh mixtures such that 5.0 ml of each mixture contains 2.0 ml of the solution of the toxin and one of a series of graded volumes of the preparation under examination, separated from each other by steps of not more than 20 per cent and covering the expected end-point. Prepare further mixtures of 5.0 ml containing 2.0 ml of the solution of the toxin and graded volumes of the standard preparation to confirm the test dose of the toxin.

Allow the mixtures to stand at room temperature, protected from light, for 60 minutes. Inject a dose of 0.5 ml of each mixture intravenously into each of not less than two mice and observe the mice for 72 hours. The mixture of the antitoxin under examination which contains 0.2 Unit in 0.5 ml is that mixture which causes the death of the same or almost the same number of mice as the mixture containing 0.2 Unit of the Standard preparation in 0.5 ml. Repeat the determinations at least once and calculate the average of all valid estimates. Estimates are not valid unless the Standard preparation gives a result within 20 per cent of the expected value.

Limits of error. For the suggested method, the limits of error (P = 0.95) have been estimated to be (a) 85 per cent and 114 per cent when 2 animals per dose are used; (b) 91.5 per cent and 109 per cent when 4 animals per dose are used; (c) 93 per cent and 108 per cent when 6 animals per dose are used.

The vaccine passes the test if the pooled serum contains 2.5 IU of *C. septicum* antitoxins.

Labelling. The label states (1) whether the preparation is a toxoid or a vaccine prepared from a whole inactivated culture, or a mixture of the two; (2) that the preparation is to be shaken before use; (3) for each target species, the immunising effect produced (for example, antibody production, protection against signs of disease or infection).

Duck Pasteurella Vaccine, Inactivated

Duck Pasteurella Vaccine, Inactivated consists of an emulsion or suspension of a virulent strain of *Pasteurella multocida* which has been inactivated in such a manner that the pathogenicity is eliminated and the immunogenic activity is retained.

Identification

Protects susceptible ducks against infection with *P. multocida.*

Tests

Safety. Either test A or test B may be carried out.

A. Inject 5 ml subcutaneously into each of four healthy rabbits, weighing between 1.0 and 1.5 kg. Observe the animals for 7 days. No untoward reaction except slight and transient local swelling occurs.

B. Inject 5 ml subcutaneously into each of two healthy rabbits, each weighing between 1.0 and 1.5 kg, and 0.5 ml subcutaneously into each of six mice, each weighing between 25 and 30 g. Observe the animals for 7 days. No untoward reaction except slight and transient local swelling occurs in both species of animals.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either test A or test B may be carried out.

A. Inject subcutaneously with the minimum dose of the vaccine stated on the label three healthy susceptible ducks, between 4 and 6 weeks old. Use another two ducks of the same stock and age as unvaccinated controls. Three weeks later, challenge each of the vaccinated and control ducks, subcutaneously with 10^2 mouse LD_{50} viable organisms in 0.2 ml of a suitably diluted 18-hour broth culture of the homologous virulent strain of *P. multocida*. Observe the ducks for 7 days. Not less than two of the vaccinated ducks remain in normal health and both the controls die of pasteurellosis.

B. Inject subcutaneously each of six mice, each weighing between 25 and 30 g, with 0.2 ml of the vaccine under examination. Use another six mice of the same stock and weight range as unvaccinated controls. Three weeks later, challenge each of the vaccinated and control mice subcutaneously with 0.2 ml of a suitably diluted 18-hour broth culture of the homologous virulent strain of *P. multocida* containing 50 mouse LD_{50} viable organisms. Observe the animals for 7 days.

All the vaccinated mice survive. The test is not valid unless all the control mice die of pasteurellosis during the observation period.

Labelling. The label states (1) the type of strain; (2) the recommended age for vaccination.

Duck Plague Vaccine, Live

Duck Plague Vaccine, Live is a preparation of attenuated strain of duck plague virus. This monograph applies to vaccines intended for administration to duck for active immunisation against duck plague disease.

Production

The vaccine virus is grown in SPF eggs (2.7.7) or in cell cultures. The master seed lot complies with the tests for extreneous agents in seed lot (2.7.10).

Substrate for virus propagation

The vaccine virus is grown in embryonated hens' eggs or in cell cultures obtained from flocks free from specified pathogens SPF (2.7.7). If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines. The vaccine virus is filled with suitable stabilizing agent and freeze dried.

Identification

Protects ducks against duck plague.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Safety. Inject subcutaneously each of four healthy susceptible ducks, between 8 and 12 weeks old and each weighing not less than 600 g, with 1 ml of a 1 : 10 dilution of the reconstituted vaccine. Observe the ducks for 14 days. None of the ducks shows any untoward reaction.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject subcutaneously each of four healthy susceptible ducks, between 8 and 12 weeks old and each weighing not less than 600 g, with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum dose stated on the label. Fourteen days later, challenge each of the vaccinated ducks and each of two control ducks of the same stock and weight range, subcutaneously with 10^2 ID_{50} of virulent duck plague virus. Observe the ducks for 14 days. None of the vaccinated ducks dies or shows any clinical symptoms of plague. The test is not valid unless the control ducks die from duck plague or show typical signs of serious infection during the observation period.

If potancy test has been performed with satisfactoery results on a representative batch of the vaccine, it may be omitted as a vaccine test during production on the other batches of vaccine prepared from the same seed lot.

Labelling. The label states (1) the minimum virus titre per dose; (2) the recommended age of the birds in which the vaccine is to be used.

Egg Drop Syndrome'76 (Adenovirus) Vaccine, Inactivated

Egg Drop Syndorme'76 (Adenovirus) Vaccine,

Egg Drop Syndrome'76 (Adenovirus) Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of egg drop syndrome'76 virus (haemagglutinating avian adenovirus) which has been inactivated in such a manner that immunogenic activity is retained.

Production

The vaccine strain is propagated in embryonated duck eggs from healthy flocks or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Test for inactivation

The test for inactivation is carried out in fertilized duck eggs from a flock free from egg drop syndrome '76 virus infection or hen eggs from a flock free from specified pathogens, or in suitable cell culture derived from SPF eggs (2.7.7), whichever is the most sensitive for the vaccine strain; the quantity of virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

The vaccine may contain a suitable adjuvant.

Identification

When inoculated into chicken, the development of specific neutralizing antibodies against egg drop syndrome '76 (adenovirus) can be demonstrated by suitable serological tests.

Tests

Safety. Inject each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens between 2 and 4 weeks old, with two doses and by the route stated on the label. Observe the chicken for 14 days. None of the chicken shows any abnormal local or systemic reaction.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of twenty SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the dose and by the route stated on the label. After 21 days, collect serum samples from each of the birds as well as ten-control chickens of the same stock and perform haemagglutination inhibition test on each serum using 4 haemagglutinating units of antigen and chicken erythrocytes. The vaccine passes the potency test if the mean antibody titre of the vaccinated group is greater than 1:128. The test is valid only if no specific antibody is found in the control chicken.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/Insert states (1) the strain used for the preparation; (2) the route of administration.

Foot-and-Mouth Disease Vaccine, Inactivated

Foot-and-Mouth Disease Vaccine, Inactivated is a liquid preparation containing one or more types of foot-and-mouth disease virus that have been inactivated in such a manner that its immunogenic activity is retained. Depending on the number of types of virus incorporated, the vaccine is described as monovalent, bivalent, trivalent or polyvalent.

Production

The virus is grown in suitable cell cultures. The virus is separated from cellular material by filtration or other suitable procedures and the virus is inactivated using *binary ethyleneimine (BEI)* in suitable conditions. The antigen may be concentrated and purified. The antigen is used for the preparation of vaccine. The vaccine contains a suitable adjuvant.

Only an inactivated antigen suspension that complies with the requirements mentioned under final bulk vaccine may be used in the preparation of the final lot.

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more inactivated antigen suspensions.

During inactivation of the virus, samples should be taken at regular intervals for the purpose of monitoring the rate and linearity of the inactivation process. Virus titre in the samples is determined by inoculation into sensitive cell culture. The infectivity of the timed samples is plotted against time, and the inactivation procedure is not considered to be satisfactory unless the extrapolation indicates that there would be less than one infectious particle per 10^4 litres of liquid preparation at the end of the inactivation period.

Only a final bulk vaccine that complies with the following requirements may be used in the preparation of the final lot.

Tests

Inactivation. A proportion of each batch of bulk inactivated antigen representing at least 200 doses is tested for freedom from infectious virus by inoculation in to sensitive cell culture. A sample of inactivated antigen is concentrated to volumes adequate for inoculation into cell cultures and it must show that the concentrated antigen does not interfere with the sensitivity or reading of the assay. The sample is passaged 3 times at an interval of 24 to 48 hours and inoculated cell cultures are examined for the presence of foot-and-mouth disease virus by suitable tests. No cytopathic changes attributable to foot-and-mouth disease virus replication should be detected. If infectious foot-and-mouth disease virus is detected, the bulk antigen is rejected.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are closed so as to avoid contamination.

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use.

Identification

The serum of a foot-and-mouth disease susceptible animal that has been immunized with the vaccine neutralizes the types of virus used to prepare the vaccine, when tested by a suitable method.

Tests

Safety. Use two cattle, not less than six months old, that do not have antibodies against foot-and-mouth disease virus. Administer to each animal a double dose of the vaccine by the prescribed route of administration stated on the label. Observe the animals daily for at least 14 days. The vaccine complies with the test if no animal shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

Sterility (2.2.11). Complies with the test for sterility.

Assay. Use three groups of not less than five cattle per group, not less than 6 months old, which have never been vaccinated and are free from antibodies neutralizing the different types of foot-and-mouth disease virus in the vaccine. Vaccinate the 3 groups by the prescribed route stated on the label. Use different doses of the vaccine for each group without diluting

the vaccine. For example, if 3 ml is one dose, a 1/3 dose of vaccine would be obtained by injecting 1 ml, and a 1/10 dose would be obtained by injecting 0.3 ml. Three weeks later, challenge all the vaccinated animals and a control group of two cattle susceptible to foot-and-mouth disease, with a suspension of virus that is fully virulent and of the same type as that used for preparation of the vaccine by inoculating 10,000 ID_{50} (50 per cent bovine infectious dose) intradermally into two sites into the tongue (0.1ml per site). The challenge of oil adjuvanted vaccines is effected 28 days post vaccination. Observe the animals for 8 days and then sacrifice them. Unprotected animals show lesions at sites other than the tongue. Protected animals may display lingual lesions. The test is not valid unless control animals show lesions on at least three feet. From the number of animals protected in each group, calculate the PD_{50} content of the vaccine. The potency of the vaccine is expressed as the number of 50 per cent cattle protective doses (PD_{50}) contained in the dose stated on the label. The vaccine must contain at least 3 PD_{50} per dose for cattle.

Alternatively, percentage of protection against generalized foot infection (PGP) test can be carried out. A group of 16 cattles of six months age which have never been vaccinated and are free from antibodies neutralizing the different types of foot-and-mouth disease virus in the vaccine are vaccinated with a full vaccine dose by the route recommended by the manufacturer. These animals and a control group of two non-vaccinated animals susceptible to foot-and-mouth disease are challenged three to four weeks after vaccination with a suspension of virus that is fully virulent and of the same type as that used for preparation of the vaccine by inoculating 10,000 ID50 (50 per cent bovine infectious dose) intradermally into two sites into the tongue (0.1ml per site). Observe the animals for 8 days and then sacrifice them. Unprotected animals show lesions at sites other than the tongue. Protected animals may display lingual lesions. The test is not valid unless control animals show lesions on at least three feet. The vaccine passes the test if a minimum of 12 animals out of 16 vaccinated are protected.

Test animals shall be bled on day 0, 21 or 28 days post vaccination for screening the animals for sero-negative status and for estimation of the antibody titres post vaccination. Indirect tests, including post vaccination measurement of virus neutralizing antibodies in cell culture or ELISA antibodies, may be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the test on the relevant vaccine serotype and the potency test in cattle.

The description applies to the testing of a monovalent vaccine. The potency of polyvalent vaccines may be tested by challenging each valency as described above.

Labelling. The label states (1) the method of preparation; (2) the serotype of the virus used to prepare the vaccine.

Fowl Cholera Vaccine, Inactivated

Pasturella multocida Vaccine for Chickens

Fowl Cholera Vaccine, Inactivated is a preparation of suitable strains of 1 or more serovars of *Pasteurella multocida*. This monograph applies to vaccines intended for the active immunisation of chickens, turkeys, ducks and geese against fowl cholera infection.

Production

The seed material is inoculated in a suitable medium. If the vaccine contains more than 1 strain of bacterium, the different strains are grown and harvested separately. The bacterial harvests are inactivated with suitable agent. The vaccine may contain suitable adjuvant.

Identification

Protects susceptible chicken against infection with *P. multocida.*

Tests

Safety. Administer double dose of vaccine subcutaneously into each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens of 4 to 6 weeks age. Observe the chickens for 7 days; none of the chicken shows untoward reaction other than slight transient local swelling.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Immunize 20 SPF chickens (2.7.7, table 3) or healthy susceptible chickens 4 to 6 weeks of age per strain incorporated in the batch with one dose of vaccine. Give booster dose of vaccine 15 to 21 days after primary immunization. Keep unvaccinated healthy susceptible control birds of similar age 10 birds per strain incorporated in vaccine. Challenge the birds with an appropriate dose of virulent 18 hour old broth culture of recently bird passaged strain of *Pasteurella multocida* that shall kill at least 80 per cent of the unvaccinated susceptible chickens. Observe birds for 14 days post challenge. There should be not less than 70 per cent protection of vaccinated birds and specific mortality of at least 80 per cent in control.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) the serovar(s) used to prepare the vaccine; (2) the route of administration.

Fowl Pox Vaccine, Live

Pigeon Pox Vaccine, Live

Fowl Pox Vaccine, Live is a preparation of a suitable strain(s) of pigeon pox virus or fowl pox virus. This monograph applies to vaccines intended for administration to chickens for active immunization against avian pox virus.

Production

The vaccine virus is grown in embryonated hens' eggs from SPF flock (2.7.7) or in cell cultures derived from SPF eggs (2.7.7) or cell lines. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for virus propagation

The vaccine virus is grown either in embryonated hens' eggs from flocks free from specified pathogens SPF (2.7.7) or in avian cell cultures obtained from flocks free from specified pathogens SPF (2.7.7) or cell lines.

Identification

Carry out an immunostaining or neutralization test in cell culture derived from SPF eggs (2.7.7) to demonstrate the presence of the vaccine virus or inoculate the vaccine into eggs and notice the characteristic lesions.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Administer 10 doses of the vaccine to each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens 6 to 8 weeks old by the route stated on the label. Observe the birds for 21 days. No chicken dies from causes attributable to the vaccine or shows signs of toxicity other than mild, transient, local reactions. If during the observation period more than two chickens die from causes not attributable to the vaccine, repeat the test.

Virus titre. Not less than 10^2 $EID_{50}/TCID_{50}$ of the virus per dose, determining the titre by inoculation into the chorio-allantoic membrane of SPF embryonated eggs, between 9-11 days old, or one or more route for virus titration depending upon the strain.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a separate potency test for each of the routes of administration stated on the label. Use not less than ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 6 to 8 weeks old. Use ten birds from the same flock and weight range as controls. Administer to each chicken a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. After 21 days, challenge each chicken by intrafollicular administration or by scarification with a virulent strain of fowl poxvirus. Observe the birds for 14 days. The vaccinated chickens survive and show no signs of disease except transient local reactions of fowl pox within 6 days following the challenge. All control chickens show lesions of fowl pox.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine it may be omitted as a routine test during production of the other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) the minimum virus titre; (2) the dose of vaccine.

Goat Pox Vaccine, Live

Goat Pox Vaccine, Live is a freeze-dried preparation of an attenuated strain of goat pox virus propagated in a suitable cell culture. It is reconstituted immediately before use by a suitable diluent.

Production

The virus is propagated in suitable cell culture. The viral suspension is harvested, titrated and may be mixed with a suitable stabilizing agents. The vaccine is then freeze-dried.

Identification

The vaccine protects susceptible animals against goat pox.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Safety

A. Inoculate 10 doses of the reconstituted vaccine in each animal of the three species mentioned below by the route stated against each. Administer 0.2 ml intraperitoneally to each of six mice and 0.5 ml and 1.0 ml subcutaneously to each of three guinea pigs and three rabbits respectively. Observe the animals for 10 days. None of the animals shows an abnormal reaction.

B. Inject 100 doses of the vaccine contained in 1 ml of the reconstituted vaccine subcutaneously into each of two

susceptible goats, 6 to 8 months old. Observe the goats for 10 days. None of the animals shows abnormalities other than local erythema of not more than 3 cm in diameter around the site of injection.

Virus titre. Not less than 10^3 TCID50 of the virus per dose, determining the titre in a suitable cell culture or by inoculation into the chorio-allantoic membrane of SPF embryonated eggs (2.7.7), 9 to 11 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Use nine susceptible goats, 8 to 10 months old. Divide them into three groups of three goats each. Inject subcutaneously 1/20th of the dose of the vaccine stated on the label into each goat of one group. Administer a quantity equivalent to the dose of the vaccine stated on the label into each goat of the second group. Use the third group as unvaccinated controls which should be kept along with the inoculated goats. Observe the animals for 14 days and record the rectal temperature daily of each goat during the observation period. None of the vaccinated goats shows any thermal reaction or local or generalised lesion. After 21 days, challenge the vaccinated and control animals with sufficient quantity of a virulent goat pox virus by intradermal injection. Observe the animals for 14 days and record the rectal temperature daily of each goat during the observation period. None of the vaccinated goats shows any thermal reaction or local or generalised lesion. The test is valid only if the control animals develop high fever or show local or generalised lesions. If the test for potency has been carried out with satisfactory results on a representative batch of vaccine, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot, subject-to agreement by the competent authority.

Labelling. The label states (1) the strain used for the preparation; (2) the virus titre; (3) the dose and age of vaccination.

Haemorrhagic Septicaemia Vaccine

Haemorrhagic Septicaemia Vaccine (Inactivated) is a preparation of *Pasteurella multocida*. The whole culture is inactivated by addition of formalin and a suitable adjuvant (gel or mineral oil).

Identification

The vaccine protects susceptible animals against infection by *P. multocida*.

Tests

Safety. Inject intraperitoneally into each of six mice, weighing not less than 18 g, with 0.5 ml of the vaccine under examination. Observe the animals for 5 days; no abnormal systemic reaction occurs.

Inject two seronegative cattle with twice the maximum dose stated on the label and observe for 10 days for adverse effects.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out any of the following three tests.

A. Test on mice. Inject intramuscularly each of fifty mice, weighing not less than 18 g, with 0.2 ml of the preparation under examination. Repeat the dose 14 days later. After 7 days of second vaccine divide the mice into ten groups of five each. Use another fifty mice of the same weight and from the same stock as controls. Divide the controls also into ten groups of five each. Challenge the vaccinated and the control mice with an 8 to 12-hour old broth culture of a virulent strain of *P. multocida* in the range of 10^{-1} to 10^{-10}. Observe the mice for 5 days and record the number of vaccinated and control mice found dead in each group. Calculate the median lethal dose (LD_{50}) for the vaccinated and control mice by standard methods.

The protection provided by the vaccine is calculated as number of protection units using following formula:

Number of protection units = LD_{50} in control animals - LD_{50} in vaccinated animals.

The vaccine passes the test if it provides minimum protection of 10^4 units.

B. Test on rabbits. Inject intramuscularly each of not less than six rabbits, each weighing not less than 2.0 kg, with 2 ml of the vaccine under examination. Use two rabbits of the same weight and of the same stock as controls. After 21 days, challenge each of the vaccinated rabbits as well as the control rabbits with an 18 hour old culture of *P. multocida* containing not less than 10 LD_{50} of virulent organisms. Observe the animals for 7 days; none of the vaccinated animals dies of pasteurellosis. The test is not valid unless both the control rabbits die of pasteurellosis.

C. Test on calves. Inject each of not less than 3 healthy susceptible calves, weighing not less than 140 kg each with 2 ml of vaccine. Three weeks later, these animals along with two healthy animals of the same type are challenged subcutaneously with 18-hours old broth culture of *P. multocida* equivalent to at least 50 million mouse minimum infective dose. Observe the animals for 7 days. Both the controls should die of pasteurellosis and at least two out of three vaccinated animals should survive.

Potency is conducted on each seed lot or for every fifth batch produced from the seed lot.

Labelling. The label states (1) the type and strains of bacteria used to prepare the vaccine; (2) the adjuvant used.

Inclusion Body Hepatitis (IBH) Vaccine, Inactivated

Hydropericardium Syndrome (HPS)

Inclusion Body Hepatitis (IBH) Vaccine, Inactivated consists of an emulsion or a suspension of avian adenovirus(es) which have been inactivated in such a manner that the immunogenic activity is retained. The vaccine may contain one or more suitable adjuvants.

Production

Substrate for virus propagation

Vaccine virus is multiplied in healthy susceptible chicks or SPF eggs (2.7.7) or in cell culture derived from SPF eggs (2.7.7).

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Test for Inactivation

To confirm inactivation an amplification test for residual live IBH/HPS virus is carried out on each batch of antigen immediately after inactivation or on the final bulk (if the vaccine contains a mixture of inactivated antigens). The test is conducted on healthy susceptible chickens demonstrated to be free from antibodies to IBH/HPS virus or in fertilized eggs derived from specific pathogen free flocks (2.7.7) if the vaccine virus has been propagated in embryos. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Identification

Protects chickens against infection of IBH/HPS.

Tests

Safety. Inject subcutaneously a quantity equivalent to 2 doses into each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the recommended age at which vaccine is to be used. Observe the chickens for 14 days, no abnormal systemic or local reaction is seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either test A or test B may be carried out.

A. Inject one dose by the route stated on label into each of 20 SPF chickens (2.7.7, table 3) or healthy susceptible chickens at the age recommended by manufacturer. Use 10 similar chickens from same source as unvaccinated controls. After 10 days of immunization challenge the birds with 10 per cent IBH positive infected liver suspension 0.5 ml per bird. Observe the birds for ten days. The vaccine passes the potency test when there is 90 per cent protection in vaccinated bird and 80 per cent deaths in unvaccinated controls.

B. At least five, 3-6 week old SPF chickens (2.7.7, table 3) or healthy susceptible chickens are vaccinated with one field dose of vaccine by intramuscular route. Blood samples are collected between 3 and 5 weeks and the antibody response measured by ELISA. The mean antibody titre should be at least 10 $\log_2$ ELISA units.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) strain used for vaccine production; (2) the route of administration.

Infectious Avian Encephalomyelitis Vaccine, Live

Encephalomyelitis Vaccine Live, Epidemic Tremor Vaccine Live

Infectious Avian Encephalomyelitis Vaccine, Live is a freeze-dried preparation of an attenuated strain of infectious avian encephalomyelitis virus.

Production

The virus is grown in SPF embryonated eggs (2.7.7) or in suitable cell culture derived from SPF eggs (2.7.7). The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

Inoculate 0.1 ml of the undiluted reconstituted vaccine into the yolk sac of SPF embryonated eggs, between 5 to 6 days old. Keep them in an incubator and transfer to the setter for hatching. Observe the hatched chickens for 7 days. Not less than 50 per cent of the chickens show the typical symptoms characteristic of infectious avian encephalomyelitis-like weakness or paralysis of legs, sitting posture on hock joints and tremors.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Administer ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens by ten doses of the vaccine by the recommended route. Observe the chickens for 21 days. No chicken develops signs of the disease or dies from causes

attributable to the vaccine. Repeat the test if more than two chickens die from causes not attributable to the vaccine during the observation period.

Virus titre. Not less than $10^{2.5}$ $TCID_{50}/EID_{50}$ of the virus per dose, determining the titre of the virus in cell culture derived from SPF eggs (2.7.7) or by inoculation into the yolk sac of SPF embryonated hen eggs (2.7.7), between 5 to 6 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a separate potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 weeks old. Administer to each chicken a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum virus titre stated on the label. Use ten chickens of the same flock and age as controls. After 21 days, challenge each chicken in the vaccinated and control groups with intracerebral injection of a suitable quantity of a virulent avian encephalomyelitis virus. Observe the chickens for another 21 days. Not less than 80 per cent of the vaccinated chickens survive or show no signs of disease and not less than 70 per cent of the controls die or develop signs or paralytic lesions of avian encephalomyelitis.

If the potency test has been performed with satisfactory results on representative batch of the vaccine from the same seed lot, it may be omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) the minimum virus titre; (2) the dose of vaccine.

Infectious Bursal Disease Vaccine, Inactivated

Infectious Bursal Disease Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of infectious bursal disease virus which has been inactivated in such a manner that immunogenic activity is retained. The vaccine may contain one or more suitable adjuvant.

Production

The virus is propagated in fertilized eggs obtained from healthy flock or in suitable cell culture derived from SPF eggs (2.7.7) or in healthy susceptible chicken.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation

An amplification test for residual live infectious bursal disease virus is carried out on each batch of antigen immediately after inactivation and the test is carried out in fertilized hen eggs obtained from SPF flocks (2.7.7) or in suitable cell culture derived from SPF eggs (2.7.7) or, where chickens have been used for production of the vaccine, in chickens from a flock free from specified pathogens. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Test for inactivation

For vaccine prepared with embryo-adapted strains of the virus. Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity or onto the chorio-allantoic membrane of the SPF embryonated hen eggs, between 9 to 11 days old, and incubate at 36±1°. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos, and that from eggs containing dead embryos, excluding those dying from non-specific causes within the first 24 hours after inoculation.

Inject into the allantoic cavity of each of the SPF embryonated hen eggs, between 9 to 11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos or membrane from the dead embryos and incubate at 36±1° for 6 days. Examine each embryo for lesions of infectious bursal disease. The vaccine complies with the test if there is no evidence of lesions of infectious bursal disease.

The test is valid only if not more than 20 per cent of the embryos die at either stage of the test. If more than 20 per cent of the embryos die at either one of the stages of the test, repeat that stage. In any repeat test, not more than 20 per cent of the embryos die from non-specific causes. Antibiotics may be used to control extraneous bacterial infection.

For vaccine prepared with strains of virus not adapted to embryos. Inject two doses intramuscularly into each of twenty chickens, between 14 and 28 days old, complying with the requirements stated under Test on chicken flocks free from pathogens for the production and quality control of vaccines (2.7.7). Four days later, kill ten of the chickens and remove bursa of fabricius from each chicken, pool the bursa and homogenise in an equal volume of a suitable liquid. Inject 1 ml of the homogenate into each of a further ten chickens of the same flock and age. After 21 days, examine microscopically the bursa of each chicken from the first group and the second group. No evidence of infectious bursal disease is seen and no abnormal local reaction develops.

For vaccine prepared with cell culture-adapted strains of the virus. The formaldehyde in the test sample is neutralizesd with sodium metabisulphite. Five ml is tested for the presence of infective Gumboro Disease virus by inoculation of at least 800 square cm primary or secondary CEF. The cultures are incubated for 3 to 4 days at a temperature of 37°. After one cycle of freezing and thawing the supernatant from these cultures is passaged onto a fresh CEF cultures. Three to four days latter this is repeated. Three to four days after final inoculation the cultures are inspected for CPE. A vital stain and overlay may be used. If no trace of CPE is detected, the inactivation of the antigen suspension is accepted to be completed.

Identification

Protects susceptible chickens against infectious bursal disease by producing specific antibodies on inoculation.

Tests

Safety. Inject each of ten healthy chickens, 14 to 28 days old with twice the minimum vaccinating dose and by one of the routes stated on the label. Observe the chickens for 14 days. No abnormal local or systemic reaction is seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the minimum dose and by the route stated on the label. Use ten chickens of the same flock and age as controls. After 21 days, collect serum samples from each bird including the ten-control chickens and perform quantitative agar gel precipitation test or serum neutralizing test on each serum sample. The mean antibody titre of sera in vaccinated group shall be 1:8 by agar gel diffusion test and 10000 units per ml by serum neutralization test and there are no IBD specific antibodies in the sera of control chickens.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) the type of strain; (2) the route of administration.

Infectious Bursal Disease Vaccine, Live

Infectious Bursal Disease Vaccine, Live is a freeze dried preparation of attenuated strain of infectious bursal disease (IBD) virus. This monograph applies to vaccines intended for administration to chickens for active immunization.

Production

Infectious Bursal Disease Vaccine, Live is a suitable strain of Infectious Bursal Disease virus. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for virus propagation

The vaccine virus is grown in embryonated eggs obtained from SPF flocks or in cell culture derived from SPF eggs (2.7.7) or susceptible cell lines.

Identification

When mixed with monospecific infectious bursal disease virus antiserum the vaccine no longer infects susceptible cell culture derived from SPF eggs (2.7.7) or embryonated hen eggs, 9 to 11 days old.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Use not less than ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 10 to 15 days old. According to the type of viral vaccine strain incorporated in the product - Invasive - Moderately invasive- it may be necessary to conduct the safety test on chicks possessing moderate level of maternal antibodies.

Administer by eye drop to each chicken ten doses of the vaccine reconstituted so as to obtain a concentration suitable for the test. Observe the chickens for 21 days. If during the period of observation more than 2 chickens die from causes not attributable to the vaccine, repeat the test. The vaccine complies with the test if non of the chickens shows signs of the disease, if no chicken dies from causes attributable to the vaccine and if 21 days after inoculation of the vaccine, no chicken shows lesions of the bursa of Fabricius.

Sterility (2.2.11). Complies with the test for sterility.

Virus titre. Not less than 10^2 $TCID_{50}/EID_{50}$ of the virus per dose. Determining the titre in cell cultures derived from SPF embryo or onto the chorio-allantoic membrane of SPF embryonated hen eggs between 9-11 days old.

Potency. Use 20 SPF chickens (2.7.7, table 3) or healthy susceptible chickens 10 to 15 day old. Administer to each chicken one dose of the vaccine by recommended route. Use 10 chickens of the same flock and age as controls. Fourteen days after immunization challenge chicken of both groups by intraocular route administration of a suitable quantity of virulent infectious bursal disease virus. Observe the birds for 10 days after challenge. Not more than 4 of vaccinated chickens die or show signs of the infectious bursal disease or on

histological examination show severe bursal lesions. The test is not valid unless not less than 50 per cent of the control birds die or show signs of IBD and all the surviving controls show severe bursal lesions on histological examination.

If at least 90 per cent of the follicles show greater than 75 per cent depletion of lymphocytes, the bird is considered as one showing severe bursal lesions.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) minimum virus titre; (2) the dose of vaccine.

Infectious Chicken Anemia Vaccine, Inactivated

Infectious Chicken Anemia Vaccine (ICAV), Inactivated is a preparation of a suitable strain of chicken anemia virus, inactivated in such a manner that the immunogenic activity is retained. This monograph applies to vaccines intended for administration to chickens for immunization.

Production

Substrate for virus propagation

The vaccine is grown in embryonated hen's egg obtained from SPF flocks or in suitable cell culture derived from SPF eggs (2.7.7) or susceptible cell line. Harvested virus is inactivated using suitable inactivating agent. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation

An amplification test for residual live chicken infectious anemia virus is carried out on each batch of antigen immediately after inactivation. The test is carried out in suitable cell culture derived from SPF eggs (2.7.7) or using susceptible cell lines. The quantity of inactivated virus used in the test is equivalent to not less than ten doses of the vaccine. No live virus is detected.

Test for Inactivation

Inoculate 10 doses of vaccine virus using suitable cell culture derived from SPF eggs (2.7.7) or in susceptible cell lines or SPF eggs (2.7.7). Incubate at 36 ±1 ° for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) or in cell lines or embryonated SPF eggs (2.7.7) and incubate at 36 ±1° for 7 days. None of the cultures shows signs of CPE.

Identification

In susceptible chicks, the vaccine stimulates the production of specific antibodies against vaccine virus detected by suitable serological tests.

Tests

Safety. Inject a double dose of vaccine by recommended route in to each of ten, 14 to 28 day-old SPF chickens (2.7.7, table 3) or healthy susceptible chickens. Observe the chickens for 21 days. No abnormal local or systemic reactions occur.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a potency test for the route of administration stated on the label. Vaccinate, ten, 21 to 28 day old SPF chickens (2.7.7, table 3) or healthy susceptible chickens with one dose of vaccine. Keep 10 unvaccinated birds of the same age group as controls. Observe the birds for 28 days. Collect serum samples from each bird including the ten-control chickens. Detect the virus specific antibodies by serological methods i.e. Enzyme Linked Immunoassay or Virus Neutralization test. The mean serum neutralization antibody titre of sera in vaccinated group shall be 5000 units per ml and there are no CAV specific antibodies in the sera of control chickens.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) strains used for preparation; (2) the route of administration.

Infectious Chicken Anemia Vaccine, Live

Infectious Chicken Anemia Vaccine, Live is a preparation of a suitable strain of chicken anemia virus. This monograph applies to vaccines intended for administration to breeder chicken for active immunization, to prevent excretion of virus, to prevent or reduce transmission through eggs.

Production

Substrate for propagation

Vaccine is grown either in embryonated hen's egg obtained from SPF flocks (2.7.7) or in cell culture obtained from flocks free from specified pathogens (2.7.7) or susceptible cell lines.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

The vaccine, diluted if necessary and mixed with a monospecific chicken anaemia virus (CAV) antiserum, no longer infects susceptible cell culture derived from SPF eggs (2.7.7) or egg from SPF flock (2.7.7) into which it is inoculated.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Use not fewer than 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens, not older than the minimum age recommended for vaccination (2.7.7). Administer by a recommended route to each chickens 10 doses of the vaccine. Observe the chickens daily for 21 days. The test is not valid if more than 20 per cent of the chickens show abnormal clinical signs or die from causes not attributable to vaccine. The vaccine complies with the test if no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine.

Virus titre. Titrate the vaccine virus by inoculating into suitable cell lines or eggs from SPF flocks (2.7.7). One dose vaccine contains not less than $10^{3.0}$ $TCID_{50}$/ EID_{50} per dose.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out potency test for each of the routes of administration stated on the label. Vaccinate ten, 21 to 28 day old SPF chickens (2.7.7, table 3) or healthy susceptible chickens with one dose of vaccine. Keep 10 unvaccinated birds of the same age group as controls. Two to three weeks post vaccination challenge both the groups by intramuscular route with 10^2 CID50 CAV virus. Observe the birds for 14 days. Bleed individual birds for haematocrit value, thymus atrophy and bone marrow tissue discolouration.

The vaccine complies with the test if during the observation period after challenge not less than 90 per cent of the vaccinated chickens survive and show no notable clinical signs of disease and/or macroscopic lesions of the bone marrow and thymus.

It is not necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum virus stated on the label.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) strain of virus used; (2) the dose of vaccine.

Infectious Coryza Vaccine

Infectious Coryza Vaccine is a suspension of inactivated culture of suitable strains of one or more serotype/s or preferably locally prevalent strain/s of *Avibacterium (Haemophilus) paragallinarum* in a suitable medium.

Production

The seed material is inoculated in a suitable medium. If the vaccine contains more than one strain of bacterium, the different strains are grown and harvested separately. The bacterial harvests are inactivated with a suitable agent. The vaccine may contain suitable adjuvant.

Identification

Protects susceptible chicken against infection with *Avibacterium paragallinarium.*

Tests

Sterility (2.2.11). Complies with the test for sterility.

Safety. Inject double dose of vaccine subcutaneously into each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens at the minimum age group at which vaccine is intended. Observe these birds for 7 days; no bird shows untoward reactions other than slight transient local swelling.

Potency. Inject subcutaneously each of 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the minimum age group at which vaccine is used, with minimum dose stated on the label. Repeat the vaccination after 2 to 4 weeks. Use 10 healthy chickens of same age group and of same stock as controls. Two to three weeks later, challenge vaccinated and control chickens by instillation with 0.2 ml of 18 hour broth culture of homologous strain of *A. paragallinarium* diluted suitably so as to contain 1×10^6 colony forming units by infra-orbital sinus instillation. Observe the chickens for 7 days for unilateral eye swelling, nasal discharge. There should be not less than 70 per cent protection of vaccinated birds. The test is not valid unless 70 per cent of control chickens exhibit typical symptoms of eye swelling and nasal discharge typical of infectious coryza.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date of potency testing.

Labelling. The label/insert states (1) strains used for preparation; (2) the route of administration.

Marek's Disease Vaccine, Live

Marek's Disease, Freeze Dried / Cell Associatated Vaccine, Live is a preparation of a suitable strain or strains of Marek's Disease Virus (Avian Herpes Virus) or combinations theirof.

Production

The vaccine virus is grown in cell cultures obtained from SPF (2.7.7) eggs. If the vaccine contains more than one type of virus, the different types are grown separately. The vaccine may be freeze-dried or stored in liquid nitrogen.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Substrate for virus propagation

Cell culture derived from SPF eggs (2.7.7) obtained from SPF hens (2.7.7) eggs.

Identification

Carry out either the test A or B.

A. The vaccine on inoculation in susceptible cell cultures derived from SPF embryos causes cytopathic effects typical of Marek's Disease virus.

B. When mixed with a specific avian herpes virus antiserum the vaccine loses its capability to produce cytopathic effects or plaques in susceptible cell cultures derived from SPF embryos.

Tests

Water (2.3.43). Not more than 3.0 per cent (For Freeze dried vaccine only).

Mycoplasams (2.7.9). Complies with the test for mycoplasmas.

Safety. Use ten one-day-old SPF chickens (2.7.7, table 3) or healthy susceptible chickens. Administer by recommended route and method to each chicken or chicken embryo 10 doses of the vaccine. Observe the chicken for 21 days. No chicken shows persistent clinical signs, dies or, at autopsy, shows macroscopic lesions from causes attributable to the vaccine. If during the observation period more than two chickens die from causes not attributable to the vaccine, repeat the test.

Sterility (2.2.11). Complies with the test for sterility.

Virus titre. Vaccine containing one type of virus: Titrate the vaccine virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7). If the virus titre is determined in plaque forming units (PFU), only primary plaques are taken into consideration. The vaccine complies with the test if one dose contains not less than 10^3 PFU per dose.

Vaccine containing more than one type of virus: For vaccine containing more than one type of virus, titrate each virus by inoculation into suitable cell culture derived from SPF eggs (2.7.7), reading the results by immunostaining using antibodies. Vaccine complies with the test if one dose contains for each vaccine virus not less than 10^3 PFU of virus per dose.

Potency. Carry out a separate potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than thirty susceptible one-day-old SPF chickens (2.7.7, table 3) or healthy susceptible chickens.

Administer each chicken a volume of the vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. Use thirty chickens of the same flock and age as controls. After 9 days, challenge each chicken by a suitable route with a suitable quantity of virulent Marek's disease virus. Observe the birds for 10 weeks. Record the deaths and kill the survivors to carry out autopsies on both dead and sacrificed chicken for specific macroscopic lesions of Marek's disease. For each of the stated routes of administration, the total number of vaccinated birds that show specific macroscopic lesions is reduced by not less than 80 per cent as compared with the control birds and the challenge virus produces specific macroscopic lesions in not less than 70 per cent of the control birds.

If the potency test has been performed with satisfactory results on representative batch of the vaccine from the same seed lot, it may be omitted as a routine control test during production of other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label states (1) the minimum virus titre; (2) the dose of vaccine.

The frozen vaccine has to be dispensed in glass ampoules suitable for liquid nitrogen storage and if the above information cannot be printed on the small size ampoule, the product should be accompanied by suitable insert which clarifies the prescribed contents of the labels.

Peste Des Petits Ruminants Vaccine, Live

Peste Des Petits Ruminants (PPR) Vaccine, Live is a preparation of a suitable strain of PPR virus that is attenuated for sheep and goats.

Production

The vaccine strain is grown in suitable cell cultures. The viral suspension is harvested, mixed with a suitable stabilizing liquid and freeze-dried.

Batch testing

If the test for potency has been carried out with satisfactory results on the representative batch of vaccine, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot, subject to agreement by a National Regulatory Authority.

Identification

When injected into the target animals, the vaccine stimulates the production of specific PPR virus neutralizing antibodies.

Tests

Safety. This test is done in rodents in order to detect any nonspecific toxicity associated with the product. The test requires reconstituted vaccine in solvent (mixed contents of five bottles), six guinea pigs, each weighing 200-250 g; ten unweaned mice (17-22 g, Swiss line or similar).

Vaccine, 0.5 ml, is injected intramuscularly into a hind limb of two guinea-pigs, 0.5 ml into the peritoneal cavity of two guinea-pigs, and 0.1 ml into the peritoneal cavity of six mice. Two guinea-pigs and four mice are kept as uninoculated controls. The animals are observed for 3 weeks. If one guinea-pig or two mice die, the test must be repeated. Dead animals undergo post-mortem examination to ascertain the cause of death. At the end of 3 weeks of observation, all animals are killed for post-mortem examination. All the results are recorded. The vaccine considered to be satisfactory if, during the first or second test, at least 80 per cent of animals remain in good health during the period of observation, and no significant post-mortem lesion is found.

Inject two susceptible goats of one year old free from antibodies to rinderpest or PPR by subcutaneous route with a 100 times the dose of vaccine stated on the label. Observe the animals for 21 days. No sign of illness attributable to PPR is noticed.

Water (2.3.43). Not more than 3.5 per cent.

Virus titre. Not less than $10^{2.5}$ $TCID_{50}$ per dose.

Extraneous viruses. The reconstituted vaccine when mixed with specific anti-PPR serum should not produce cytopathic effects in susceptible cell cultures and the cells should show no evidence of the presence of haemadsorbing agents.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Use not less than six healthy goats and six healthy sheep of 1 year old free from antibodies to rinderpest or PPR. Collect sera from the animals before the time of vaccination and 3 weeks after vaccination and just before challenge. Vaccinate two goats and two sheep subcutaneously with one dose each; vaccinate two goats and two sheep subcutaneously with 1/10 dose each. Keep the remaining animals as the in-contact controls. Monitor each animal for clinical signs, in particular respiratory symptoms and record temperature measurements daily for three weeks. Three weeks after vaccination collect sera samples from all vaccinated as well as control animals and challenge the vaccinated animals and in-contact controls group with a suspension of virus containing either 10^3 LD_{50} pathogenic PPRV or 2.5 ml of a 10% splenic suspension by subcutaneous route. The animals are observed for clinical signs and the body temperatures are recorded daily for two weeks. The vaccine passes the test if all vaccinated animals resist challenge infection and all the in-contact controls develop signs of PPR. The serum neutralization test must be positive for PPR antibody in vaccinated animal only, in samples taken three weeks after vaccination.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Labelling. The label states (1) the cell line used for vaccine manufacture; (2) the virus titre per dose; (3) the recommended age for vaccination.

Rabies Veterinary Vaccine, Inactivated (Cell Culture)

Rabies Vaccine for Veterinary Use is a preparation of rabies fixed virus adapted to and propagated in cell culture and inactivated by a suitable method. It may be issued as a liquid containing a suitable adjuvant or as a freeze-dried preparation to be reconstituted with a suitable liquid immediately before use.

Production

The vaccine is prepared from virus grown either in suitable cell lines or in primary cell cultures from healthy animals. The virus suspension is harvested on one or more occasions within 28 days of inoculation. Multiple harvests from a single production cell culture may be pooled and considered as a single harvest. The rabies virus is inactivated by a suitable method. The vaccine may contain one or more adjuvants.

Inactivation

A. The test for residual live rabies virus is carried out by inoculation of the inactivated virus into the same type of cell culture as that used in the production of the vaccine or a cell culture shown to be at least as sensitive. The quantity of inactivated virus used in the test is equivalent to not less than 25 doses of the vaccine. After incubation for 4 days, a subculture is made using trypsinised cells; after incubation for a further 4 days, the cultures are examined for residual live rabies virus by an immunofluorescence test. No live virus is detected.

B. Inject each of twenty suckling mice, each weighing between 12 and 16 g, intracerebrally with not less than 0.03 ml of the vaccine or antigen under examination. Observe the animals for 21 days. None of the mice dies or shows any abnormalities attributable to the vaccine. If more than two mice die within 48 hours, repeat the test.

Identification

When injected into animals, the vaccine stimulates production of specific neutralising antibodies.

Tests

Water (2.3.43). Not more than 3.0 per cent (for freeze dried vaccine only).

Safety. Inject each of twenty mice, each weighing between 12 and 16 g, intracerebrally with not less than 0.03 ml of the vaccine under examination. Observe the animals for 21 days. None of the mice dies or shows any abnormalities attributable to the vaccine. If more than two mice die within 48 hours repeat the test. If the vaccine is intended for more than one species including one belonging to the order of Carnivore, carry out the test in dogs. Otherwise use one of the species for which the vaccine is intended. Administer, by a recommended route, a double dose of vaccine to each of 2 animals having no antibodies against rabies virus. Observe the animals for 14 days. No abnormal local or systemic reaction occurs.

Sterility (2.2.11). Complies with the test for sterility.

Potency. The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the clinical effects of the dose of rabies virus defined below, administered intracerebrally, with the quantity of a reference preparation, calibrated in International Units, necessary to provide the same protection.

Preparation of the challenge suspension. Inoculate a group of mice intracerebrally with the CVS strain of rabies virus and when the mice show signs of rabies, but before they die, kill the mice and remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant liquid as challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below -60°. Thaw one ampoule of the suspension and make serial dilutions in a suitable diluent. Allocate each dilution to a group of 10 mice and inject intracerebrally into each mouse 0.03 ml of the dilution allocated to its group. Observe the animals for 14 days and record the number in each group that, between the fifth and the fourteenth day, develop signs of rabies. Calculate the ID_{50} of the undiluted suspension.

Determination of potency of the vaccine

Use in the test, healthy mice about 4 weeks old and from the same stock. Distribute the mice into at least 10 groups of not less than 10 mice. Prepare at least three serial dilutions of the vaccine under examination and three similar dilutions of the reference preparation. Prepare the dilutions such that those containing the largest quantity of vaccine may be expected to protect more than 50 per cent of the animals into which they are injected and those containing the smallest quantities of vaccine may be expected to protect less than 50 per cent of the animals into which they are injected. Allocate each dilution to a different group of mice and inject intraperitoneally into each mouse 0.5 ml of the dilution allocated to its group. Fourteen days after the injection prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, it contains about 50 ID_{50} in each 0.03 ml. Inject intracerebrally into each vaccinated mouse 0.03 ml of this suspension. Prepare 3 suitable serial dilutions of the challenge suspension. Allocate the challenge suspension and the 3 dilutions one to each of 4 groups of 10 unvaccinated mice and inject intracerebrally into each mouse 0.03 ml of the suspension or one of the dilutions allocated to its group. Observe the animals in each group for 14 days. The test is not valid if more than 2 mice of any group die within the first 4 days after challenge. Record the numbers in each group that show signs of rabies in the period 5 days to 14 days after challenge.

The test is not valid unless (a) for both the vaccine under examination and the reference preparation the 50 per cent protective dose lies between the smallest and the largest dose given to the mice; (b) the titration of the challenge suspension shows that 0.03 ml of the suspension contained at least 10 ID_{50} and not more than 50 ID_{50}; (c) the confidence limits ($P = 0.95$) are not less than 25 per cent and not more than 400 per cent of the estimated potency; (d) the statistical analysis shows a significant slope and no significant deviations from linearity or parallelism of the dose-response lines.

The vaccine complies with the test if the estimated potency is not less than 1 IU in the smallest prescribed dose.

Labelling. The label states (1) the strain used for the preparation; (2) the name of any added adjuvant.

Ranikhet Disease Vaccine, Inactivated

Newcastle Disease Vaccine, Inactivated

Ranikhet Disease Vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain of Newcastle disease virus (avian paramyxovirus 1) that has been inactivated in such a manner that immunogenic activity is retained.

Production

Substrate for virus propagation

The vaccine virus is grown either in embryonated hens' eggs or in cell culture derived from SPF eggs (2.7.7) or suitable cell line.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation

Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of each of 10 embryonated 9 to 11 days old SPF eggs (2.7.7), and incubate. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos and that from eggs containing dead embryos, excluding those dying within 24 hours of the injection. Examine embryos that die after 24 hours of injection for the presence of Newcastle disease virus. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.

Inject into the allantoic cavity of each of 10 SPF eggs (2.7.7), 9 to 11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos and, into each of 10 similar eggs, 0.2 ml of the pooled fluid from the dead embryos and incubate for 5 to 6 days. Test the allantoic fluid from each egg for the presence of haemagglutinins using chicken erythrocytes.

The vaccine complies with the test if there is no evidence of haemagglutinating activity and if not more than 20 per cent of the embryos die at either stage. If more than 20 per cent of the embryos die at one of the stages, repeat that stage; the vaccine complies with the test if there is no evidence of haemagglutinating activity and not more than 20 per cent of the embryos die at that stage.

Antibiotics may be used in the test to control extraneous bacterial infection.

Identification

When injected into susceptible healthy chicken, the vaccine stimulates the production of specific antibodies against Newcastle disease virus.

Tests

Safety. Inject ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the age stated on the label with twice the dose and by the route stated on the label. Observe the birds for 21 days. No abnormal local or systemic reactions are observed.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Either test A or test B may be carried out.

A. Inject intramuscularly each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, between 3 - 4 weeks old, with a volume of the vaccine equivalent to one-fiftieth of a dose. Use ten chickens of the same stock and age group as controls. After 21 days, collect serum samples from each of the vaccinated and unvaccinated chicken. Perform haemagglutination inhibition test using the method described below. Use the positive control serum calibrated against a Standard preparation of anti-Newcastle disease serum. The vaccine passes the test if a mean HI titre of the vaccinated group is equal to or greater than 1:16 and that of the unvaccinated controls is equal to or less than 1:4.

If the HI titre are not satisfactory, carry out the test B.

Standard preparation

The Standard preparation is the 1st International reference preparation, established in 1966, consisting of freeze-dried chicken serum (supplied in ampoules containing 320 Units), or another suitable preparation, the potency of which has been determined in relation to the International reference preparation.

Suggested method of haemagglutination inhibition test. Inactivate the serum samples by heating at 56° for 30 minutes. Add 0.05 ml of *saline solution* to all the wells in a microtitre plate and 0.05 ml of the test sera to the first row of wells. Prepare two-fold dilutions of the serum samples across the plate. Add 0.05 ml of a suspension of Newcastle disease virus containing 4 haemagglutinating units of inactivated Newcastle disease virus. Incubate the plate at 4° for one hour. Add 0.05 ml of a 1 per cent suspension of erythrocytes collected from chicken, between 3-4 weeks old, susceptible to Newcastle disease.

Incubate the plate at 4° for one hour. It must be ensured that negative and positive control sera are included in the test. The positive control serum must show a titre of 300 to 400 Units determined by calibration against the Standard reference Preparation.

B. Inject intramuscularly each of three groups of twenty SPF chickens (2.7.7, table 3) or healthy susceptible chickens, between 3 - 4 weeks old, with five fold dilution of vaccine. Use minimum three dilutions. Allocate a different volume to

each vaccination group. Vaccinate each chicken by the intramuscular route with the volume of vaccine allocated to its group. Maintain not less than 10 chickens as controls. Challenge each chicken after 21 days by the intramuscular route with 10^6 chick LD_{50} of the virulent strain of avian Paramyxovirus 1. Observe the chickens at least daily for 7 days after challenge. At the end of the observation period, calculate the PD_{50} by standard statistical methods from the number of chickens that survive in each vaccinated group without showing any signs of Newcastle disease during the 7 days. The vaccine complies with the test if the smallest dose stated on the label corresponds to not less than 50 PD_{50} and the lower confidence limit is not less than 35 PD_{50} per dose. If the lower confidence limit is less than 35 PD_{50} per dose, repeat the test; the vaccine must be shown to contain not less than 50 PD_{50} in the repeat test. The test is not valid unless all the control birds die within 6 days of challenge.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) strain of virus used; (2) the route of administration.

Ranikhet Disease Vaccine, Live (Lentogenic Strain)

Newcastle Disease Vaccine, Live (Lentogenic strain)

Ranikhet Disease Vaccine Live (Lentogenic Strain) is a preparation of a suitable strain of Newcastle disease/Ranikhet disease virus (avian paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens and/or other avian species for active immunization.

Production

Substrate for virus propagation

The vaccine virus is grown in embryonated SPF eggs (2.7.7) or in cell cultures derived from SPF flocks (2.7.7).

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

The vaccine, diluted if necessary and mixed with a monospecific Newcastle disease virus antiserum, no longer provokes haemagglutination of chicken red blood cells or infects embryonated hens' eggs from SPF flock or susceptible cell culture derived from SPF eggs (2.7.7) into which it is inoculated.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. For vaccines recommended for use in healthy susceptible chickens, use not less than 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens demonstrated to be free from antibodies to Newcastle disease virus and of the youngest age recommended for vaccination. For vaccines recommended for use only in avian species other than the chicken, use not less than 10 birds of the species likely to be most sensitive to Newcastle disease, which do not have antibodies against Newcastle disease virus and of the minimum age recommended for vaccination. Administer to each bird by eye-drop, or parenterally if only parenteral administration is recommended, 10 doses of the vaccine in a volume suitable for the test. Observe the birds at least daily for 21 days. The test is not valid if more than 20 per cent of the birds show abnormal clinical signs or die from causes not attributable to the vaccine. The vaccine complies with the test if no bird shows notable clinical signs of disease or dies from causes attributable to the vaccine.

Virus titre. Not less than 10^6 $TCID_{50}/EID_{50}$ of the virus per dose, determining the titre in suitable cell culture derived from SPF eggs (2.7.7) or by inoculation into the allantoic cavity of SPF embryonated eggs, 9 to 11 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out a potency test for each of the routes of administration stated on the label. For each of the stated routes, use at least ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens and of the minimum age recommended for vaccination.

Administer each chicken with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. Use ten chickens of the same flock and age as controls. After 14 to 21 days, challenge each chicken by intramuscular injection with 10^5 LD_{50}, of a virulent strain of Newcastle disease virus. Observe the chickens for 14 days. The vaccine complies with the test if not more than two of the vaccinated chickens die or show signs of disease. The test is valid only if all the control birds die within 6 days of inoculation of the virulent challenge strain.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined.

The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) strain of virus used; (2) the dose of vaccine.

Ranikhet Disease Vaccine, Live (Mesogenic Strain)

Ranikhet Disease Vaccine, Live (Mesogenic Strain) is a preparation of a suitable strain of Newcastle disease virus (naturally modified avian Paramyxovirus 1). This monograph applies to vaccines intended for administration to chickens for active immunization.

Production

Substrate for virus propagation

The vaccine virus is grown in embryonated SPF eggs (2.7.7) or in cell cultures derived from SPF flocks (2.7.7) or susceptible cell lines. The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Identification

The vaccine, diluted if necessary and mixed with a monospecific Newcastle disease virus antiserum, no longer provokes haemagglutination of chicken red blood cells or infects embryonated hens' eggs from SPF flock (2.7.7) or susceptible cell culture derived from SPF eggs (2.7.7) into which it is inoculated.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Administer fifteen SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 8 to 9 weeks old, with a minimum 10 doses and by the route stated on the label. Observe the chickens for 21 days. Not more than 2 chicken show abnormal clinical signs or die due to causes attributable to the vaccine. If more than two chickens die during the period of observation due to causes other than those attributable to the vaccine, repeat the test.

Virus titre. Not less than 10^5 $TCID_{50}/EID_{50}$ of the virus per dose, determining the titre in suitable cell culture derived from SPF eggs (2.7.7) or by inoculation into the allantoic cavity of SPF embryonated eggs (2.7.7), between 9-11 days old.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out potency test for each of the routes of administration stated on the label. For each of the stated routes, use not less than ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the minimum age recommended for vaccination. Administer each chicken with a volume of the reconstituted vaccine containing a quantity of the virus equivalent to the minimum titre stated on the label. Use ten chickens of the same flock and age as controls. After 14 to 21 days, challenge each chicken by intramuscular injection with 10^5 LD_{50} of a virulent strain of Newcastle disease virus. Observe the birds for 14 days. The vaccine complies with the test if not more than two of the vaccinated chickens die or show signs of disease. The test is valid only if all the control chickens die within 6 days of inoculation of the virulent challenge strain.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) strain of virus used; (2) the dose of vaccine.

Reo Virus Vaccine, Inactivated

Reo virus vaccine, Inactivated consists of an emulsion or a suspension of a suitable strain /s of Reo virus which has been inactivated in such a manner that immunogenic activity is retained. The vaccine may contain one or more strains and a suitable adjuvant.

Production

Substrate for virus propagation

The virus is propagated in fertilized eggs obtained from healthy flock or in suitable cell culture derived from SPF flocks (2.7.7) or susceptible cell line.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines (2.7.10).

Inactivation

An amplification test for residual live infectious avian reo virus is carried out on each batch of antigen immediately after inactivation and the test is carried out in fertilized SPF hen eggs or in suitable cell culture derived from SPF eggs (2.7.7). The quantity of inactivated virus used in the test is equivalent

to not less than ten doses of the vaccine. No live virus is detected.

A. **In cell culture derived from SPF eggs** (2.7.7). Inoculate 10 doses of vaccine into suitable cell culture derived from SPF eggs (2.7.7). Incubate at 36±1° for 7 days. Make a passage on another set of cell culture derived from SPF eggs (2.7.7) and incubate at 36±1° for 7 days. None of the cultures shows signs of infection i.e. CPE.

B. **In embryonated eggs**. Inject quantity of inactivated virus equivalent to 10 doses of vaccine into the allantoic cavity of the SPF embryonated hen eggs, between 9-11 days old, and incubate at 36±1°. Observe for 6 days and pool separately the allantoic fluid from eggs containing live embryos, and that from eggs containing dead embryos, excluding those dying from non-specific causes within the first 24 hours after inoculation. Inject into the allantoic cavity of each of the SPF embryonated hen eggs, between 9-11 days old, 0.2 ml of the pooled allantoic fluid from the live embryos or membrane from the dead embryos and incubate at 36±1° for 6 days. Examine each embryo for lesions of Reo virus. The vaccine complies with the test if there is no evidence of lesions of Reo virus. The test is valid only if not more than 20 per cent of the embryos die at either stage of the test. If more than 20 per cent of the embryos die at either one of the stages of the test, repeat that stage. In any repeat test, not more than 20 per cent of the embryos die from non-specific causes. Antibiotics may be used to control extraneous bacterial infection.

Identification

In susceptible chickens, the vaccine stimulates the production of specific antibodies against each of the virus serotypes in the vaccine detected by virus neutralization.

Tests

Safety. Inject each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 14 to 28 days old with twice the minimum vaccinating dose and by one of the routes stated on the label. Observe the chickens for 14 days. No abnormal local or systemic reaction should be seen.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of twenty SPF chickens (2.7.7, table 3) or healthy susceptible chickens, 3 to 4 weeks old, with the minimum dose and by the route stated on the label. Use ten chickens of the same flock and age as controls. After 21 days, collect serum samples from each bird including the ten-control chickens and perform quantitative agar gel precipitation test or serum neutralization test on each serum sample. The mean antibody titre of sera in vaccinated group shall be 1:8 by Agar gel diffusion test and 10000 units per ml by serum neutralization test and there should be no specific antibodies in the sera of control chicken.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined.

Labelling. The label/insert states (1) strains used for preparation; (2) the route of administration.

Reo Virus Vaccine, Live

Reo Virus Vaccine, Live is a preparation of a suitable strain(s) of Reo virus known to be safe and immunogenic. This monograph applies to vaccines intended for administration to chickens for protection against Malabsorption Syndrome and /or proventriculitis and /or Tenosynovitis in birds.

Production

Substrate for virus propagation

The vaccine virus is grown in embryonated SPF hens' eggs or in cell cultures derived from SPF flocks (2.7.7) or suitable cell line.

The master seed lot complies with the tests for extraneous agents as described in the General monograph for Veterinary Vaccines.

Identification

When mixed with monospecific Reo virus antiserum, the vaccine no longer induces cytopathic effect in susceptible cell culture derived from SPF eggs (2.7.7) or carry out immunostaining test in cell culture derived from SPF eggs (2.7.7) to identify the vaccine virus.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.9). Complies with the test for mycoplasmas.

Safety. Final container samples of completed product from each serial shall be tested as follows:

A. For vaccines intended for use in very young chickens, each of 10, one day old SPF chickens (2.7.7, table 3) or healthy tenosynovitis/malabsorption/proventriculitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

B. For vaccines intended for use in older chickens, each of ten, 4-week-old or older SPF chickens (2.7.7, table 3) or healthy tenosynovitis susceptible chickens shall be vaccinated with the equivalent of 10 doses by one method recommended on the label.

The vaccinates shall be observed each day for 21 days. If unfavourable reactions occur which are attributable to the product, the serial is unsatisfactory. If unfavorable reactions

occur in more than two vaccinates which are not attributable to the product, the test is inconclusive and may be repeated. If the test is not repeated, the serial is unsatisfactory.

Virus titre. Titrate the vaccine in cell cultures derived from SPF embryos or in SPF eggs (2.7.7). One dose of the vaccine contains not less than 10^3 $TCID_{50}$ / EID50 per dose.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Reo susceptible healthy chickens of same age and from the same source shall be used as test birds. Vaccine intended for use in very young chickens shall be administered to chickens of the youngest age for which vaccine is recommended. Vaccines intended for use in older chickens shall be administered to 4 weeks or older birds. Ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens vaccinates shall be used for each method of administration. One dose will be injected to vaccinates. Ten chicks shall be held as unvaccinated controls.

Potency test of each age group shall be conducted separately. Twenty one days post vaccination each vaccinate and control shall be challenged by injecting virulent virus into one foot pad. The vaccinates & controls shall be observed for 14 days post challenge. If at least 90 per cent of the controls do not develop swelling and discolouration in the phalangeal joint area of injected foot pad typical of infection of Reo virus, the test is inconclusive and may be repeated. If at least 18 out of 20 vaccinates do not remain free of these signs, disregarding transient swelling which subsides within 5 days post challenge, the serial is unsatisfactory.

The serial is satisfactory when it gives 90 per cent protection to vaccinated group and 90 per cent controls develop positive Reo virus lesions on challenge.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 18 months from the date the virus titre was determined. The reconstituted vaccine should be used immediately after preparation.

Labelling. The label/insert states (1) strain of virus used; (2) the dose of vaccine.

Rinderpest Vaccine, Live

Rinderpest Vaccine, Live is a freeze-dried preparation of a live attenuated strain of rinderpest virus that has been modified by adaptation to and propagation in suitable cell cultures in such a manner that it remains avirulent but retains its immunogenicity in cattle. It is reconstituted immediately before use with a suitable diluent.

Production

SEED LOT

The seed lots should be validated for the following tests:

a) Purity. It should be free from contaminations with viruses, bacteria, fungi and mycoplasmas;

b) Should not induce any abnormal clinical reaction on inoculation into rinderpest susceptible cattle;

c) Efficacy. It should induce an immunity to rinderpest in the susceptible cattle.

CELLS. The primary cells/subcultured cells/continuous cell lines when used should be free from BVD and other contaminating viruses.

Identification

A. The vaccine protects cattle against virulent rinderpest virus.

B. The seed and the vaccine must be titrated in a suitable cell culture system capable of supporting the multiplication of the rinderpest virus.

C. When neutralised with a specific rinderpest antiserum, the vaccine is no longer capable of protecting cattle against rinderpest infection.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Mycoplasmas (2.7.4). Complies with the test for absence of mycoplasmas.

Extraneous pathogens. Complies with the requirements stated under Veterinary Vaccines.

Safety

A. Use four healthy guinea-pigs, each weighing not less than 400 g. Inject two of them intramuscularly and two intraperitoneally with 0.5 ml of the vaccine under examination. In addition, inject intraperitoneally each of six mice, each weighing between 18 and 25 g, with 0.1 ml of the vaccine. Observe the animals for 21 days. All the animals remain healthy during the observation period. At the end of the observation period sacrifice the animals and perform autopsy on each. None of the animals shows any unusual changes.

B. Inject subcutaneously each of two susceptible cattle, free from specific antibodies, with a quantity of the vaccine containing not less than 100 times the minimum dose stated on the label, using pooled reconstituted contents of not less than ten containers taken at random. Observe the animals for

21 days. No sign of disease attributable to the vaccine other than mild transient pyrexia is seen.

Virus titer. Not less than 10^3 $TCID_{50}$ per dose of cell culture vaccine determining the virus content of the reconstituted vaccine in a suitable cell culture system.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject subcutaneously each of two susceptible cattle, free from rinderpest specific antibodies, with a field dose and 1/10th of the minimum dose respectively stated on the label, considering 10^3 $TCID_{50}$ of cell culture vaccine. Use two animals of the same stock and age as controls. Observe the animals for 21 days. Challenge intramuscularly each animal with a dose of not less than 10^4 ID_{50} of virulent rinderpest virus. Observe the animals for 14 days. None of the vaccinated animals shows any clinical signs suggestive of rinderpest. The test is not valid unless both the control animals develop signs of rinderpest.

If the potency test has been performed with satisfactory results on a representative batch of the vaccine from the seed lot, it may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot, provided the National Regulatory Authority permits.

Labelling. The label states (1) the strain of the virus used; (2) the number of doses in the container; (3) that the vaccine should be used immediately after reconstitution.

Salmonella Abortus Equi Vaccine

Salmonella abortus equi Vaccine is a suspension of killed mixture of equal parts of pure formalized cultures of smooth laboratory strains of *Salmonella abortus equi.*

Production

The whole culture or its filtrate or a mixture is inactivated in such a manner that pathogenecity is eliminated and immunogenic activity is retained. The inactivated cultures may be treated with a suitable adjuvant.

Identification

It protects susceptible animals against infection with *Salmonella abortus equi.*

Tests

Safety. Inject 0.5 ml of the vaccine intraperitoneally to each of six mice, each weighing not less than 18 g. Observe the mice for 96 hours, none of the mice dies of salmonellosis.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of twelve mice, each weighing not less than 18 g, subcutaneously with 0.5 ml of the preparation under examination. Use another twelve mice of the same weight range and from the same stock as controls. Three weeks later, challenge the mice from both groups by injecting intraperitoneally each animal with 0.5 ml of a suspension of an 18-hour old culture containing 10 LD_{50} virulent organisms of *S. abortus equi.* Observe the mice for 7 days. The vaccine passes the test if not less than nine mice of the vaccinated group survive. The test is not valid unless not less than nine of the control mice succumb to the challenge.

Labelling. The label states (1) the method of preparation; (2) the strains of bacteria used to prepare the vaccine.

Salmonella Vaccine, Inactivated

Salmonella Vaccine, Inactivated is a preparation of 1 or more suitable strains of 1 or more serovars of *Salmonella* organism, inactivated while maintaining adequate immunogenic properties.

This monograph applies to vaccines intended for the active immunization of chickens against infection/s of *Salmonella* in chickens and reducing *Salmonella* colonization and fecal excretion in chickens.

Production

The seed material is inoculated in a suitable medium. If the vaccine contains more than 1 strains of bacterium, the different strains are grown and harvested separately. During production, parameters such as growth rate, purity and identity is verified on harvests using suitable culture. The bacterial harvests are inactivated with suitable agent. The vaccine may contain suitable adjuvant.

Identification

Vaccine stimulates production of strain specific antibodies against *Salmonella* organisms in susceptible birds.

Tests

Safety. Administer double dose of vaccine subcutaneously into each of ten SPF chickens (2.7.7, table 3) or healthy susceptible chickens of minimum age recommended for vaccination. Observe the birds at least for 21 days. The test is not valid if more than 20 per cent of the chickens show abnormal signs or die from causes not attributable to the vaccine. The vaccine complies with the test if no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Carry out separate potency test for each strain of *Salmonella* organism incorporated in the vaccine preparation. Use not less than 10 SPF chickens (2.7.7, table 3) or healthy susceptible chickens of the minimum age recommended for vaccination. Administer 1 dose of vaccine by a recommended route. Maintain 10 chickens as unvaccinated controls from the same source and flock used for vaccination for each strain used in vaccine. Repeat the vaccination with the same dose and route after 21 days to vaccinated birds. Challenge both the groups, 2 weeks after last administration of vaccine, by oral administration to each chicken a sufficient quantity of a homologous strains of *Salmonella* organisms that is able to colonize chickens. Observe the birds daily for 14 days. Collect fecal samples on 14th day for detection of presence of *Salmonella* organisms by direct plating. The vaccine complies with the test, if the number of *Salmonella* organisms in fresh fecal samples after challenge is significantly lower in vaccinated birds than in unvaccinated controls.

Storage. When stored under the prescribed conditions, the vaccine may be expected to retain its potency for not less than 2 years from the date the potency was determined

Labelling. The label states (1) strains used for preparation; (2) the route of administration.

Sheep Pox Vaccine, Live Attenuated

Sheep Pox Vaccine, Live Attenuated is a freeze dried preparation obtained by producing attenuated sheep poxvirus in a suitable cell culture and mixed with a suitable stabilizer and freeze dried. The freeze dried vial is reconstituted with a suitable diluent and used immediately.

Production

The vaccine reconstituted with a suitable liquid and diluted if necessary to provide a concentration appropriate to the particular test, complies with the requirements stated under Veterinary Vaccines with the following modifications.

The seed lots used for vaccine preparation must be free from extraneous pathogens.

If the immunogenicity tests have been performed with satisfactory results on a representative batch of the vaccine from the seed lot, they may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Identification

The vaccine specifically protects sheep against sheep pox.

Tests

Water (2.3.43). Not more than 3.0 per cent.

Safety. Inoculate not less than 2 sheep of 8 to 12 months old, free from neutralizing antibodies against sheep pox virus, with ten times the field dose of the vaccine contained in 1 ml by subcutaneous route. Observe the animals for 14 days. The vaccine complies the test if none of the vaccinated animals show deep necrotic lesion and generalization.

Virus titre. Not less than 10 $TCID_{50}$ of the virus per dose as determined by the titre of the vaccine in a suitable cell culture system.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Administer each of three sheep, between 8 and 12 months old, free from sheep pox neutralizing antibodies, with the dose of the vaccine and by the route stated on the label. Use two sheep of the same stock and age as un-vaccinated controls. Shave the animals closely on the flank from the shoulder to the proctodal area. Challenge each animal after 21 days post-vaccination by inoculating intradermally with 0.1 ml of a suspension six ten fold dilution of the sheep pox challenge virus. Make five separate inoculations in a vertical line for each serial dilution from the anterior to the posterior of the animals. The titer of the challenge virus is calculated using a standard statistical method for the vaccinated and control sheep by the number of pox lesions observed in each dilution. The titer of the challenge virus is calculated for the vaccinated and control animals. The vaccine passes the test if there is a difference of log titer of more than $\log_{10}$ 2.5.

Labelling. The label states (1) the strain of virus used in preparing the vaccine; (2) the virus titre; (3) the minimum dose and the routes of administration; (4) the volume of the liquid to be used for reconstitution of the vaccine.

Sterile Diluent for Live Vaccines

Sterile diluents are required for reconstitution of freeze dried and frozen vaccines. The sterile diluents may be a special liquid solution.

Each diluent batch shall be given a number which shall be used in records, test reports and final containers.

Tests

Sterility (2.2.11). Complies with the test for sterility.

Viral stability. Each batch should be tested for viral stability by holding two hours after reconstitution of vaccine at recommended temperature.

pH. Hydrogen Ion concentration shall be determined with a pH meter which has been standardized with buffer.

Clarity. Each batch should be free from visible particulate matter.

Swine Fever Vaccine, Live

Swine Fever Vaccine, Live is a preparation of a modified strain of classical swine fever virus, which is devoid of pathogenicity for the pig by adaptation either to cell cultures or to the rabbit. It is prepared immediately before use by reconstitution from the dried vaccine with a suitable diluent.

Production

The virus is propagated in suitable cell culture. The viral suspension is harvested, titrated and may be mixed with a suitable stabilizing agents. The vaccine is then freeze-dried

Identification

LAPINISED VACCINE. Administer 0.5 ml intravenously into one or more non-immunised rabbits , immunized either with an identical dose of a vaccine of the same type injected by the same route between 10 and 60 days before hand or with a sufficient dose of antiserum administered a few hours before the injection of the vaccine. Twenty-four hours after the injection, start recording the temperature of the rabbits in the mornings and the evenings until the fifth day after the injection. The immunised rabbits do not exhibit a rise in temperature of more than 1.5°. The test is not valid unless the nonimmunised rabbits exhibit a rise in temperature of not less than 1.5°.

CELL CULTURE VACCINE. For non-lapinised vaccines prepared in cell cultures, on administration to pigs immunised with the vaccine specific neutralizing antibodies develop.

Tests

Test for extraneous pathogens. The vaccine mixed with a mono specific antiserum does not cause cytopathic effects in susceptible cell cultures. The cells also show no evidence of the presence of haemadsorbing agents and the cell-culture fluids are free of haemagglutinating agents when tested with chicken erythrocytes.

Water (2.3.43) Not more than 3.0 per cent.

Safety. Inject intramuscularly 10 times the minimum dose stated on the label into each of three healthy piglets, between 6 and 7 weeks old, free from swine fever virus antibodies. Observe the animals for 21 days. Temperature curve should be normal and animals remain in apparent good health and display normal growth.

Inject intracerebrally 0.03 ml of the vaccine, reconstituted in a manner that 1.0 ml contains 1 ml dose, into each of ten mice, weighing between 11g and 15g. Observe the mice for 21 days. If more than two mice die within the first 48 hours repeat the test. The mice show no abnormalities attributable to the vaccine within the third and twenty-first day after the injection.

Virus titre. Not less than minimum virus titre per dose stated on the label, determining the titre in a suitable cell culture.

Sterility (2.2.11). Complies with the test for sterility.

Potency. All the animals are healthy and must have had no contact with swine fever virus and serologically must be free from CSF and BVDV antibodies. Use four healthy piglets, between 6 and 7 weeks old, for each of the 1:50, 1:200 and 1:400 dilutions of the vaccine prepared in a suitable diluent or buffer. Inject intramuscularly 1 ml of these dilutions into each of the piglets in respective groups. Use two healthy susceptible piglets of the same stock and age as control animal group. After 21 days, inoculate intramuscularly with a sufficient quantity of the challenge virus in each vaccinated piglet and in each of the two unvaccinated control animals so that at least one of the two unvaccinated control animals die within 7 to 14 days. Observe the vaccinated animals for 14 days. Calculate the number of PD_{50} contained in the vaccine by standard statistical methods from the number of animals, which survive without showing any signs of swine fever. The vaccine contains not less than 100 PD_{50} per dose. The test is not valid unless the control animals die within 7 to 14 days after inoculation. PD_{50} correlation studies with virus titres can replace the potency test on routine basis.

If the test for potency has been carried out with satisfactory results on a representative batch of vaccine, this test may be omitted as a routine control on other batches of vaccine prepared from the same seed lot, subject-to agreement by the competent authority.

Labelling. The label states (1) the minimum dose; (2) the recommended routes of administration; (3) the name of any added adjuvant.

Tetanus Veterinary Vaccine

Tetanus Vaccine for Veterinary Use is a preparation of the neurotoxin of *Clostridium tetani* treated in a manner that eliminates toxicity while maintaining adequate immunogenic properties.

Production

The *C. tetani* strain used for production is cultured in a suitable medium. The toxin is purified and then detoxified or it may be detoxified before purification. The antigenic purity is determined in Lf units of tetanus toxoid per milligram of protein and shown to be not less than the value approved for the particular product.

Choice of vaccine composition

The *C. tetani* strain used in the preparation of the vaccine is shown to be satisfactory with respect to the production of the

neurotoxin. The vaccine is shown to be satisfactory with respect to safety and immunogenicity for each species of animal for which it is intended. As part of the studies to demonstrate these characteristics, the tests described below may be used.

Production of antigens. The production of the neurotoxin of *C. tetani* is verified by a suitable immunochemical method carried out on the neurotoxin obtained from the vaccine strain under the conditions used for the production of the vaccine.

Safety. Carry out the test for each recommended route of administration and species of animal for which the vaccine is intended; use animals of the minimum age recommended for vaccination and of the most sensitive category of the species.

Use not less than 15 animals, free from antitoxic antibodies for each test. Administer a double dose of vaccine to each animal. Administer a single dose of vaccine to each animal after the interval stated on the label. Observe the animals until 14 days after the last administration. The vaccine complies with the test if no animal shows abnormal local or systemic signs of disease or dies from causes attributable to the vaccine.

DETOXIFIED HARVEST

Absence of toxin and irreversibility of toxoid. Carry out a test for reversion to toxicity on the detoxified harvest using 2 groups of 5 guinea-pigs, each weighing between 350 to 450 g; if the vaccine is adsorbed, carry out the test with the shortest practical time interval before adsorption. Prepare a dilution of the detoxified harvest so that the guinea-pigs each receive 10 times the amount of toxoid (measured in Lf units) that will be present in a dose of vaccine. Divide the dilution into 2 equal parts. Keep one part at 5 ± 3° and the other at 37° for 6 weeks. Attribute each dilution to a separate group of guinea-pigs and inject into each guinea-pig the dilution attributed to its group. Observe the animals for 21 days. The toxoid complies with the test if no guinea-pig shows clinical signs of disease or dies from causes attributable to the neurotoxin of *C. tetani*.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers. The containers are closed so as to avoid contamination.

Identification

Carry out test A if permitted by the nature of the adjuvant. Otherwise carry out test B.

A. Separate the toxoid from the adjuvant. For vaccines adsorbed on aluminium hydroxide, the following treatment is suitable. Dissolve sufficient sodium citrate in the vaccine under examination to give a 10 per cent w/v concentration. Maintain at 37° for about 16 hours and centrifuge. The clear supernatant liquid reacts with a suitable tetanus antitoxin and yields a precipitate.

B. When inoculated into healthy susceptible animals, the vaccine stimulates the formation of antitoxin to the neurotoxin of *C. tetani* or protects the animals against the paralytic effects of the toxin.

Tests

Safety. Inject 5 ml of the vaccine subcutaneously as two equally divided doses at separate sites into each of five guinea pigs, each weighing between 350 and 450g. Observe the guinea pigs for 21 days. None of the animals shows any symptoms of tetanus or dies from tetanus. If more than one animal dies of non-specific causes, repeat the test. No animal dies in the second test.

Sterility (2.2.11). Complies with the test for sterility.

Potency. *Test A may be omitted if test B is carried out. Test B may be ommited if test A is carried out.*

A. Inject subcutaneously each of ten guinea pigs, each weighing between 350 and 450 g, with a quantity of the vaccine not more than the minimum dose stated on the label as the primary dose, and 28 days later with a quantity of the vaccine not more than the minimum dose stated on the label as the secondary dose. Fourteen days after the second dose, collect the blood from each guinea pig, pool the sera and determine the antitoxin titre by the biological assay of *C. tetani* antitoxin described below.

1 ml of serum contains not less than 7.5 IU per ml or, for vaccine intended for use in equine, not less than 30 IU per ml.

When *C. tetani* vaccine is presented as a component of a mixed vaccine intended for use in animals other than equine and the potency test of the other component or components normally carried out using rabbits, the potency test described above may be carried out using ten healthy rabbits, between 3 and 6 months old. 1 ml of serum contains not less than 2.5 Units.

Biological assay of *C. tetani* antitoxin

The potency of *C. tetani* antitoxin is determined by comparing the dose necessary to protect mice or other suitable animals against the toxic effects of a fixed dose of *C. tetani* toxin with the quantity of a Standard preparation of *C. tetani* antitoxin necessary to give the same protection. For this purpose, the Standard preparation of *C. tetani* antitoxin and a suitable preparation of *C. tetani* toxin are required.

The test dose of the toxin is determined in relation to the Standard preparation of antitoxin and the potency of the preparation under examination is then determined in relation to the Standard preparation using the test toxin.

Standard preparation

The Standard preparation is the 2nd International standard, established in 1969, consisting of freeze-dried hyperimmune horse serum (supplied in ampoules containing 1400 Units) or another suitable preparation, the potency of which has been determined in relation to the International standard.

Suggested method

NOTE *— The severity of tetanic paralysis to be regarded as the end-point is such that the paralysis is readily recognised but not sufficiently extensive to cause significant suffering. For human reasons the animals should be examined at least twice a day and should be killed as soon as the end-point is reached.*

In practice, when using high levels of toxin to determine the test dose, or when using low levels of antitoxin in the preliminary and final tests, the development of paralysis is so rapid that the defined end-point is usually synchronous with death. Where death occurs, the combined totals of animals dying or reaching the paralytic end-point are used in the calculations.

Preparation of test toxin. Prepare *C. tetani* toxin by growing *C. tetani* in liquid culture for 8 to 10 days and then adding 1 volume of a sterile filtrate of the culture to 1 or 2 volumes of *glycerine*. Store at 0° or at temperatures slightly below it. The toxin may be dried by a suitable method.

Selection of test toxin. Select toxin for use as the test toxin by determining the following quantities:

LP/10 dose (Limes paralyticum). This is the smallest quantity of toxin that when mixed with 0.1 Unit of antitoxin and injected subcutaneously into mice (or guinea pigs) causes tetanic paralysis in the animals on or by the fourth day after injection.

Paralytic dose 50. This is the quantity of toxin that when injected subcutaneously into mice (or guinea pigs) causes tetanic paralysis in one-half of the animals injected on or by the fourth day after injection. A suitable toxin is one that contains not less than 1000 paralytic dose 50 in an LP/10 dose.

Determination of test dose of toxin. Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid. Reconstitute or dilute the Standard preparation with a suitable liquid to give a solution containing 0.5 Unit in 1 ml.

Prepare mixtures of the solution of the Standard preparation and the solution of the test toxin such that each mixture contains 0.1 Unit of antitoxin in the volume selected for injection and one of a series of graded volumes of the solution of the toxin, separated from each other by steps of not more than 20 per cent and covering the expected end-point. Adjust each mixture to the same final volume (0.4 to 0.6 ml if mice are used or 4.0 ml if guinea-pigs are used) with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes and then inject a dose of the selected volume of each mixture subcutaneously into each of not less than 2 animals of the group to which each mixture has been allocated. Observe the animals for 4 days and record daily the degree of tetanus developing in each group of animals. Repeat the determination at least once, add together the results of the separate tests that have been made with mixtures of the same composition such that a series of totals is obtained and determine the mean values. The test dose of the toxin is the amount present in that mixture that causes tetanic paralysis in one-half of the total number of animals injected with it. When the test dose of the test toxin has been determined, a concentrated solution of the test toxin may be prepared in a mixture consisting of 1 volume of *saline solution* and 1 or 2 volumes of *glycerine*. This concentrated solution may be stored frozen and diluted as required. The specific activity of such a solution must be determined at frequent intervals.

Determination of potency of the antitoxin.

Preliminary test. Measure or weigh a quantity of the test toxin and dilute with or dissolve in a suitable liquid such that the solution contains 5 test doses per ml. Prepare mixtures of the solution of the test toxin and the preparation under examination such that for each mixture the volume selected for injection contains the test dose of toxin and one of a series of graded volumes of the preparation under examination. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixtures to stand at room temperature, protected from light, for 60 minutes. Inject a dose of the selected volumes of each mixture subcutaneously into each of not less than two animals of the group to which each mixture has been allocated. Observe the animals for 4 days and record daily the degree of tetanus developing in each group of animals. From the results select suitable mixtures for the final test.

Final test. Prepare similar fresh mixtures of the test toxin and the preparation under examination such that for each mixture the volume selected for injection contains the test dose of toxin and one of a series of graded volumes of the preparation under examination, separated from each other by steps of not more than 20 per cent and covering the expected end-point as determined in the preliminary test. Prepare further mixtures with the same amount of test toxin and graded volumes of the Standard preparation, centered on 0.1 Unit in the volume selected for injection to confirm the test dose of the toxin. Adjust each mixture to the same final volume with a suitable liquid. Allow the mixture to stand at room temperature, protected from light, for 6 minutes. Inject a dose of the selected volume of each mixture subcutaneously into each of not less than two animals of the group to which each mixture has been allocated. Observe the animals for 4 days and record daily the degree of

tetanus developing in each group of animals. The mixture of antitoxin under examination that contains 0.1 Unit in the volume injected is that mixture which causes tetanic paralysis in the same, or almost the same number of animals as the mixture containing 0.1 Unit of the Standard preparation in the volume injected. Repeat the determination at least once and calculate the average of all valid estimates. Estimates are not valid unless the Standard preparation gives a result within 20 per cent of the expected value.

Limits of error. For the suggested method, the limits of error (P = 0.95) have been estimated to be 85 to 114 per cent when two animals are used per dose, 91.5 to 109 per cent when three animals are used per dose, and 93 to 108 per cent when six animals are used per dose.

B. Carry out the biologycal assay of adsorbed tetanus vaccine as stated under Tetanus Vaccine (Adsorbed).

This method may only be used for those preparations for which it has been shown to be suitable and in particular may not be suitable for vaccine with an oil adjuvant. Where this alternative method is used the estimated potency is not less than 150 IU in the smallest dose stated on the label.

Labelling. The label states (1) the name of the adjuvant used; (2) the preparation should be shaken before use.

Theileriosis Vaccine, Live

Theileriosis Vaccine, Live is a lymphoblast cell culture containing *Theileria annulata* macroschizonts attenuated by passage in such a manner that it remains avirulent while it retains its immunogenicity. The concentrate of the vaccine may be diluted with a suitable diluent after thawing.

Production

Production is approved by a seed lot system. Each lot of the seed stock is tested for safety and potency by methods described below. If the immunogenicity tests have been performed with satisfactory results on a representative batch of the vaccine from the seed lot, they may be omitted as a routine control test during production on other batches of the vaccine prepared from the same seed lot.

Identification

Protects susceptible cattle against theileriosis.

Tests

Safety. Inject each of two healthy susceptible cattle not less than 9 months old with twice the dose as recommended on the label. Observe the animals for 30 days. None of the animals shows systemic reactions other than mild pyrexia and mild swelling of superficial lymph nodes. No schizonts/piroplasms should be seen in the blood smears/lymph node smear.

Cell count. Contains not less than 2 million live lymphoblast cells in each dose.

Sterility (2.2.11). Complies with the test for sterility.

Potency. Inject each of three susceptible cattle not less than 9 months old with the minimum dose by the route stated on the label. Use two cattle of the same stock and age as controls. After 30 days, challenge each of the vaccinated as well as the control animals with a preparation of gut homogenate of ticks containing suitable quantity of sporozoites to infect adult cattle. Observe the animals for 30 days; none of the vaccinated animals shows any abnormal signs. The test is not valid unless both the control animals show typical signs of theileriosis. If these tests have been performed with satisfactory results on a representative batch of the vaccine from the seed lot, they may be omitted by the manufacturer as a routine control on other batches of the vaccine prepared from the same seed lot.

Labelling. The label states (1) the number of doses in the container; (2) the recommended dose; (3) the method of thawing and reconstitution; (4) that the reconstituted vaccine should be used within 3 hours after thawing and reconstitution.

VETERINARY DIAGNOSTICS MONOGRAPHS

Avian Mycoplasma Antigen

Mycoplasma Antigen shall be prepared either from *Mycoplasma gallisepticum* or *Mycoplasma synoviae*, grown in broth cultures that are inactivated and standardized. Plate antigen shall be stained with an acceptable dye. Each intermediate antigen lot shall be tested for purity, density, and preservative.

Purity. Intermediate antigen lot sample should be free from extraneous organisms as determined by microscopic examination and Gram staining.

Density. A 2.5 ml of sample of intermediate lot shall be diluted with 2.5ml of buffer solution, formulated in the same manner as the vehicle of the antigen being tested in a modified Hopkin's tube and then sedimented by centrifugation. If the packed cell volume of the sample is not 1.2± 0.4 per cent, the intermediate antigen lot is unsatisfactory.

Preservative

Phenol contents of antigen lot shall be 0.25±0.05 per cent.

A batch of finished product should be tested for Identification, Homogeneity and Hydrogen Ion Concentration. The batch of finished product found unsatisfactory for any prescribed test shall not be released.

Identification

Gives specific agglutination when mixed with the serum of birds infected with *M. gallisepticum* or *M. synoviae* but fails to react with serum from healthy birds.

Tests

Homogeneity. Antigen shall show no evidence of auto agglutination or unusual appearance such as presence of large visible particles.

pH (2.4.24). Hydrogen ion concentration shall be determined with a pH meter which has been standardized with buffer just prior to use. The pH of *Mycoplasma gallisepticum* antigen shall be 6.0±0.2. The pH of *Mycoplasma synoviae* antigen shall be 7.0±0.2.

Storage. When stored under the prescribed conditions, the antigen may be expected to retain its potency for not less than 1 year from the date the potency was determined.

Labelling. The label states (1) strains used for preparation; (2) the dose of test.

Brucella Abortus Coloured Antigen

Brucella abortus Coloured Antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99, which are coloured by the addition of crystal violet and brilliant green, in phenolised glycerine saline.

Identification

Gives specific agglutination when mixed with the serum of animals infected with *Brucella* organisms.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Assay. Mix 0.5 ml with 4.5 ml of saline solution contained in a Hopkin's graduated tube and centrifuge at 3,000 rpm for 60 minutes. The volume of the packed bacterial cells is not less than 11 per cent of the total volume.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Brucella Abortus Milk Ring Test Antigen, Hematoxylin Stained

Brucella abortus Milk Ring Test Antigen, Hematoxylin Stained is a suspension of a pure smooth culture of *Brucella abortus* strain 99 bacteria stained with hematoxylin and suspended in *saline solution* containing 0.5 per cent w/v of *phenol*, the reaction of which is adjusted to pH 4.0 with *0.1 M citric acid* or with *0.5 M disodium hydrogen phosphate,* as appropriate.

For standardisation, the stained suspension is washed by centrifugation in a solution containing 6.4 g of *sodium chloride*, 1.5 ml of *lactic acid* and 4.4 ml of 10 per cent w/v solution of *sodium hydroxide* in 1,600 ml of *distilled water*, the pH of the solution being adjusted to 4.0. The washed cells are resuspended in *phenol saline solution* and the packed cell volume of the final product is adjusted to 4 per cent v/v.

Identification

The antigen forms a blue-coloured ring in the cream layer when mixed with milk from animals suffering from Brucellosis.

Tests

Sterility (2.2.11). Complies with the tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Sensitivity. It has the same sensitivity as that of a standard antigen when tested by the milk ring test.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Brucella Abortus Milk Ring Test Antigen, Tetrazolium-Stained

Brucella abortus Milk Ring Test Antigen, Tetrazolium-Stained is a suspension of a pure smooth culture of *Brucella abortus* strain 99 bacteria stained supravitally with *2,3,5-triphenyl-tetrazolium chloride* in *saline solution* containing 1 per cent v/v of *glycerin* and 1 per cent w/v of *phenol.* Smooth strain of *Brucella abortus* strain 99 is grown on *potato infusion agar* for 48 to 72 hours in roux flasks, at 37°. Condensation fluid if any is pipetted off before washing. Each flask is washed with about 20 ml of *normal saline*. The pooled washing is filtered through a gauze and the filtrate is collected in a measuring cylinder. To every 500 ml of the filtrate 1g of *2, 3, 5 triphenyl-tetrazolium chloride* is added immediately. The container is shaken for thirty minutes till the *tetrazolium salt* is dissolved. The product is taken out and kept in at 37° for two hours. After incubation the product is heated at 65° in a *water* bath for thirty minutes. It is cooled and centrifuged at 3,000 rpm for one hour. The supernatant is pipetted off and sediment is suspended in *normal saline* containing 1 per cent *glycerol* and 1 per cent *phenol* and filtered through sterile cotton wool. This forms concentrated antigen.

For the standardisation of the stained antigen, one ml of an aliquot of the suspension representing the initial undiluted suspension is taken in each of 6 test-tubes to which increasing quantities (0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml) of *saline solution* containing 1 per cent v/v of *glycerin* and 1per cent w/v of *phenol* are added. The contents of each tube are then diluted 10-fold with the same diluent and serve as antigen for the tube agglutination test with the reference standard antiserum. Thus six sero-reactions will be carried out. During this procedure the concentrated stained microbial suspension is kept at a temperature between 2° and 8°. The agglutination reactions are read after 48 hours. The dilution which gives 50 per cent agglutination with a 1 in 500 final dilution of the standard antiserum is taken as the final dilution for the preparation.

Identification

The antigen forms a cherry-red ring in the cream layer when mixed with milk from animals infected with Brucellosis.

Tests

Sterility (2.2.11). Complies with the tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Brucella Abortus Plain Antigen

Brucella abortus Plain Antigen is a suspension of a pure smooth culture of killed *Brucella abortus* strain 99 bacteria in *phenol-saline solution.*

Identification

Gives specific agglutination when mixed with the serum of animals infected with *Brucella abortus* organisms.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Sensitivity. Gives 50 per cent agglutination on incubation at 37° for 20 ± 1 hours with a 1 in 500 dilution of a standard *Brucella antiserum* containing 1,000 International Units.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Brucella Abortus Rose Bengal Plate Test Antigen (Strain 99)

Brucella abortus Rose Bengal Plate Test Antigen (Strain 99) is a suspension of inactivated bacteria from a pure smooth culture of *Brucella abortus* strain 99. The bacteria being stained with *Rose Bengal*, in a *buffered solution* prepared by adding 540 ml of *lactic acid* to 2,000 ml of *phenol saline solution* and diluting to 6,000 ml.

The antigen is used when an approximate idea of the extent of the infection in a herd is required to be assessed with minimum effort and maximum speed or as a screening test to assess whether an outbreak of abortions is due to Brucellosis.

Identification

Gives the specific agglutination when mixed with the serum of animals infected with *Brucella* organisms.

Tests

pH (2.4.24). 3.6 to 3.7.

Sterility (2.2.11). Complies with tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Assay. To 0.5 ml quantities taken in each of six Hopkin's graduated tubes or graduated haematocrit tubes, add 4.5 ml of saline solution in each tube, mix and centrifuge at 3,000 rpm

for 60 minutes. The packed bacterial cell volume is not less than 8 per cent.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Brucella Abortus Working Standard Serum

Brucella abortus Working Standard Serum is serum of cattle infected with *Brucella abortus* biotype I, or serum raised in rabbits against smooth cultures of *B. abortus* strain 99 or strain 544 which is suitably diluted with healthy cattle serum or rabbit serum as appropriate. It contains 0.01 per cent w/v of *thiomersal* as antibacterial preservative.

The serum is suitably standardised so that a 1 in 500 dilution gives 50 per cent agglutination in tube agglutination test in comparison with the *Brucella abortus* standard serum.

Identification

Gives specific agglutination when mixed with a pure smooth culture of *Brucella abortus* organisms.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Assay. When tested with standardised *Brucella abortus* tube test antigen, gives 50 per cent agglutination at 1 in 500 final serum dilution in tube agglutination test in comparison with the *Brucella abortus* standard serum.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Johnin Purified Protein Derivative

Johnin Purified Protein Derivative is a preparation of a fluid synthetic medium in which *Mycobacterium paratuberculosis* has been grown and which has been freed of the bacilli by filtration. The active fraction of the filtrate, which is predominantly protein in nature, is isolated by precipitation, washed and re-dissolved. It is then distributed in sterile glass containers and sealed so as to exclude micro-organisms. It may contain a suitable preservative. It reveals delayed hypersensitivity in animals sensitised by *M. paratuberculosis*.

Description. A yellowish-brown liquid.

Identification

Inject intradermally small doses of the preparation into suitable guinea-pigs that have been sensitised with *M. paratuberculosis;* hot, painful oedematous swellings occur at the sites of inoculation after 48 hours.

Tests

pH (2.4.24). 6.5 to 7.5.

Phenol (2.3.36) (if present). Not more than 0.5 per cent w/v.

Sterility (2.2.11). Complies with the tests of sterility, Method A.

Abnormal toxicity (2.2.1). Inject 0.5 ml subcutaneously into each of two guinea-pigs. Observe the animals for 7 days; none of the guinea pigs shows significant local or systemic reaction.

Potency. Carry out the biological assay of Johnin Purified Protein Derivative described below:

Biological assay of Johnin purified protein derivative

The potency of Johnin purified protein derivative is determined by comparing the reactions produced in sensitised guinea-pigs by intradermal injection of a series of dilutions of the preparation under examination with those produced by known concentrations of the Standard preparation.

Standard preparation

The Standard preparation is Johnin purified protein derivative, maintained by the Indian Veterinary Research Institute, Izatnagar, or another suitable preparation, the potency of which has been determined in relation to the Standard preparation.

Suggested method

Sensitise five guinea-pigs, each weighing between 300 and 450 g, by deep intramuscular injection of 0.5 ml of a suspension in *saline solution* containing 0.1 µg of moist growth from solid slants of live *M. paratuberculosis*. After a period of not less than 3 weeks carry out the following test. Use two healthy animals of the same weight range and from the same stock as controls. Shave the flanks to provide space for not more than 4 injection sites on each side. Prepare 1:500, 1:1000, 1:2000 and 1:4000 dilutions of each of the Standard preparation and the preparation under examination in *phosphate-buffered saline pH 7.4* containing 0.005 per cent w/v of *polysorbate* 80. Using not less than 2 doses of each dilution of the Standard preparation and the preparation under examination, inject each dose intradermally in the same volume (0.1 to 0.2 ml) to the available sites in a random manner in Latin-square design.

The sensitised guinea-pigs exhibit hot, painful and oedematous swellings typical of *M. paratuberculosis* at the sites of injection persisting for not less than 72 hours. The test is not valid unless the control animals fail to produce

such reactions. With the help of callipers, measure the skin thickness around the sites of injection, 72 hours after inoculation.

Calculate the potency using standard statistical methods on the basis that the skin thickness are directly proportional to the logarithms of the concentrations of the Johnin Purified Protein Derivative.

Assay. To 5 ml add 2.5 ml of water and 2.5 ml of a 40 per cent w/v solution of trichloro acetic acid, mix, allow to stand for 30 minutes and centrifuge for 15 minutes. Discard the supernatant liquid and dissolve the residue in 0.5 ml of *5 M sodium hydroxide* solution. Transfer the solution to a Kjeldhal flask with the aid of 6 ml of *water*. Add about 0.1 g of a mixture of 100 parts of potassium sulphate, 10 parts of cupric sulphate and 5 parts of selenium dioxide and 1 ml of nitrogen-free sulphuric acid. Heat until the water evaporates. Continue the heating until a brown deposit appears. Dissolve the deposit in 0.5 ml of *hydrogen peroxide* solution, continue heating until white fumes appear and boil rapidly for at least 10 minutes. If a brown deposit again appears add a further 0.5 ml of hydrogen peroxide solution. Transfer to an ammonia distillation apparatus with the aid of 5 ml of water and add 5 ml of a 50 per cent w/v solution of sodium hydroxide to form a lower layer. Distil for 3 minutes, collecting the distillate in a mixture of 5 ml of a 2 per cent w/v solution of boric acid and 0.05 ml of a solution containing 0.066 per cent w/v of methyl red and 0.033 per cent w/v of bromocresol green in ethanol (95 per cent). Titrate with 0.00447M sulphuric acid. Repeat the operation using 5 ml of the water in place of the preparation under examination. The difference between the titrations represents the ammonia liberated by the substance under examination.

1 ml of *0.00447 M sulphuric acid* is equivalent to 0.875 mg of purified protein derivative.

Storage. As stated under Veterinary Diagnostics.

Labelling. The label complies with the requirements stated under Veterinary Diagnostics and also states (1) the total volume in the container; (2) the name and percentage of any added preservative.

Mallein Purified Protein Derivative

Mallein Purified Protein Derivative is a preparation of a fluid synthetic medium in which *Pseudomonas mallei* (*Burkholderia mallei*) has been grown and which has been freed of the bacilli by filtration. The active fraction of the filtrate, which is predominantly protein in nature, is isolated by precipitation, washed and re-dissolved in *phosphate buffered saline* at about neutral pH. It is then distributed in sterile containers that are inert towards the contents and sealed so as to exclude micro-organisms.

For standardisation, four ponies previously sensitised with *P. mallei* and two healthy ponies are injected intradermo-palpebrally with 0.2 ml of the preparation near the rim of the lower eye-lid of one eye. Typical reaction such as painful swelling of the palpebral tissue with mucopurulent discharge from the eye of sensitised animals and no such reaction in the healthy ponies should be seen. A similar test is performed with the Standard preparation maintained by the Indian Veterinary Research Institute, Izatnagar. When the reactions of the two preparations are comparable the batch is considered fit for use.

Mallein Purified Protein Derivative contains not less than 0.95 mg per ml and not more than 1.05 mg per ml of purified protein derivative.

Caution - Mallein Purified Protein Derivative is not dangerous to humans, but the organism from which it is prepared is pathogenic to man and may be fatal if an infection is not treated properly. Treatment should begin promptly if an infection is suspected.

Description. A yellowish to brown, viscous liquid.

Identification

Inject intradermally small doses of the preparation into suitable guinea-pigs that have been sensitised with killed *P. mallei* in an oily adjuvant; hot, tense, painful oedematous swellings occur at the sites of inoculation after 48 hours.

Tests

pH (2.4.24). 6.5 to 7.5.

Phenol (2.3.36). (*if present*). Not more than 0.5 per cent w/v.

Sterility (2.2.11). Complies with the test of sterility, with modifications stated under Johnin Purified Protein Derivative.

Abnormal toxicity (2.2.1). Inject 0.5 ml subcutaneously into each of two guinea pigs. Observe the animals for 7 days; none of the guinea pigs shows significant local or systemic reaction.

Assay. Carry out the Assay described under Johnin Purified Protein Derivative using 2.5 ml.

Storage. As stated under Veterinary Diagnostics.

Labelling. The label complies with the requirements stated under Veterinary Diagnostics and also states (1) the total volume in the container; (2) the name and percentage of any added preservative.

Purified Protein Derivative (PPD), Bovine Tuberculin

Purified Protein Derivative (PPD), Bovine Tuberculin is a preparation of a fluid synthetic medium in which *M. bovis*

AN5 has been grown and which has been freed of the bacilli by filtration. The final sterile product is distributed in sterile, tamper-evident glass containers, which are then sealed to prevent contamination with extraneous microorganisms.

Description. A yellowish-brown viscous liquid, or dry yellowish-brown powder or pellet.

Identification

Inject intradermally a range of graded doses at different sites into suitable albino guinea pigs sensitised with tuberculosis. Depending upon the allergic status of the animal, the magnitude of dose and specificity of the product, reactions occur at the points of injection as diffused oedematous swellings with erythema with or without necrosis. When similar injections are given to non-sensitised guinea pigs no such reactions occur.

Tests

The preparation, reconstituted if necessary with a suitable liquid and diluted to provide a concentration appropriate to the particular test, complies with the requirements stated under Veterinary Diagnostics with the following modifications.

pH (2.4.24). 6.5 to 7.5.

Phenol (*if present*) (2.3.36). Not more than 0.5 per cent w/v.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Abnormal toxicity (2.2.1). Inject subcutaneously 0.5 ml of the preparation under examination into each of two guinea-pigs weighing not less than 250 g. No abnormal effects are produced within 7 days.

Potency. Carry out the biological assay of bovine tuberculin purified protein derivative described below:

Biological assay of bovine tuberculin purified protein derivative

The potency of bovine tuberculin purified protein derivative is determined by comparing the reactions produced in sensitised guinea-pigs by intradermal injection of a series of dilutions of the preparation under examination with those produced by known concentrations of the Standard preparation calibrated in International Units. Sensitise not less than 9 albino guinea-pigs each weighing between 300 and 450 g by deep intramuscular injection of 0.0001 mg of wet mass of living *M. bovis* of strain AN5 suspended in 0.5 ml of *normal saline solution*. Not less than 4 weeks after the sensitisation of the guinea-pigs, shave their flanks to provide space for not more than 4 injection sites on each side.

Standard preparation

The International Standard for Purified Protein derivative (PPD) of *Mycobacterium bovis* Tuberculin was donated to National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK by Central Diergeneeskundig, Netherlands. With effect from 1st June 1998, the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK is the custodian and distributor of this material. Each ampoule contains 58,000 International Units of PPD and when reconstituted with 1.8 ml of diluting fluid will contain 1 mg PPD and 32,500 I.U. per ml.

Suggested method

Sensitise nine guinea-pigs, each weighing not less than 400 g. Inject each animal intramuscularly on the medial side of thigh with 0.0001 mg wet weight of *M. bovis*, live strain of *Mycobacterium bovis* suspended in 0.5 ml *physiological saline*. Test three dilutions of each of 3 preparations. Since it is practicable to give only 8 injections to an individual animals, a balanced incomplete Latin Square design is used, in which a different one of the 9 dilution is omitted from each animal. The remaining 8 dilutions are allocated to 4 sites.

Diluting fluid for assay

The diluent consists of isotonic *phosphate-buffered saline pH 7.3*, containing *tween 80* (0.0005 per cent) and is prepared by adding 0.5 ml of 1 per cent w/v solution of *tween 80* in *distilled water* to 1 litre of the following solution: $Na_2HPO_4 2H_2O$ - 7.60 g; KH_2PO_4 - 1.45 g; NaCl - 4.80 g; *distilled water* - 1 litre. Tuberculin diluted 1 in 100, 1 in 500 and 1 in 2,500 with 0.1 ml inoculum or 1 in 200, 1 in 1,000 and 1 in 5,000 ml with 0.2 ml inoculum generally will produce satisfactory results in guinea pigs.

Measure the skin thickness at each site at the time of injection and after 72 hours. Calculate the results using standard statistical methods on the basis that the diameters of the lesions are directly proportional to the logarithms of the concentrations of the tuberculin.

Storage. As stated under Veterinary Diagnostics.

Labelling. The label insert states (1) the number of units per dose of 0.1 ml or per ml or per mg; (2) the total volume in the container (for liquid preparation); (3) the name and proportion of any added substances; (5) the strain used; (6) the storage conditions; (7) the date after which the contents are not intended to be used.

Salmonella Abortus Equi H Antigen

Salmonella abortus Equi H Antigen is a suspension of killed organisms derived from a pure smooth culture of actively motile *Salmonella abortus equi*.

Identification

Gives specific agglutination when mixed with the serum of animals infected with *S. abortus equi* organisms.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Opalescence of suspension. The opalescence of the preparation under examiantion corresponds to Brown's opacity standard tube No. 2.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Storage. As stated under Veterinary Diagnostics.

Salmonella Pullorum Antigen

Salmonella pullorum Antigen is a suspension of a pure smooth culture of representative strains of *Salmonella pullorum* which are of known antigenic composition, high agglutinability, but are not sensitive to negative and non-specific serum. Each intermediate lot shall be tested for purity, density, and preservative.

Purity. Intermediate lot sample should be free from extraneous organisms as determined by microscopic examination and Gram staining.

Density. The bacterial density shall be 80±15 times Mac-Farland Number 01 for stained antigen and 50 ±10 times Mac-Farland Number 01 for tube antigen.

Preservative

Formalin contents of the intermediate lot of coloured antigen shall be 1.0±0.2 per cent. Phenol contents of plain antigen shall be 0.55±0.5 per cent.

A batch of finished product should be tested for Identification, Homogeneity and Hydrogen Ion Concentration. The batch of finished product found unsatisfactory for any prescribed test shall not be released.

Identification

Gives specific agglutination when mixed with the serum of birds infected with *S. pullorum* or *S. gallinarum* but fails to react with serum from healthy birds.

Tests

Homogeneity. Antigen shall show no evidence of auto agglutination or unusual appearance such as presence of flakes.

pH (2.4.24). Hydrogen ion concentration shall be determined with a pH meter which has been standardized with pH 4.0 buffer just prior to use. The pH of stained antigen shall be 4.6±0.4. No pH level is specified for pullorum tube antigen but after dilution, as recommended for use it shall have a pH of 8.2 to 8.5.

Storage. When stored under the prescribed conditions, the antigen may be expected to retain its potency for not less than 1 year from the date the potency was determined.

Labelling. The label states (1) strains used for preparation; (2) the dose of test.

Salmonella Pullorum Plain Antigen

Salmonella pullorum Plain Antigen is a suspension of dead bacterial cells of a pure smooth culture of a suitable strain of *Salmonella pullorum* in *saline solution* containing 0.5 per cent w/v of *phenol.*

Identification

Gives specific agglutination when mixed with the serum of birds infected with of *S. pullorum* or *S. gallinarium* but fails to react with serum from healthy birds.

Tests

Opalescence of suspension. The opalescence of the preparation under examination corresponds to Brown's opacity standard tube No. 1.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

Salmonella Pullorum Positive Serum

Salmonella pullorum Positive Serum is a liquid or freeze-dried antiserum raised against a suitable smooth strain of *S. pullorum* in rabbits. The liquid preparation contains 0.01 per cent w/v of *thiomersal* or other suitable preservative.

Identification

Gives specific agglutination when mixed with a smooth strain of *S. pullorum* and gives a titre 1:1000 with tube test antigen.

Tests

Sterility (2.2.11). Complies with tests for sterility.

Other tests. Complies with the tests stated under Veterinary Diagnostics.

Storage. As stated under Veterinary Diagnostics.

Labelling. As stated under Veterinary Diagnostics.

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