INDIAN PHARMACOPOEIA 6

GUIDE TO FORMATS

INDIAN PHARMACOPOEIA COMMISSSION

INDIAN PHARMACOPOEIA 6

Introduction: The Indian Pharmacopoeia (IP) is a compilation of official standards for drugs manufactured in India. The full name or title of the book is Indian Pharmacopoeia 6, abbreviated to IP 6. Addenda or Supplements to the pharmacopoeia will be numbered with additional characters starting with 1 e.g. Indian Pharmacopoeia 6.1 or IP 6.1 for the first addendum or supplement, Indian Pharmacopoeia 6.2 or IP 6.2 for the second addendum, and so on.

Standards in the IP are expressed in the form of specifications and test methods for determining compliance with such standards. Specifications that are applicable to any pharmaceutical article are compiled in a monograph. A monograph states the quality or test parameters, the acceptance criteria and details of the tests that are to be performed to determine compliance with the criteria. In other words, a pharmacopoeial monograph provides a reliable basis for making an independent and objective judgement as to the quality of a pharmaceutical substance.

As IP standards are statutory, it is important that the contents of monographs are unambiguous, acceptance criteria are clearly spelt out and the methods of evaluation provide all the details for carrying out the tests and assays, including the equipment, reagents and other ancillary materials that are to be used. To ensure that this requirement is uniformly and consistently met, guidance is provided in the following pages on the manner of drawing up of monographs and test methods and other relevant information. The exact manner of describing the tests, standards and reference to the general testing method numbers are also given.

It shall be ensured that statements made in the monographs do not conflict with those stated in the General Notices, General Texts and with any information given in other sections of the Pharmacopoeia.

Contents of the Pharmacopoeia

The technical part of the pharmacopoeia shall be broadly divided into the following sections:

1. Introduction
2. General Notices
3. Monographs
4. Test methods
5. Reagents and Solutions
6. General Texts
7. Index

1. Introduction

The Scientific Director of the Indian Pharmacopoeia Commission (IPC) shall write this part after all the contents of the pharmacopoeia have been finalised. It shall briefly give the background to the edition and describe the salient features including the additions to and deletions from the previous edition.

2. General Notices

The purpose of the General Notices is to provide the basic guidelines to the interpretation and application of the standards, tests, assays and other specifications of the pharmacopoeia, as well as to the statements made in the monographs, test methods and appendices. Included, among other things, is the system of nomenclature of chemical compounds that is to be adopted. Recommendations on storage of drugs and specific labelling requirements may also be given.

3. Formats and Contents of Monographs

General

1. A one-column format shall be used for all the pages of the monographs.
2. The font shall be Arial and the size for the text matter shall be 10 pt as given in the program 'Microsoft Word'.
3. Capital letters, bold and italic types shall not be used indiscriminately since they have a special significance in the Pharmacopoeia.
4. Reagents, buffer solutions, chemicals other substances that are described or defined in the Pharmacopoeia shall be in italics. However, where a specific reagent is prepared for a specific test in a monograph and reference to it is made subsequently in the monograph it need not be in italics.
5. Italic types shall also be used for the systematic names of plants and micro-organisms, and for some sub-headings of tests and texts (such as precautions to be observed while performing the tests, or which identification tests may be omitted etc) and for some parts of the chemical names.
6. The title of any monograph i.e. the name of the Pharmacopoeial substance shall be printed with initial letters in capitals and other letters in small case
7. Titles of monographs and headings of tests shall be in bold letters. The title shall be aligned on the left with the text. Synonyms, if any, shall be printed two spaces below the main title and shall not be in bold letters.
8. Single-line spacing shall be followed and the alignment of the text of the monograph shall be 'justified'. Each test parameter and the accompanying text shall be separated from the other by a space of 1.5 lines.
9. Given in the following pages are directions on the manner in which the various tests and assays are to be described. Where the instructions are in red, the texts shall appear in the monographs in exactly the same way as shown.

A. Active Pharmaceutical Ingredients (APIs) (Bulk Drug Substances) Chemical Excipients	
Title of the Monograph	The following, and in a few cases, some of the following information in this column shall be included in the monographs in the order given below 1. Name of the item printed in bold letters in font Arial size 12 pt (MS Word). The International Non-proprietary Name (INN) approved by the World Health Organization (WHO) shall be used Subsidiary or abbreviated title or synonym (if any) may be shown two spaces below the main title (in ordinary letters) The main monograph headings viz. Identification and Tests etc. shall be in Arial size 11 pt, and the headings of the individual tests in size 10 pt, and all in bold letters.
Example	**Sodium Aminosalicylate** Sodium PAS
Formula	1. Structural (Graphic) Formula 2. The molecular formula on the left and the molecular weight expressed to one decimal place on the right, two spaces below the graphic formula
Chemical name	A statement of the chemical name, two spaces below the molecular formula, where the substance is a distinctly definable chemical entity, as follows: XXX is YYY, where XXX is the name of the item as given in the title of the monograph, and YYY is the chemical name sanctioned and employed by the International Union of Pure and Applied Chemistry (IUPAC).
Examples	Ethionamide is 2-ethylpyridine-4-carbothioamide. Carbamazepine is 5*H*-dibenz(*b*,*f*)azepine-5-carboxamide *Note- Guidance on steriochemical configuration, the sign of the optical rotation of enantiomers etc shall be given in the General Notices of the Pharmacopoeia.*
Statement of purity	A definitive statement of the purity of the article, two spaces below Chemical name, and expressed in the following manner: AB contains not less than X per cent and not more than Y per cent of the chemical entity expressed as the molecular formula, calculated on the dried basis (where a test for loss on drying is specified), or on the anhydrous basis (where a test for water is specified), where AB is the pharmacopoeial name of the article, X and Y are the lower and higher percentage figures, respectively, expressed to one decimal place only.

Examples	Ethionamide contains not less than 98.5 per cent and not more than 101.0 per cent of $C_8H_{10}N_2S$, calculated on the dried basis. Cyclophosphamide contains not less than 98.0 per cent and not more than 102.0 per cent of $C_7H_{15}Cl_2N_2O_2P$, calculated on the anhydrous basis. *Note: With certain articles the measure of purity may not be the content of the chemical entity but some other factor such as potency or Unit of activity.*
Examples	Erythromycin has a potency not less than 920 Units per mg, calculated on the anhydrous basis. Bacitracin Zinc has a potency of not less than 60 Units of bacitracin activity per mg, calculated on the dried basis.
Description	A brief description of the physical form of the material, including colour, texture, whether hygroscopic, odour, if readily apparent, and any other characteristic.
Examples	**Description**. A white, crystalline powder or colourless, transparent crystals, efflorescent. **Description**. A pale yellow oil with slight, but not rancid odour **Description**. A colourless and odourless gas. *Note- The indefinite article 'a' shall be used before the description* *Note:* The following sections deal with the tests to be performed. Where reference is made to a general test procedure, the relevant number of the procedure is mentioned in brackets immediately after the test heading. However, in the following cases, the brackets may appear where the reference to the appendix is needed or at the end of the statement a) thin-layer chromatography, infrared absorption spectrophotometry etc., b) if the general test procedure is amended, the one to be adopted, c) when limits are to be given. In the former case, a dot should be put after the bracket and in the latter, a dot or comma depending on whether the text ends or continues, respectively. There should not be a comma before the brackets.
Examples	**pH** (…….). 3.0 to 4.0 **Arsenic** (……). 5 ml of solution S complies with the limit test for Arsenic (2 ppm) **Related substances**. Determine by thin layer chromatography (…..), coating the plate with zzz
Identification	At least two or three identification tests, starting with physical and instrumental tests and ending with general chemical reactions shall be given. The tests shall be marked with the letters A, B, C and so on followed by a dot and then the text after one space. The texts will naturally vary from test to test but given below is the mode of expression (indicated in red) of certain common tests:

	Infrared absorption spectrophotometry- This shall normally be the first identification test, where applicable. Determine by infrared absorption spectrophotometry (…..) (test method number to be put within the brackets). Where necessary, the specific manner of preparing the sample may be given. Compare the spectrum with that obtained with *yyyRS* (where *yyyRS* is the Reference Substance) or with the reference spectrum of yyy. *Note*- The acceptance criterion need not be repeated in the monographs. It shall be given in the test method itself.
Example	**Identification**. A. Determine by infrared absorption spectrophotometry (2.2.40). Compare the spectrum with that obtained with *ceftazidime RS* or with the reference spectrum of ceftazidime.
	UV Light Absorption (…….). The manner of preparing the solution of the substance under examination shall be given. The specific absorbance determined at the maximum at……nm is ….. to……
Examples	**Identification** Dissolve 20.0 mg in *water* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water*. The specific absorbance (2.2.25) determined at 235 nm is 360 to 390.
	When examined between 230 nm and 360 nm (…….), a 1.0 per cent w/v solution of xxxx shows an absorption maximum at about ….nm
	Thin layer chromatography- *Determine* by thin-layer chromatography (…..), coating *the* plate with *zzz*. *Mobile phase*. The proportions of the constituents of the solvent mixture shall be given *Test solution*. The method of preparation shall be described. *Reference solution* The method of preparation shall be described. Procedure The method shall be described, starting with the volumes of solutions to be applied on the plate, the distance over which the mobile phase shall be allowed to run, treatment of the plate after development and manner of examination of the plate. The criterion for acceptance shall be indicated.
Example	**Identification**. C. Determine by thin-layer chromatography (2.2.27), coating the plate with *silica gel F254*. *Mobile phase*. A mixture of 6 volumes of *butanol*, 26 volumes of *sodium acetate buffer pH 4.5*, 32 volumes of *butyl acetate* and 32 volumes of *glacial acetic acid*. *Test solution*. Dissolve 0.10 g of the substance under examination in a 36 g/l solution of *disodium phosphate* and dilute to 2.0 ml with the same solution. *Reference solution*. Dilute 1 ml of the test solution to 200 ml with 36 g/l solution of *disodium phosphate*.

	Apply to the plate 2µl of each solution. Allow the mobile phase to rise 12 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 * The height to which the mobile phase should be allowed to rise should be given only if it is different from 15 cm, the figure given in the general method. *HPLC test*- Usually when this procedure is used for the identification test the assay is also done by the same procedure. In such cases, the identification test shall state the agreement between the principal peaks in the chromatograms of the test and reference solutions.
Example	**Identification**. D. 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. Where the HPLC test is used only for the identification test and not for the assay the manner of describing the test shall be similar to that given below in the examples for Related substances or Assay where such a test is to be employed, but the acceptance criterion will be as given in the example above except that the words " In the Assay" shall be omitted.
	Chemical reactions- Texts depend on the nature of the tests.
Examples	Dissolve about 10 mg in 1 ml of *sulphuric acid*. An intense yellow colour develops. Dissolve about 10 mg in 2 ml of *dilute hydrochloric acid* and heat on a water-bath for three minutes. Add 3 ml of *sodium carbonate solution* and 1 ml of a 20g/l solution of *sodium nitroprusside*. A violet-red colour develops.
	General chemical reactions-
Examples	**Identification**. E. It gives reaction (a) of sodium (2.3.1) **Identification**. E. The solution prepared for identification test A gives reactions (a) and (b) of potassium (2.3.1).
Appearance of solution	Method of preparing the test solution to be given. The solution is clear (…….) and not more intensely coloured than reference solution xx or The solution is not more opalescent than opalescence standard……..(……..)
Example	**Appearance of solution**. Dissolve 4.0 g in 10 ml of *water*. The solution is clear (2.2.1) and not more intensely coloured than reference solution Y5 (2.2.1) or The solution is not more opalescent than opalescence standard OS2 (2.2.1).

pH	Method of preparation of solution to be given. The pH of the solution is ….. to …..
Examples	**pH**. Dissolve 0.2 g in *water* and dilute to 20 ml with *water*. The pH of the solution is 4.5 to 5.5 (2.2.4) **pH**. The pH of solution S is 4.0 to 5.0 (2.2.4). **pH** (…..). 3.5 to 4.5.
Specific optical rotation	+xxx° to +xxx° or -yyy° to -yyy°. The method of preparing the test solution may be given in some cases. Results to be reported to only one decimal place.
Example	**Specific optical rotation** (2.2.7).+ 70.0° to + 73.0°. Weigh accurately about 0.5 g, dissolve in *water* and dilute to 20.0 ml with the same solvent.
Light-absorbing impurities	Method of preparation of test solution to be given. The absorbance (…….) of the resulting solution, determined at ….nm is not more than…… calculated on the…….basis.
Examples	**Light-absorbing impurities**. Dissolve 0.10 g in a mixture of 1 volume of *1M hydrochloric acid* and 99 volumes of *methanol* and dilute to 10.0 ml with the same mixture of solvents. The absorbance (2.2.25) of the resulting solution, determined within 1 hour of preparing the solution, at 490 nm is not more than 0.07.
Related substances	Details of the method-usually by thin-layer, or liquid chromatography or gas chromatography shall be given.
Example	For Ethosuximide: **Related substances**. Determine by gas chromatography (2.2.28). See details in the Annexure.
Arsenic	Method of preparing the test solution shall be given. The resulting solution complies with the limit test for arsenic (…….) (x ppm).
Example	For Boric Acid **Arsenic**. Dissolve 1.0 g in 50 ml of *water* containing 2 g of *citric acid* and add 0.1 ml of *stannous chloride AsT* and 10 ml of *hydrochloric acid*. The resulting solution complies with the limit test for arsenic (2.4.2) (10 ppm).
Heavy metals	X g complies with limit test Y for heavy metals (a ppm). Prepare the standard using b ml of lead standard solution (..ppm)

Example	**Heavy metals** (2.4.8) 2.0 g complies with limit test C for heavy metals (20 ppm). Prepare the standard using 4 ml of *lead standard solution (10 ppm Pb)*
	Where the method of preparing the test solution is different from the standard one the method shall be described and the manner of doing the test shall then be given.
Example	**Heavy metals**. Dissolve 2.0 g in 4 ml of a 40 g/l solution of *sodium hydroxide* and dilute to 20 ml with *water*. 12 ml of the solution complies with limit test A for heavy metals (2.4.8). Prepare the standard using 1 ml of *lead standard solution (10 ppm Pb)*.
Iron	Method of preparation of test solution to be given The solution complies with the limit test for iron (……ppm)
Example	**Iron** (2.4.9) Dissolve 2.0 g in 20 ml of *water*. The solution complies with the limit test for iron (20 ppm).
Chlorides	Method of preparing the test solution to be given. The solution complies with the limit test for chlorides (……ppm).
Example	**Chlorides** (2.4.4) Dissolve 1.0 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 (50 ppm).
Sulphates	Method of preparing the test solution to be given. The solution complies with the limit test for sulphates (…..ppm).
Example	**Sulphates** (2.4.13) Dissolve 5.0 g in distilled *water* and dilute to 15 ml with *distilled water.* The solution complies with the limit test for sulphates (200 ppm)
Non-volatile substances	Not more than…….per cent. The method of performing the test may be given.
Example	**Non-volatile substances**. Not more than 0.002 per cent. Evaporate 100 g to dryness on a water-bath after having verified that it complies with the test for peroxides, and dry in an oven at 100° to 105°. The residue weighs not more than 2 mg.
Residual solvents	Determine by head-space gas chromatography (…….) using the ABC method. The content of xxxx is not more than …ppm, and the content of yyyy is not more than…..ppm. For ABC the specific method given in the test method section shall be stated, and for xxxx and yyyy, the names of the solvents shall be mentioned. Chromatographic system. The details shall be given
Microbial contamination	Total viable aerobic count (……) not more than $Y \times 10^3$ micro-organisms per g, determined by plate count.

Bacterial endotoxins	**Bacterial endotoxins**. If intended for use in the manufacture of sterile dosage forms without a further procedure for the removal of bacterial endotoxins, not more than XX Endotoxin Units per mg (2.6.14)
Sterility	**Sterility**. If intended for use in the manufacture of sterile dosage forms without a further appropriate sterilisation procedure, it complies with the test for sterility (2.6.1)
Pyrogens	It complies with the test for pyrogens. The quantity to be injected shall be given.
Example	**Pyrogens** It complies with the test for pyrogens (2.6.8). Inject per kilogram of the rabbit's weight a volume equivalent to 0.5 g of immunoglobulin but not more than 10 ml per kilogram of body weight.
Sulphated ash	Not more than ….. per cent, determined on …..g.
Example	**Sulphated ash** (2.4.14) Not more than 0.1 per cent, determined on 2.0 g.
Water	Not more than …. per cent, determined on …… g
Example	**Water** (2.5.12). Not more than 2.0 per cent, determined on 0.5 g.
Loss on drying	Not more than……per cent, determined on XXX g by drying in an oven at xxx^{o} to yyy^{o}.
Example	**Loss on drying** (2.2.32) Not more than 1.0 per cent, determined on 5.0 g by drying in an oven at 100° to 105°.
Assay	Although **there** are many types of assay, they broadly fall into one or the other of the ones given here. The mode of writing them is: *HPLC method* Determine by liquid chromatography (……..). *Test solution*. Directions for preparing to be given *Reference solution.* – do – Chromatographic system: - details of the column, - mobile phase composition and flow rate, - detector and wavelength setting, - injection device (if any), and - any other detail. *Note*- Commas are to be put after each item except the last where a full stop is to be given. Instructions for carrying out the determination, including the volumes

	to be injected, sequence of injections etc Calculate the percentage content of xxxx, where xxxx is the chemical entity mentioned in the opening purity statement.
Example	**Assay**. Determine by liquid chromatography (2.2.29) *Test solution.* Dissolve 25.0 mg of the substance under examination in *water* and dilute to 25.0 ml with the same solvent. *Reference solution (a).* Dissolve 25.0 mg of *cefuroxime RS* in *water* and dilute to 25.0 ml with the same solvent. *Reference solution (b).* Warm 20.0 ml of reference solution (a) at 60º for 10 min. Cool and inject immediately. *Reference solution (c).* Dilute 1.0 ml if the test solution to 100.0 ml with *water*. Chromatographic system - a column 12.5 cm x 4.6 mm packed with hexylsilyl *silica gel* (5 µm), - mobile phase: 1 volume of *acetonitrile* and 99 volumes of acetate buffer solution pH 3.4, prepared by dissolving 6.01 g of *glacial acetic acid* and 0.68 g of *sodium acetate* in *water* and diluting to 1000 ml with the same solvent, - flow rate 1.5 ml per minute, - spectrophotometer set at 273 nm, - a 20 µl loop injector. . Inject reference solution (b). The chromatogram shows peaks corresponding to cefuroxime and descarboylcefuroxime. The test is not valid unless the resolution between these peaks is at least 2.0. Adjust the concentration of acetonitrile in the mobile phase, if necessary. Inject reference solution (c). Adjust the sensitivity of the detector so that the height of the principal peak is at least one quarter of the full scale of the recorder. The assay is not valid unless the symmetry factor of the cefuroxime peak is at most 1.5. Inject reference solution (a) six times. The assay is not valid unless the relative standard deviation of the peak area of cefuroxime is at most 1.0 per cent. Inject alternately the test solution and reference solution (a). Calculate the percentage content of cefuroxime sodium, $C_{16}H_{15}N_4NaO_8S$.
Example	*By UV Light Absorption* Method for preparing the test solution to be given. Measure the absorbance (……..) at the maximum at …..nm. Calculate the content of ……..taking the specific absorbance to be ….. **Assay**. Weigh accurately about 50 mg in *ethanol* and dilute to 100.0 ml with the same solvent. Dilute 2.0 ml of the solution to 50.0 ml with *ethanol*. Measure the absorbance (……) at the maximum at about 240 nm.
	By titrimetry Method for preparing the test solution to be given. Titrate with ……. Determine the end-point potentiometrically (……) Carry out a blank titration. 1 ml of titrant is equivalent to g of…….

Example	Weigh accurately about 0.15 g and dissolve in a mixture of 10 ml of *anhydrous acetic acid* and 40 ml of *glacial acetic acid*. Titrate with *0.1M perchloric acid*. Determine the end-point potentiometrically (……) Carry out a blank titration. 1 ml of *0.1 M perchloric acid* is equivalent to 0.01827 g of $C_6H_{15}ClN_2O_2$
Storage	Special storage conditions, if any shall be specified. Note: The type of container need not be given except in very rare cases. e.g. Store in single dose or multiple dose containers.
Examples	Store at a temperature not exceeding 30°. If the substance is sterile, store in a sterile, airtight, tamper-proof container. Store protected from light and moisture Store the sealed container protected from light and at a temperature not exceeding 25°
	Manner of storing to be specified
Examples	Store protected from light. Store in a tightly closed container, protected from light. Store protected from light and at a temperature not exceeding 25°.
Labelling	Any special labelling statements specific to the product and also not stipulated in the Drugs Rules shall be given.
Examples	The label states whether or not the material is intended for the manufacture of sterile preparations. The label states, where applicable, that the substance is sterile.

B. Inactive Ingredients other than Chemicals **Drugs of Plant Origin**	
	The following, and in a few cases, some of the following information in this column shall be included in the monographs in the order given below:
Title of the Monograph	Name of the item printed in bold letters in font size 13 (MSWord). Alternate titles, if any shall be given one space below the main title.
Opening Statement	The statement shall define the article.
Examples	Emulsifying Wax is a waxy solid containing 90 parts of Cetostearyl Alcohol, 10 parts of Sodium Lauryl Sulphate or sodium salts of similar sulphated higher primary aliphatic alcohols, and 4 parts of Purified Water. Activated Charcoal is obtained from vegetable matter by suitable carbonisation processes intended to confer a high adsorbing power.
Description, Identification and other tests, including Assay	These shall be expressed in the manner detailed above under Section A. ***Note-*** In the case of plant materials (not products derived from them), after Description the following shall be added, where applicable: It has (or they have) the macroscopic and microscopic characters described under Identification tests…..
Relative density	xxx to yyy. x and y shall be reported to three decimal places only.
Weight per ml	xxxx g to yyyy g. X and Y shall be reported to three decimal places
Refractive index	xxxx to yyyy. X and y shall be reported to three decimal places, unless otherwise stated.
Melting point	xxx° to yyy°
Freezing point	Not less than xxx°
Viscosity	x mPa.s to y mPa.s
Peroxide value, Acid value, Ester value	Not more than xxx. Result shall be reported to one decimal place only.

Unsaponi-fiable matter Acetyl value, Hydroxyl value, Saponi-fication value	Not less than xxxx. Values shall be rounded to the next higher integer. No decimals to be used.
Iodine value	xxx to yyy. Values shall be rounded to the next higher integer. No decimals to be used.
Acidity	Method of preparing the test solution and the indicator to be used to be given. Not more than x ml of yM sodium hydroxide is required to change the colour of the solution.
Example	To 1.0 g add 10 ml of *ethanol* and 0.1 ml of *phenol red solution*. Not more than ml of *0.01M sodium hydroxide* is required to change the colour of the solution.
Foreign matter, Total ash, Ash insoluble in hydrochloric acid	Not more than……. per cent.
Storage	Special storage conditions, if any shall be specified.
Labelling	Any special labelling statements specific to the product and also not stipulated in the Drugs Rules shall be given.
Example	The label states whether it is Sumatra Benzoin or Siam Benzoin.

C. Dosage Forms	
Title of the Monograph	The following or some of the following information in this column shall be included in the monographs of dosage forms that contain synthetic APIs Name of the item printed in bold letters in font size 13 pt (MS Word). Alternate titles, if any shall be given one space below the main title.
Example	**Trimethoprim and Sulphamethoxazole Tablets** Sulphamethoxazole and Trimethoprim Tablets. Co-trimoxazole Tablets
Definition/ Description	A definition of the preparation in terms of the active ingredient(s) together with information on its presentation except where the nature of the product is evident from the title. For parenteral preparations information shall be provided whether it is a solution, a suspension, a dry powder or a concentrate for dilution. Also to be mentioned is information on the nature of any additives (buffers, antimicrobial preservatives etc.) present; for other sterile preparations the nature of the vehicle shall also be stated. For semi-solid preparations information on the type of base (water-in-oil, oil-in-water) etc. shall be given. For tablets information on whether or not the tablets are coated and, if so, the type of coating shall be given.
Examples	Betamethasone Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injections. Clotrimazole Cream contains Clotrimazole in a suitable base. Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified Water. Aciclovir Oral Suspension is a suspension of Aciclovir in a suitable flavoured vehicle. For injections that are supplied as solids that are to be constituted before use: Cefotaxime Injection is a sterile solution of Cefotaxime in Water for Injections. It is prepared by dissolving Cefotaxime Sodium for Injection in the requisite amount of Water for Injections before use.

Content statement	XXXX contain not less than……per cent and not more than……per cent of the stated amount of YYY (active ingredient), ZZZZZ (molecular formula).
Examples	Alprazolam Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of alprazolam, $C_{17}H_{13}ClN_4$. Salbutamol Inhaler contains not less than 80.0 per cent and not more than120.0 per cent of the amount of salbutamol, $C_{13}H_{21}NO_3$ stated to be delivered by actuation of the valve.
Identification	Method of treating the sample and preparing the test solution shall be given, where required, followed by the details of the test in the manner shown in Section A.
Examples	**Identification**. A. On the contents of the capsules determine by infrared absorption spectrophotometry (2.2.40). Compare the spectrum with that obtained with *chlorthalidone RS* or with the reference spectrum of chlorthalidone. B. Dissolve 20.0 mg of the contents of the capsules in *water* and dilute to 100.0 ml with the same solvent. Dilute 10.0 ml of this solution to 100.0 ml with *water*. The specific absorbance (2.2.25) determined at 235 nm is 360 to 390. C. In the Assay, the principal peak in the chromatogram obtained with solution (2) corresponds to the peak due to beclomethasone dipropionate in the chromatogram obtained with solution (1).
Related substances/ Impurities	Tests for related substances or impurities arising on manufacture or storage of the dosage form shall be included. The tests applied to the bulk drug substance shall be applied, wherever possible with necessary modifications.
Examples	**Related substances**. Determine by thin-layer chromatography (2.2.27), coating the plate with *silica gel F254*. Mobile phase. A mixture of 60 volumes of 2-*butanone*, 20 volumes of *2-methoxyethanol* and 20 volumes of *strong ammonia solution.* Test solution. Shake a quantity of the powdered tablets containing 0.25 g of Allopurinol with 10 ml of *strong ammonia solution* and filter. Reference solution. A 0.005 per cent w/v solution of *5-aminopyrazole-4-carboxamide hemisulphate RS.* Procedure. Apply to the plate 10 µl of each solution. After development, dry the plate in a current of 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 the reference solution.

	Related substances. Determine by liquid chromatography (2.2.29) *Test solution.* Shake a quantity of the powdered tablets with a suitable quantity of the mobile phase to obtain a suspension containing 0.05 per cent w/v of Nevirapine and filter through a membrane filter and reject the first few ml of the filtrate. *Reference solution..* Dissolve 25.0 mg of nevirapine *RS* in sufficient mobile phase to produce 50.0.0 ml. Chromatographic system - a column 25 cm x 4.6 mm octadecylsilane *bonded to silica (5 µm)*, - mobile phase: 20 volumes of *acetonitrile,* 20 volumes of *methanol* and 60 volumes of a buffer solution 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*, filter and degas. - flow rate 1.2 ml per minute, - spectrophotometer set at 230 nm, - a 20 µl loop injector. Inject the reference solution. Record the chromatograms. 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 per cent. Inject the test solution and record the chromatograms 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 2.0 per cent.
Specific tests	Depending on the dosage form, details of any test parameter and the method of testing for it that is not included in the General Monograph on a Dosage Form, or is specific to a particular dosage form shall be given.
Examples	For Ascorbic Acid Injection: **Oxalic acid**. Dissolve 0.25 g in 5 ml of *water* and neutralise to *litmus paper* with *2M sodium hydroxide*. Add 1 ml of *2M acetic acid* and *0.5M calcium chloride*. Any opalescence, after 60 minutes, is not more intense than that produced by treating 5 ml of a solution prepared by dissolving 70 mg of *oxalic acid* in 500 ml of *water* in a similar manner (0.3 per cent). For Aspirin Tablets: **Salicylic acid**. Shake a quantity of the powdered tablets containing 0.2 g of Aspirin with 4 ml of *ethanol*, dilute to 100.0 ml with *water*, filter immediately, transfer 50 ml of the filtrate to a Nessler cylinder, add 1.0 ml of freshly prepared *acid ferric ammonium sulphate solution*, mix and allow to stand for one minute; the violet colour produced is not more intense than that produced by adding freshly prepared *acid ferric ammonium sulphate solution* to a mixture of 3 ml of a freshly prepared 0.010 per cent w/v solution of *salicylic acid*, 2 ml of *ethanol* and sufficient *water* to produce 50 ml contained in a second Nessler cylinder (0.3 per cent).

	For Propantheline Bromide Tablets: **Xanthanoic acid**. Shake the combined ether extracts reserved in the Assay with two quantities, each of 30 ml, of *0.1M sodium hydroxide* containing 1.5 per cent w/v of *sodium chloride*. Remove the ether from the combined aqueous extracts by heating on a water-bath, add sufficient *0.1M sodium hydroxide* to produce 100.0 ml and dilute 25.0 ml to 100.0 ml with *0.1M sodium hydroxide*. The absorbance (2.2.25) of the resulting solution at 248 nm is not more than 0.31. For Protamine Sulphate Injection: **Optical rotation** (2.2.7). –0.52° to –0.68°, determined in a solution prepared by diluting the injection with *0.5M hydrochloric acid* so as to contain 0.8 per cent w/v of Protamine Sulphate. **Abnormal toxicity**. Complies with the test for abnormal toxicity (2.6.9), using a volume containing 10 mg of Protamine Sulphate per kg of the rabbit's weight. **Pyrogens**. Complies with the test for pyrogens (2.6.8), using a volume containing 10 mg of Protamine Sulphate per kg of the rabbit's weight.
Disintegration	For Tablets and Capsules: **Disintegration**. The time for which the disintegration test apparatus is to be operated is to be given only when it is different from that in the General Monograph on Tablets. For Cyclophosphamide Tablets: **Disintegration** (2.9.1). 30 minutes. *Note*- Where the test for disintegration is not applicable, the following statement shall be included: **Disintegration**. The test for Disintegration does not apply to tablets that are intended to be chewed before swallowing.
Example	For Piperazine Phosphate Tablets **Disintegration**. The test does not apply to Piperazine Phosphate tablets intended to be chewed before swallowing.
Dissolution	**Dissolution** (……). - apparatus: No, - medium volume and composition, - speed and time of rotation of spindle. The volume of medium to be withdrawn and subsequent operations for treating the aliquot and the manner of calculating the content shall be given. For Quinidine Sulphate Tablets:
Example	**Dissolution** (2.9.3). Apparatus. No 2

	Medium. 900 ml of *0.1M hydrochloric acid* Speed and time. 100 rpm and 30 minutes. Withdraw a suitable volume of the medium and filter. Measure the absorbance (2.2.25) of the filtrate, suitably diluted if necessary, at 248 nm. 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*.
Other tests	Complies with the tests stated under xxxx, where xxxx stands for the title of the general monograph on the specific dosage form (where all the tests apply)
Examples	**Other tests**. Comply with the tests stated under Tablets **Other tests**. The injection complies with the tests stated under Injectable Preparations (Powders for Injection).
Assay	Details of the method of preparation of the test solution shall be given followed by the further treatment of the solution for determining the content of active ingredient in the drug product. Where the method of assay is similar to that followed for the relevant API reference shall be made to that effect. In other cases, the actual details of the method shall be given.
Examples	For Carbenicillin Injection: **Assay**. Carry out the Assay described under Carbenicillin Sodium using the mixed contents of 10 containers. For Cyclophosphamide Tablets: **Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.1 g of anhydrous cyclophosphamide, add 30 ml of *chloroform*, shake vigorously for 15 minutes, filter and wash the filter with 15 ml of *chloroform*. Complete the Assay described under Cyclophosphamide Injection beginning at the words “Evaporate the combined filtrate…..”.
Examples	For Atenolol Tablets: **Assay**. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.2 g of Atenolol, transfer to a 500-ml volumetric flask using 300 ml of *methanol*, heat the resulting suspension to 60° and shake for 15 minutes. Cool, dilute to 500.0 ml with *methanol*, filter through a sintered glass funnel and dilute a suitable volume of the filtrate with sufficient methanol to produce a solution containing 0.01 per cent w/v of Atenolol. Measure the absorbance (2.2.25) of the resulting solution at 275 nm. Calculate the content of $C_{14}H_{22}N_2O_3$ taking 53.7 as the specific absorbance at 275 nm.
Storage	Directions for storing the product with particular reference to the nature of the pack and storage temperatures (as appropriate) shall be stated.

Examples	Protect the sealed container from light and store at a temperature not exceeding 25°. Store in a well-closed, light-resistant container. Store in a light-resistant container, protected from moisture and against attack by insects and rodents.
Labelling	Any specific requirement relating to the standard of the product or the storage directions shall be given. The label states the quantity of the active ingredient in terms of the equivalent amount of amoxicillin.
Examples	The label states (1) the number of Units per ml; (2) the species of animal from which the preparation has been made; (3) the name and proportion of any added preservative; (4) that the preparation, if liquid, should not be allowed to freeze; (5) that the preparation, if dried, should be used immediately after reconstitution in the stated quantity of the diluent.

D. Vaccines, Immunosera and Products of Plant Origin

The texts of the monographs shall be arranged in the following order:

1. An opening statement that defines the preparation

Examples

Diphtheria Antitoxin is a preparation containing the specific antitoxic globulins or their derivatives and having the specific activity of neutralising the toxin formed by Corynebacterium diphtherae. The liquid preparation may contain a suitable antimicrobial preservative.

Tetanus Vaccine (Adsorbed) is a sterile suspension prepared from tetanus toxoid containing not less than 1000 Limes flocculationis (Lf) per mg of protein nitrogen adsorbed on a mineral carrier. It contains a suitable antimicrobial preservative.

Belladonna Dry Extract is a dried and powdered ethanolic extract of Belladonna Herb.

2. Production. The details of the method of producing the product shall be described.

Example

For Diphtheria and Tetanus vaccine (Adsorbed)

Purified diphtheria formol toxoid and purified tetanus formol toxoid are mixed with a suspension of a mineral carrier, which is 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 in suitable media of *Corynebacterium diphtheriae* and *Clostridium tetani* respectively. The toxins are converted to toxoids by treatment with formaldehyde solution by methods, which avoid reversibility of the toxoids. 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.

3. Identification. Details of tests designed to specifically identify the
article shall be given.

Examples

Specifically neutralises and renders the toxin formed by *Cl. Tetani* harmless to susceptible animals or by any other suitable *in-vitro* test.

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.

4. Tests. Details of specific tests including sterility, toxicity, potency
or assay shall be given.

Examples

Sterility. Complies with the test for sterility (2.6.1)

Abnormal toxicity. Complies with the test for abnormal toxicity, Method B (2.6.9).

Potency. Carry out the biological assay of rabies vaccine (……..).

E. General Monographs on Dosage Forms

The dosage forms for which General Monographs may be written are as follows:

1. Capsules
2. Ear preparations
3. Eye preparations
4. Granules
5. Liquids for oral use
6. Nasal Preparations
7. Parenteral preparations
8. Oral powders
9. Preparations for inhalation
10. Creams and Ointments
11. Rectal and vaginal preparations
12. Tablets

The General Monographs shall be generally in three sections:

1. General description or definition of the dosage form and its different types.
2. Specific aspects of production that impact on the quality of the product.
3. Tests to be done in addition to the ones set out in the individual monographs.

Examples

Tablets

Description. Tablets are solid preparations each containing a single dose of one or more active substances. Tablets are intended for oral administration. Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before administration. Some tablets are retained in the mouth where the active substance is liberated……..

Tablets for oral use may be categorised as follows:

- uncoated tablets,
- coated tablets,
- effervescent tablets,
- soluble tablets,
- dispersible tablets,
- modified-release tablets,
- gastro-resistant tablets.

Production. Tablets are usually prepared by compressing uniform volumes of particles or particle aggregates (granules) by applying high pressures and using punches and dies…….

In the manufacture, packaging, storage and distribution of tablets, suitable measures are taken to ensure their microbiological quality.

	Tests **Dissolution.** Tablets comply with the Dissolution test for solid dosage forms (2.9.3). *Note*- Where a dissolution test is prescribed, a disintegration test may not be required. **Uniformity of content** (2.9.6). Unless otherwise stated, tablets with a content of active substance less than 2 mg or less than 2 per cent of the total weight comply with the test for uniformity of content of single-dose preparations. If the preparation has more than one active substance, the requirement applies only to those substances which correspond to the above conditions. **Uniformity of weight** (2.9.5) Uncoated tablets and, unless otherwise stated, film-coated tablets comply with the test for uniformity of weight of single-dose preparations. If the test for uniformity of content is prescribed for all the active substances, the test for uniformity of weight is not required. **Uncoated tablets** **Description**.Uncoated tablets include single-layer tablets resulting from a single compression of particles and multi-layer tablets consisting of concentric or parallel layers obtained by successive compression of different composition. The excipients used are not specifically intended to modify the release of the active substance in the digestive fluids……. **Tests** **Disintegration.** Uncoated tablets comply with the test for disintegration of tablets and capsules (2.9.1). Use *water* as the liquid. Add a disc to each tube. Operate the apparatus for 15 minutes, unless otherwise directed. Examine the state of the tablets. If the tablets fail to comply because of adherence to the discs, repeat the test on a further 6 tablets. The tablets comply with the test if all the 6 have disintegrated.

4.Test Methods

Test Methods shall be broadly divided into the following sections.

a. Apparatus
b. Physical and physicochemical methods
c. Identification tests
d. Limit tests
e. Chemical assays
f. Biological tests
g. Pharmaceutical tests

5. Reagents and Solutions

This section shall provide details of the quality and of preparation of reagents and solutions that are to be used in the tests and assays of the pharmacopoeia. It shall also include information on Reference Substances that are required for specific tests.

6. General Texts

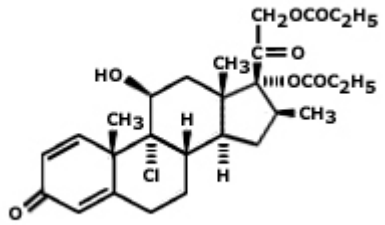
These shall consist of general information, not specific to any product, but pertaining to aspects of production and testing of pharmaceuticals impacting on quality, such as sterilisation, the quality of water for pharmaceutical use, containers (including closures) for packing drugs and drug products etc.

7. Index

The Index shall be in alphabetical order of the titles of monographs, titles and sub-titles of test methods and of general texts, as well as of reagents and special solutions mentioned in any of the pages of the Pharmacopoeia except the cover page.

The Annexures that follow show specimens of monographs in different formats for APIs, excipients, dosage forms etc.

Beclomethasone Dipropionate



$C_{28}H_{37}ClO_7$ Mol. Wt. 521.1

Beclomethasone Dipropionate is 9α -chloro-11β -hydroxy- 16β -methyl-3, 20-dioxo-1, 4-pregnadiene-17, 21-diyl dipropionate.

Beclomethasone Dipropionate contains not less than 96.0 per cent and not more than 103.0 per cent of $C_{28}H_{37}ClO_7$, calculated on the dried basis.

Description. A white to creamy-white, crystalline powder; 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 (.....). Compare the spectrum with that obtained with *beclomethasone dipropionate RS.*

B. Determine by thin-layer chromatography (……), 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 *beclomethasone dipropionate 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 120º for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120º 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. Determine by the oxygen flask method, (.....), on 25 mg and use a mixture of 20 ml of *water* and 1 ml of *1M sodium hydroxide* as the absorbing liquid. The liquid gives reaction A of chlorides (.....).

Light absorption. Dissolve 50 mg in sufficient *ethanol (95 per cent)* to produce 100 ml and dilute 2 ml of this solution to 50 ml with the same solvent. Absorbance of the resulting solution at the maximum at about 238 nm, 0.57 to 0.60, (.....).

Specific optical rotation (.....). +88° to +94°, determined in a 1 per cent w/v solution in *dioxan*.

Related foreign steroids (…..). Use mobile phase (a) and reference solution (c).

Test solution. Dissolve 0.15 g of the substance under examination in a mixture of 9 ml of *chloroform* and 1 volume of *methanol*.

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

Loss on drying (.....). Not more than 0.5 per cent, determined on 1 g by drying in an oven at 105° for 3 hours.

Assay (…….). *Test solution*. Weigh accurately about 0.1 g and dissolve in sufficient of *aldehyde-free ethanol* to produce 50.0 ml. To 5.0 ml of this solution add sufficient *aldehyde-free ethanol* to produce50.0 ml. Dilute 5.0 ml of the resulting solution to 100.0 ml with *aldehyde-free ethanol*.

Standard solution. Weigh accurately about 10 mg of *beclomethasone dipropionate RS* and dissolve in sufficient of *aldehyde-free ethanol* to produce 50.0 ml. To 5.0 ml of this solution add sufficient *aldehyde-free ethanol* to produce 100.0 ml.

Storage. Store protected from light.

Ethosuximide

(Graphic Formula)

$C_7H_{11}NO_2$ Mol. Wt. 141.2

Ethosuximide is (RS)-2-ethyl-2-methylsuccinimide.

Ethosuximide contains not less than 98.0 per cent and not more than101.0 per cent of $C_7H_{11}NO_2$, calculated on the anhydrous basis.

Description. A white or almost white powder or waxy solid.

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 (2.2.40). Melt a sufficient quantity at 50°, prepare a thin film between two previously warmed bromide plates and record the spectrum immediately. Compare the spectrum with that obtained with *ethosuximide RS.*

.

B. When examined between 230 nm and 360 nm (……..) a 0.1 per cent w/v solution in *ethanol (95 per cent)* shows an absorption maximum at about 248 nm; absorbance at 248 nm, about 0.85 (……..).

C. Dissolve 0.1 g in 3 ml of *methanol* and add 0.05 ml of a 10 per cent w/v solution of *cobalt chloride*, 0.05 ml of a 10 per cent w/v solution of *calcium chloride* and 0.1 ml of *2M sodium hydroxide*; a purple colour develops and no precipitate is produced.

D. Melting point (……..) 45° to 50°.

Tests

Appearance of solution. Dissolve 2.5 g in sufficient *water* to produce 25 ml. The solution is clear (…….) and colourless (……..)

Acidity. Dissolve 5.0 g in 50 ml of *water* by warming on a water-bath for 5 minutes. Cool and titrate with *0.1M sodium hydroxide* using *bromocresol green solution* as indicator. Not more than 0.7 ml of *0.1M sodium hydroxide* is required.

Cyanide. Dissolve 1 g in 10 ml of *ethanol (90 per cent)* and add 0.5 ml of *ferrous sulphate solution*, 1 ml of *2M sodium hydroxide* and 0.1 ml of *ferric chloride solution*. Heat to boiling, cool and acidify using 3 ml of *1M sulphuric acid*. After 15 minutes, there is no blue colour and no blue precipitate is produced.

Related substances. Determine by gas chromatography (…….).

Test solution (a) Dissolve 1.0 g of the substance under examination in sufficient *chloroform* to produce 10 ml

Test solution (b). Dilute 5 ml of test solution (a) to 10 ml with a 0.010 per cent w/v solution of *anthracene* (internal standard) in *chloroform*.

Reference solution (a). Dissolve 10 mg of *2-ethyl-2-methylsuccinic acid* in sufficient *chloroform* to produce 10 ml.
Reference solution (b). Dilute 1 ml of test solution (a) to 100 ml with *chloroform*. To 1 ml of this solution add 5 ml of internal standard solution and sufficient *chloroform* to produce 10 ml.

Reference solution © Dilute 1 ml of test solution (b) to 50 ml with *chloroform*. Add 1 ml of this solution to 1 ml of reference solution (a), add 5 ml of internal standard solution and sufficient *chloroform* to produce 10 ml.

Chromatographic system

- a glass column 2m x 2 mm packed with silanised diatomaceous support (125 to 180 mesh) impregnated with 3 per cent w/w polycyanopropylmethylphenylmethyl siloxane,
- temperature:
  column.165°,
  inlet port and detector. 240°,
- flow rate 30 ml per minute of the carrier gas.

Inject 1 μl of reference solution (c) and adjust the sensitivity of the detector so that the heights of the three principal peaks are not less than 70 per cent of full-scale deflection. The peaks in order of emergence, are due to 2-ethyl-2-methylsuccinic acid, ethosuximide, and anthracene.

The test is not valid unless the resolution factor between the peaks corresponding to 2-ethyl-2-methylsuccinic acid and ethosuximide in the chromatogram obtained with test solution (a) is at least 4.

Inject 1 μl of test solution (a) and verify that there is no peak with the same retention time as the internal standard. Inject separately 1 μl of test solution (b) and reference solution (b) and record the chromatogram for twice the retention time of ethosuximide. Calculate the ratio ® of the area of the peak due to ethosuximide to the area of the peak due to the internal standard in the chromatogram obtained with reference solution (b). In the chromatogram obtained with test solution (b) the ratio of the sum of the areas of any secondary peaks to the area of the peak due to the internal standard is not greater than R.

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

Water (…..). Not more than 0.5 per cent, determined on 1 g.

Assay. Weigh accurately about 0.12 g, dissolve 20 ml of *dimethylformamide*, add 0.2 ml of a 0.5 per cent w/v solution of *thymolphthalein* in *dimethylformamide* and carry out Method B for non-aqueous titration (……..) using *0.1M tetrabutylammonium hydroxide* as titrant until a distinct blue colour is produced.

1 ml of *0.1M tetrabutylammonium hydroxide* is equivalent to 0.01412 g of $C_7H_{11}NO_2$.

Storage. Store protected from light.

Sodium Acetate

$C_2H_3NaO_2,3H_2O$ 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.

Description. A white, crystalline powder or colourless crystals; odourless.

Identification

Dissolve 10 g in sufficient *carbon dioxide-free water* to produce 100 ml (solution A). 1 ml of solution A gives reaction A of *sodium salts* (……..).

Tests

Appearance of solution. Solution A is clear (…….) and colourless (……..).

pH. (……). 7.5 to 9.0, determined in a 5 per cent w/v solution.

Arsenic. Dissolve 5 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 *0.05M zinc chloride*. Add dropwise *0.02M 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 *0.02M disodium edetate* until the blue colour is restored. Not more than 0.65 ml of *0.02M disodium edetate* is required.

Heavy metals (….). 12 ml of solution A complies with limit test D for heavy metals (10 ppm).

Iron. 20 ml of solution A complies with the limit test for iron (……..) (20 ppm).

Chlorides. 10 ml of solution A complies with the limit test for chlorides (……..).

Sulphates. 15 ml of solution A complies with the limit test for sulphates (……).

Reducing substances. Dissolve 1.0 g in 100 ml of boiling *water*, add 5 ml of *1M sulphuric acid* and 0.5 ml of *0.002M potassium permanganate*, mix and boil gently for 5 minutes; the pink colour is not completely discharged.

Loss on drying. 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 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. Carry out Method B for non-aqueous titration (…….), using 0.3 ml of *1-naphtholbenzein* as indicator, until a green colour is produced. Carry out a blank titration.

1 ml of *0.01M 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 *1M 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* 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).

Caramel

Burnt Sugar

Caramel is a concentrated solution of the product obtained by heating Sucrose or Dextrose until the sweet taste is destroyed.

Description. A thick, free-flowing, dark brown liquid; odour, slight and characteristic.

Identification

To 20 ml of a 5 per cent w/v solution add 0.5 ml of *phosphoric acid;* no precipitate is produced.

Tests

Relative density (……) Not less than 1.3

pH (……..) 3.0 to 5.5, determined in a 10 per cent w/v solution.

Acid-stability. Dilute 50 ml of a 1 per cent w/v solution to 250 ml with *water*, add 5 ml of *hydrochloric acid* and heat gently to boiling under reflux. Allow to cool and set aside for 24 hours; the solution remains clear. Repeat the test on the same test solution but boil for 30 minutes; the solution remains clear.

Heavy metals (…….). 2.0 g complies with limit test B for heavy metals (10 ppm). Prepare the standard using 2.0 ml of *lead standard solution (10 ppm Pb).*

Iron. Evaporate 0.40 g to dryness, ad 0.2 ml of nitric acid, ignite and dissolve the residue in 1 ml of *dilute nitric acid*; the solution complies with the limit test for iron (…….).

Sulphated ash (……..). Not more than 2.0 per cent.

Microbial contamination (……..) 1 g is free from *Escherichia coli* and salmonellae.

Senna Leaf

Senna leaf consists of the dried leaflets of Cassia senna *L. (*C. acutifolia *Delile), known as Alexandrian senna, or* Cassia angustifolia *Vahl, known as Tinnevelly senna, or a mixture of the two species.*

Senna Leaf contains not less than 2.5 per cent of hydroxyanthracene glycosides, calculated as sennoside B ($C_{42}H_{38}O_{20}$; Mol. Wt. 863), calculated on the dried basis.

Description. Senna Leaf has a slight characteristic odour.

It has the macroscopic and microscopic characteristics described under identification tests A and B.

Identification

A. C.senna occurs as greyish-green to brownish-green, thin, fragile leaflets, lanceolate, mucronate, asymmetrical at the base, usually 15 mm to 40 mm long and 5 mm to 15 mm wide, the maximum width being at a point slightly below the centre; the lamina is slightly undulant with both surfaces covered with fine, short trichomes. Pinnate venation is visible
mainly on the lower surface, with lateral veins leaving the midrib at an angle of about 60° and anastamosing to form a ridge near the margin.
Stomatal index: 10-12.5-15.

C. angustifolia occurs as yellowish-green to brownish-green leaflets, elongated and lanceolate, slightly asymmetrical at the base, usually 20 mm to 50 mm long and 7 mm to 20 mm wide at the centre. Both surfaces are smooth with a very small number of short trichomes and are frequently marked with transverse or oblique lines.
Stomatal index: 14-17.5-20

B. Reduce to a powder. The powder is light green to greenish-yellow. Examine under a microscope using *chloral hydrate solution*. The powder shows the following diagnostic characteristics: polygonal epidermal cells showing paracytic stomata; unicellular trichomes, conical in shape, with warted walls, isolated or attached to fragments of epidermis; fragments of vascular bundles with a crystal sheath of prismatic crystals of calcium oxalate; cluster crystals isolated or in fragments of parenchyma.

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

Mobile phase. A mixture of 1 volume of *glacial acetic acid*, 30 volumes of *water*, 40 volumes of *ethyl acetate* and 40 volumes of *propanol*.

Test solution. To 0.5 g of the powdered drug add 5 ml of a mixture of equal volumes of *ethanol* and *water* and heat to boiling. Centrifuge and use the supernatant liquid.

Reference solution. Dissolve 10 mg of senna extract RS in 1 ml of a mixture of equal volumes of *ethanol* and *water* (a slight residue remains).

Procedure. Apply to the plate 10 µl of each solution as bands 20 mm by 2 mm. Allow the mobile phase to rise 10 cm. Dry the plate in air, spray with a 20 per cent v/v solution of *nitric acid* and heat at 120° for 10 minutes. Allow to cool and spray with a 50 g/l solution of *potassium hydroxide* in *ethanol (50 per cent v/v)* until the zones appear. The principal zones in the chromatogram obtained with the test solution are similar in position (sennosides B, A, D and C in the order of increasing Rf value), colour and size to the principal zones obtained with the reference solution. Between the zones corresponding to sennosides D and C a red zone corresponding to rhein-8-glucoside may be visible.

D. Place about 25 mg of the powdered drug in a conical flask and 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 the ether, dry over *anhydrous sodium sulphate*, evaporate 5 ml to dryness and 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

Foreign matter (……..). Not more than 3 per cent of foreign organs and not more than 1 per cent of foreign elements.

Total ash (……). Not more than 12.0 per cent.

Ash soluble in hydrochloric acid (……..). Not more than 2.5 per cent.

Assay. *Carry out the assay protected from bright light.*
Weigh accurately 0.15 g of the powdered drug, add 30 ml of *water*……..

Storage. Store protected from light moisture and against attack by insects and rodents.

Amoxycillin Capsules

Amoxycillin Trihydrate Capsules; Amoxicillin Trihydrate Capsules; Amoxicillin Capsules

Amoxycillin Trihydrate Capsules contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

Identification

Shake a quantity of the contents of the capsules containing 0.5 g of amoxycillin with 5 ml of *water* for 5 minutes, filter, wash the residue first with *ethanol* and then with *ether* and dry at a pressure not exceeding 0.7 kPa for 1 hour. The residue complies with the following tests.

Test A may be omitted if test B is carried out.

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

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

Mobile phase. A mixture of 90 volumes of a 15.4 per cent w/v solution of *ammonium acetate* and 10 volumes of *acetone*, the pH of which has been adjusted to 5.0 with *glacial acetic acid*.

Test solution. Dissolve 0.25 g in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Reference solution (a). Dissolve 0.25 g of *amoxycillin trihydrate RS* in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Reference solution (b) Dissolve 0.25 g each of amoxycillin trihydrate RS and ampicillin trihydrate RS in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Apply to the plate 1 µl of each of the solutions. Allow the mobile phase to rise 10 cm. Dry the plate in a current of air, expose it to iodine vapour until spots appear and examine.

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 principal spots.

Tests

Dissolution (……).
Apparatus. No 1
Medium. 900 ml of *water*
Speed and time. 100 rpm and 60 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 272 nm ( ). Similarly measure the absorbance of a standard solution of known concentration of *amoxycillin RS* at about 272 nm and calculate the content of $C_{16}H_{19}N_3O_5S$.

D. Not less than 80 per cent of the stated amount of $C_{16}H_{19}N_3O_5S$.

Other tests. Comply with the tests stated under Capsules.

Assay. Weigh accurately a quantity of the mixed contents of 20 capsules containing 0.15 g of amoxycillin, add sufficient *water* to produce 500.0 ml, shake for 30 minutes and filter. To 10.0 ml of the filtrate add 10 ml of *alkaline borate buffer pH 9.0* followed by 1 ml of *acetic anhydride-dioxan solution*, allow to stand for 5 minutes and add sufficient *water* to produce 100.0 ml. Transfer 2.0 ml of the resulting solution into each of two stoppered tubes. To one tube add 10 ml of *imidazole-mercury reagent*, mix, stopper the tube and immerse in a water-bath at 60° for exactly 25 minutes with occasional swirling. Remove the tube from the water-bath and cool rapidly to 20° (solution A). To the second tube add 10 ml of *water* and mix (solution B). Without delay measure the absorbances of solutions A and B at the maximum at about 325 nm, ( ) using as the blank a mixture of 2 ml of *water* and 10 ml of *imidazole-mercury reagent* for solution A and *water* for solution B. Calculate the content of $C_{16}H_{19}N_3O_5S$ from the difference between the absorbances of solution A and solution B, from the difference obtained by repeating the operation using 0.17 g of *amoxycillin trihydrate RS* instead of the substance being examined and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxycillin trihydrate*

Storage. Store protected from moisture.

Labelling. The label states the quantity of the active ingredient in terms of the equivalent amount of amoxycillin.

Chloramphenicol Eye Drops

Chloramphenicol Eye Drops are a sterile solution of Chloramphenicol in Purified Water.

Chloramphenicol Eye Drops contain not less than 90.0 per cent and not more than 130.0 per cent of the stated amount of chloramphenicol, $C_{11}H_{12}Cl_2N_2O_5$.

Identification

To a volume containing 50 mg of Chloramphenicol add 15 ml of *water* and extract with four quantities, each of 25 ml, of *ether*. Combine the extracts and evaporate to dryness. The dried residue complies with the following tests.

A. Determine by thin-layer chromatography (…….), coating the plate with *silica gel G F254*.

Mobile phase. A mixture of 90 volumes of *chloroform*, 10 volumes of *methanol* and 1 volume of *water*.

Test solution. Dissolve 0.10 g of the residue in sufficient *ethanol (95 per cent)* to produce 10 ml.

Reference solution. Dissolve 0.10 g of *chloramphenicol RS* in sufficient *ethanol (95 per cent)* to produce 10 ml.

Apply to the plate 1µl of each solution. Allow the mobile phase to rise 10 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. Dissolve 10 mg in 2 ml of *ethanol (50 per cent)*, add 4.5 ml of *1M sulphuric acid* and 50 mg of *zinc powder* and allow to stand for 10 minutes. Decant the supernatant liquid or filter, if necessary. Cool the resulting solution in ice and add 0.5 ml of *sodium nitrite solution* and, after 2 minutes, 1 g of *urea* followed by 1 ml of *2-naphthol solution* and 2 ml of *10M sodium hydroxide*; a red colour is produced. Repeat the test omitting the zinc powder; no red colour is produced.

Tests

pH (……**)**. 7.0 to 7.5.

2-Amino-1-(4-nitrophenyl)-1,3-propanediol. Not more than 8.0 per cent of the declared content of Chloramphenicol.

Determine by liquid chromatography (……..).

Test solution. Dilute the eye drops with the mobile phase to contain 0.050 per cent w/v of Chloramphenicol.

Reference solution. A 0.004 per cent w/v solution of *2-amino-1-1-(4-nitrophenyl)-1,3-propanediol RS* in 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: 85 volumes of a 0.21 per cent w/v solution of *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,
- a 20 µl loop injector.

In the chromatogram obtained with the test solution, the area of any peak corresponding to 2-amino-1-(4-nitrophenyl)-1,3-propanediol is not greater than the area of the peak in the chromatogram obtained with the reference solution.

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

Assay. Dilute a volume containing 25 mg of Chloramphenicol to 250.0 ml with *water*, dilute 10.0 ml to 100.0 ml with *water* and measure the absorbance of the resulting solution at 278 nm (…….). Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the specific absorbance at 278 nm.

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 101.0 per cent of the stated amount of propranolol, $C_{16}H_{21}NO_2$,HCl.

Identification

A. Make alkaline with *1M 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 spectrometry (……..). Compare the spectrum with that obtained with *propranolol hydrochloride RS* or with the *reference spectrum* of propranolol.

B. When examined between 230 nm and 360 nm (……..) the solution obtained in the Assay shows absorption maxima at about 290nm, 306 nm and 319 nm.

Tests

pH (…….) 3.0 to 3.5.

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

Assay. To an accurately measured volume containing 2 mg of Propranolol Hydrochloride add sufficient *methanol* to produce 100.0 ml. Measure the absorbance of the resulting solution at 290 nm (……..). 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 single-dose containers.

Amoxycillin Injection

Amoxicillin Injection; Amoxycillin Sodium Injection; Amoxicillin Sodium Injection

Amoxycillin Sodium Injection is a sterile solution of Amoxycillin Sodium in Water for Injection. It is prepared by dissolving Amoxycillin Sodium for Injection in the requisite amount of Water for Injection immediately before use.

The injection complies with the requirements stated under Parenteral Preparations.

Storage. Amoxycillin Injection should be used immediately after preparation but, in any case, within the period recommended on the label when prepared and stored strictly according to the instructions on the label.

Amoxycillin Sodium for Injection

Amoxycillin Sodium for Injection is a sterile material consisting of Amoxycillin Sodium with or without excipients. It is filled in a sealed container.

Amoxycillin for Injection contains not less than 90.0 per cent and not more than 120.0 per cent of the stated amount of amoxycillin, $C_{16}H_{19}N_3O_5S$.

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

The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.

Identification

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

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

B. Carry out the method for thin-layer chromatography ( ), coating the plate with *silanised silica gel H*.

Mobile phase. A mixture of 90 volumes of a 15.4 per cent w/v solution of *ammonium acetate* and 10 volumes of *acetone*, the pH of which has been adjusted to 5.0 with *glacial acetic acid*.

Test solution. Dissolve 0.25 g in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Reference solution (a). Dissolve 0.25 g of *amoxycillin trihydrate RS* in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Reference solution (b) Dissolve 0.25 g each of amoxycillin trihydrate RS and ampicillin trihydrate RS in sufficient *0.5M sodium bicarbonate* to produce 100 ml.

Apply separately to the plate 1µl of each of the solutions. Allow the mobile phase to rise 10 cm. Dry the plate in a current of air, expose it to iodine vapour until spots appear and examine.

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 principal spots.

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

Tests

pH (.....). 8.0 to 10.0, determined in a 10 per cent w/v solution.

Appearance of solution. A 10 per cent w/v solution is not more opalescent than opalescence standard OS2, ( ) when examined immediately after preparation. The solution may initially show a pink colour and its absorbance after 5 minutes at about 430 nm is not more than 0.20, ( )

Specific optical rotation (…….). +240° to +290°, determined in a 0.25 per cent w/v solution in a 0.4 per cent w/v solution of *potassium hydrogen phthalate*.

Heavy metals (……). Not more than 20 ppm, determined on 1.0 g by Method B.

N, N-Dimethylaniline (…….). Not more than 20 ppm, determined by Method A.

Sodium chloride. Not more than 2.0 per cent, calculated on the anhydrous basis, determined by the following method. Weigh accurately about 1g, dissolve in 50 ml of *distilled water*; add 10 ml of *2M nitric acid* and titrate with *0.1M silver nitrate*, determining the end-point potentiometrically using a silver indicator electrode and a mercury-mercurous sulphate reference electrode or any other suitable electrode.

1ml of *0.1M silver nitrate* is equivalent to 0.005845 g of NaCl

Degradation products. Not more than 9.0 per cent, determined by the following method. Weigh accurately about 0.25g, dissolve in a mixture of 25 ml of *alkaline borate buffer pH 9.0* and 0.5 ml of *acetic anhydride* by stirring for 3 minutes, add 10 ml of *acetate buffer pH 4.6* and titrate immediately with *0.02M mercuric nitrate*, determining the end-point potentiometrically with a platinum or mercury indicator electrode and a mercury-mercurous sulphate reference electrode. Ignore any preliminary inflection in the titration curve.

1ml of *0.02M mercuric nitrate* is equivalent to 0.007748 g of degradation products calculated as $C_{16}H_{18}N_3NaO_5S$.

Water (.....). Not more than 4.0 per cent w/w, determined on 0.4 g.

Bacterial endotoxins (……). Not more than 0.25 Endotoxin Unit per mg of amoxycillin.

Sterility (…….). Comply with the test for sterility (……..).

Assay. Determine the weight of the contents of 10 containers. Dissolve 0.17 g of the mixed contents of the 10 containers in sufficient *water* to produce 500.0 ml. Transfer 10.0 ml of the resulting solution to a 100-ml volumetric flask, add 10 ml of *alkaline borate buffer pH 9.0* followed by 1 ml of *acetic anhydride-dioxan solution*, allow to stand for 5 minutes and add sufficient *water* to produce 100.0 ml. Place two quantities, each of 2 ml, of the solution in separate stoppered tubes. To one tube add 10 ml of *imidazole-mercury reagent*, stopper the tube and place in a water-bath at 60° for exactly 20 minutes, swirling occasionally. Remove the tube from the water-bath and cool rapidly to 20° (solution A). To the second tube add 10 ml of *water* and mix (solution B). Without delay measure the absorbances of solutions A and B at the maximum at about 325 nm( ), using as the blank a mixture of 2 ml of *water* and 10 ml of *imidazole-mercury reagent* for solution A and *water* for solution B.

Calculate the content of $C_{16}H_{19}N_3O_5S$ in a container of average content weight from the difference between the absorbances of solutions A and B, from the difference obtained by repeating the operation using 0.17 g of *amoxycillin trihydrate RS* in place of the preparation being examined and from the declared content of $C_{16}H_{19}N_3O_5S$ in *amoxycillin trihydrate RS.*

Storage. Store protected from moisture, in a sterile, tamper-evident container sealed so as to exclude micro-organisms, at a temperature not exceeding 30°. The reconstituted solution should be used immediately after preparation.

Labelling. The label states the quantity of Amoxycillin Sodium contained in the sealed container in terms of the equivalent amount of amoxycillin.

Betamethasone Tablets

Betamethasone Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amount of betamethasone, $C_{22}H_{29}FO_5$.

Identification

Powder a few tablets and extract with *chloroform*. Evaporate the extract to dryness. The residue complies with the following tests.

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

B. Place 2 ml of a 0.01 per cent w/v solution in *ethanol* in a stoppered tube, add 10 ml of *phenylhydrazine solution*, mix, warm in a water-bath at 60° for 20 minutes and cool immediately; absorbance of the resulting solution at about 450 nm, not more than 0.25 (…).

C. On the residue obtained in test A determine by thin-layer chromatography (……), 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 *betamethasone RS* in 10 ml of the solvent mixture.

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

Reference solution (c). Mix equal volumes of the test solution and a 0.25 per cent w/v solution of *dexamethasone RS* in the solvent mixture.

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 120º for 15 minutes and spray the hot plate with *ethanolic sulphuric acid (20 per cent v/v).* Heat at 120º 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. The chromatogram obtained with reference solution (c) shows two closely running spots.

Tests

Related substances. Transfer a quantity of the powdered tablets containing about 2 mg of Betamethasone to a glass-stoppered 50-ml centrifuge tube. Pipette 20 ml of *ethanol (95 per cent)*

into the tube, shake for 2 minutes and allow to stand for 20 minutes with occasional shaking. Centrifuge the mixture for 5 minutes. Pipette 10 ml of the clear supernatant liquid into a glass-stoppered tube and evaporate the ethanol on a water-bath with the aid of a current of air to about 0.5 ml, then evaporate without heat to dryness. Pipette 1 ml of a mixture of 9 volumes of *chloroform* and 1 volume of *methanol*, insert the stopper and mix. Centrifuge, if necessary, to remove any insoluble material. Use this solution as the test solution.

Determine by thin-layer chromatography (.....), coating the plate with a suitable silica gel containing a fluorescent indicator with an optimal intensity at 254 nm (such as Merck silica gel 60 F254).

Mobile phase. A mixture of 77 volumes of *dichloromethane*, 15 volumes of *ether*, 8 volumes of *methanol* and 1.2 volumes of *water.*

Reference solution (a). A 0.020 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 0.010 per cent w/v solution of the substance under examination in the same solvent mixture.

Reference solution (c). A 0.1 per cent w/v solution of each of the substance under examination and *prednisone RS* in the same solvent mixture.

Apply to the plate 5 µl of each solution. After development, dry the plate in air until the odour of solvents is no longer detectable 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). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated principal spots.

Dissolution (……..).
Apparatus. No 1
Medium. 900 ml of *water* and 1 ml of 0.05 per cent w/v solution of *testosterone RS* (internal standard) in *methanol*.
Speed and time. 50 rpm and 45 minutes.
Use one tablet in the vessel for each test.

Withdraw a suitable volume of the medium and filter. Determine by liquid chromatography (……)

Test solution. The filtrate obtained as given above.

Reference solution. Dilute a mixture of 1 ml of each of a 0.05 per cent w/v solution of *betamethasone RS* in *methanol* and 1 ml of a 0.05 per cent w/v solution of *testosterone RS* in *methanol* to 900 ml with *water*.

Chromatographic system

- a stainless steel column 30 cm x 3.9 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 60 volumes of *methanol* and 40 volumes of *water,*
- flow rate 2 ml per minute,
- spectrophotometer set at 254 nm,
- a 20 µl loop injector.

D: Not less than 75 per cent of the stated amount of $C_{22}H_{29}FO_5$.

Uniformity of content. Comply with the test stated under Tablets. Determine by liquid chromatography (……..).

Test solution. Finely crush one tablet, add 20 ml of a 0.002 per cent w/v solution of *hydrocortisone* (internal standard) in *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre filter paper.

Reference solution. A solution containing 0.0025 per cent w/v of *betamethasone RS* and 0.002 per cent w/v of *hydrocortisone*.

Note- Protect *the solutions from light.*

Chromatographic system
- a stainless steel column 20 cm x 5 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 53 volumes of *water* and 47 volumes of *methanol.*
- flow rate 1.4 ml per minute,
- spectrophotometer set at 238 nm,
- a 20 µl loop injector.

Calculate the content of $C_{22}H_{29}FO_5$ in each tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (…)

Test solution. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 2.5 mg of Betamethasone, add 20 ml of *methanol (50 per cent)*, shake for 10 minutes and filter through a glass-fibre paper.

Reference solution (a). A solution containing 0.0125 per cent w/v of *betamethasone RS* and 0.010 per cent w/v of *hydrocortisone RS* (internal standard).

Reference solution (b). Prepare in the same manner as the test solution but use 20 ml of 0.01 per cent w/v solution of *hydrocortisone* in *methanol (50 per cent)* in place of 20 ml of *methanol (50 per cent)*.

Note- Protect the solutions from light.
Carry out the chromatographic procedure described under Uniformity of content. Calculate the content of $C_{22}H_{29}FO_5$ in the tablets.

Storage. Store protected from light.

Dapsone Tablets

Dapsone Tablets contain not less than 93.0 per cent and not more than 107.0 per cent of the stated amount of dapsone, $C_{12}H_{12}N_2O_2S$.

Identification

A. Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of *acetone*, filter and evaporate the filtrate to dryness. On the residue determine by *i*nfrared absorption spectrophotometry (……). Compare the spectrum with that obtained with *dapsone RS* or with the reference spectrum of dapsone.

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).

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

Mobile phase. A mixture of 20 volumes of *n-heptane*, 20 volumes of *ethyl acetate*, 6 volumes of *methanol* and 1 volume of *strong ammonia solution*.

Test solution (a). Shake a quantity of the powdered tablets containing 0.1 g of Dapsone with 10 ml of *methanol* and filter.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *methanol*.

Reference solution (a). Dilute 1 ml of test solution (b) to 10 ml with *methanol*.

Reference solution (b). Dilute 2 ml of reference solution (b) to 10 ml with *methanol*.

Reference solution (c). A 0.1 per cent w/v solution of *dapsone* RS in *methanol*.

Apply to the plate 10 μl of each solution. After development, dry the plate in air, spray with a 0.1 per cent w/v solution of *4-dimethylaminocinnamaldehyde* in a mixture of 99 volumes of *ethanol (95 per cent)* and 1 volume of *hydrochloric acid* 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 solution (b).

Dissolution (……).

Apparatus. No 1.
Medium. 900 ml of a 2 per cent w/v solution of *hydrochloric acid*.
Speed and time. 100 rpm and 60 minutes.
Place one tablet for each test.

Withdraw a suitable volume of the solution 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. Transfer an accurately measured volume of the filtrate containing about 0.2 mg of Dapsone to a 25-ml volumetric flask, add 5 ml of *1M sodium hydroxide*, dilute to volume with *water* and mix. Measure the absorbance of the resulting solution at the maximum at about 290 nm, ( ). Calculate the content of $C_{12}H_{12}N_2O_2S$ from the absorbance obtained from a solution prepared by adding 5 ml of *1M sodium hydroxide* to 20 ml of a 2 per cent v/v solution of *hydrochloric acid* containing 0.2 mg of *dapsone RS* and adding sufficient water to produce 25.0 ml.

D. Not less than 75 per cent of the stated amount of $C_{12}H_{12}N_2O_2S$.

Other tests. Comply with the tests stated under Tablets.

Assay. Weigh and powder 20 tablets. Weigh accurately a quantity of the powder containing 0.25 g of Dapsone and dissolve in a mixture of 15 ml of *water* and 15 ml of *2M hydrochloric acid*. Cool the solution to about 15^{o} and determine by the nitrite titration (.....). Carry out a blank titration.

1ml of *0.1M sodium nitrite* is equivalent to 0.01242 g of $C_{12}H_{12}N_2O_2S$.

Storage. Store protected from light.

Diphtheria Antitoxin

Diphtheria Antitoxin is a preparation containing the specific antitoxic globulins or their derivatives and having the specific activity of neutralising the toxin formed by Corynebacterium diphtherae. The liquid preparation may contain a suitable antimicrobial preservative.

Production

The antitoxin is obtained by purification of hyperimmune serum or plasma of healthy horses or other suitable animals and having the required specific activity. The final preparation may be a liquid or a freeze-dried solid.

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 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

Complies with the tests stated under Antisera.

Potency. Carry out the biological assay of diphtheria antitoxin(……).

Biological assay of Diphtheria Antitoxin

The potency is determined by comparing the dose necessary to protect ……etc.

Storage. Store at 2º to 8º. Do not freeze.

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 name and proportion of any added preservative; (4) that the preparation, if liquid, should not be allowed to freeze; (5) that the preparation, if dried, should be used immediately after reconstitution in the stated quantity of the diluent.

Tetanus Vaccine (Adsorbed)

Tetanus Toxoid (Adsorbed)

Tetanus Vaccine (Adsorbed) is a sterile suspension prepared from tetanus toxoid containing not less than 1000 Likes flocculationis (Lf), (…….), per mg of protein nitrogen adsorbed on a mineral carrier in an appropriate solution.

Production

The toxoid is adsorbed on hydrated aluminium hydroxide, aluminium phosphate or calcium phosphate, in saline solution or other appropriate solution isotonic with blood. The formol toxoid is prepared from the toxin produced by the growth in suitable media of *Clostridium tetani*. The toxin is converted to toxoid by treatment with formaldehyde solution by a method which avoids reversibility of the toxoid. 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.

Tetanus Vaccine (Adsorbed) contains not more than 25 Lf of tetanus toxoid per dose of 0.5 ml.

Description. A whitish turbid liquid in which the mineral carrier tends to settle down on keeping.

Identification

Dissolve sufficient *sodium citrate* in the vaccine 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.

Tests

pH (…….). 6.0 to 7.0.

Specific toxicity. Use 5 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.

Other tests. Complies with the tests described under Vaccines.

Potency. 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.

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 (…….). The lower fiducial limit of error of the estimated potency is not less than 40 Units per dose.

Storage. Store at 2º to 8º. Do not freeze.

Labelling. The label states (1) the human dose; (2) the name of the mineral carrier; (3) the name and proportion of any added preservative; (4) that the vaccine should not be allowed to freeze.