Thai Pharmacopoeia II 2011

Volume I Part 1 Supplement 2024

กรมวิทยาศาสตร์การแพทย์

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กรมวิทยาศาสตร์การแพทย์
Department of Medical Sciences

PREFACE

This Thai Pharmacopoeia II Volume I Part I, Supplement 2024 contains newly developed monographs on a biopharmaceutical product and a drug preparation that is not included in other official pharmacopoeiae, as well as revised monographs on drugs derived from non-indigenous plants, and general information on advanced therapy medicinal products (ATMPs).

The general information on ATMPs is first introduced in this volume to response to their upward trend in Thailand's health system. The chapter aims at providing overall information on how to ensure safety, efficacy, and consistency of the products.

The monograph on a drug preparation that is not included in other official pharmacopoeiae is established in response to the stakeholders' voices.

It is worth noting that the Thai Pharmacopoeia II Volume I Part I, Supplement 2024 is published as an electronic book downloadable from the Bureau of Drug and Narcotic, Department of Medical Sciences website to encourage convenient access and environmental responsibility. The goal of the pharmacopoeia is to set national standards for quality control of medicinal products marketed in the country.

Yongyot Thammavudhi

Dr. Yongyot Thammavudhi
Director-General,
Department of Medical Sciences,
Ministry of Public Health

THE THAI PHARMACOPOEIA COMMITTEE AND SUBCOMMITTEES

The Thai Pharmacopoeia Committee was first appointed by the Ministerial Cabinet in 1979. The functions of the Thai Pharmacopoeia Commission are to

1. select therapeutically useful drugs for inclusion in the Pharmacopoeia;
2. establish compendial limits, tolerances and specifications for selected drugs;
3. specify appropriate tests and methods for quality control of the drugs;
4. compile complete text of the Thai Pharmacopoeia for publication;
5. issue supplements in compliance with the text;
6. revise the Thai Pharmacopoeia periodically;
7. appoint appropriate subcommittees to undertake the functions as entrusted;
8. perform any other tasks assigned by the Minister.

The Thai Pharmacopoeia Committee recruited assistance from the following subcommittees in carrying out its aforementioned functions;

1. The Subcommittee on Editorial Style

This subcommittee is responsible for:

1.1 designing the format and style for printing;

1.2 editing the text;

1.3 keeping conformity of the molecular formula, chemical name, molecular weight, and expressions of the symbols of units throughout the text;

1.4 organizing the information compiled by the subcommittees into a pharmacopoeial form and completing the final draft of the Thai Pharmacopoeia;

1.5 attending to all matters related to editing the Pharmacopoeia.

2. The Subcommittee on Standards for Thai Herbal Drug Preparations

This subcommittee is responsible for:

2.1 producing draft specifications for Thai herbal drug preparations preselected by the Thai Pharmacopoeia Committee and compiling these specifications in monographs in the Thai Herbal Pharmacopoeia;

2.2 submitting the drafts to the Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia for approval;

2.3 attending to all matters related to establishing the specifications for Thai herbal drug preparations;

2.4 preparing appendices of the tests related to the Thai herbal monographs.

3. The Subcommittee on Standards and Analytical Methods

This subcommittee is responsible for:

3.1 selecting drugs to be included in or excluded from the Thai Pharmacopoeia;

3.2 preparing draft specifications, including analytical procedure, for drug monographs in the Thai Pharmacopoeia;

3.3 preparing the appendices regarding testing methods and reagents;

3.4 performing any other assigned tasks.

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INTRODUCTION

Most of the reagents, test solutions and methods of analysis are referred to those specified in the Thai Pharmacopoeia (TP). The appendices are revised to serve the need of those involved. Any appendices not reproduced in this edition can be found in the Thai Pharmacopoeia II 2011.

However, the appendices bearing "H" after their numerical values in the Thai Herbal Pharmacopoeia (THP), a companion book to the TP, are also adopted to facilitate the users in performing testings of crude drugs. It is to be noted that the words "crude drug" in the TP and "herbal drug" in the THP can be used interchangeably to refer to a medicinal plant-based drug.

To avoid ambiguity, the more concise tests in the monographs are introduced; the details of which are grouped under the corresponding subheadings. Another noteworthy change is the grouping of non-mandatory topics, such as safety, stability and additional information, under "Other Relevant Information".

AMENDMENTS

AMENDMENTS

APPENDIX 1 GENERAL INFORMATION

1.11 POWDER FINENESS AND SIEVES pp. 339-340

Replace the text with the following version:

Powders

The degree of coarseness or fineness of a powder is differentiated and expressed by reference to the nominal mesh aperture size of the sieves used.

The following terms are used in the description of powders:

COARSE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 µm.

MODERATELY COARSE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 710 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 250 µm.

MODERATELY FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 355 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 180 µm.

FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 180 µm.

VERY FINE POWDER A powder all the particles of which pass through a sieve with a nominal mesh aperture of 125 µm.

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder shall pass through a sieve of which the nominal mesh aperture, in µm, is equal to that number.

When a batch of a vegetable drug is being ground and sifted, no portion of the drug shall be rejected, but it is permissible, except in the case of assays, to withhold the final tailings, if an approximately equal amount of tailings from a preceding batch of the same drug has been added before grinding.

When the use of sieves is inappropriate, the definition is expressed in terms of the particle size as determined by suitable microscopical examination.

Sieves

Wire mesh sieves used in sifting powdered drugs are identified by numbers indicating the nominal mesh aperture.

The sieves should be made of wires of uniform circular cross-section. The wires may be of stainless steel or of other suitable material except that plated wire is not permitted. Sieves should conform to the specifications which are concordant with the recommended International Standard ISO 3310-1: 2000 (E), shown in the table below.

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the evaluation of the effective opening of test sieves in the size range of 212 to 850 µm, Standard Glass Spheres are available from the national or international organization, e.g., NIST[1]. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and a relative humidity between 20 and 70 per cent.

CLEANING TEST SIEVES Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort. Washing sieves in hot water is not recommended since the sieves can distort and rupture during heating and cooling. If it is necessary to use water, it should be used at ambient temperature and the sieve dried by first using a volatile water-miscible solvent to remove the water and then a low-pressure air jet to remove the solvent. This procedure should be carried out in a fume hood or cabinet that conforms to local regulations.

Method for Determining Powder Fineness

Place the specified quantity of the test powder upon the appropriate sieve having a close-fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction and vertically by tapping on a hard surface for not less than the specified time or until sifting is practically complete. Avoid prolonged shaking that would result in increasing the fineness of the powder during the testing. In the case of oily or other powders that tend to clog the openings, carefully brush the screen at intervals during the test. Breaking up lumps that form during the sifting. Weigh accurately the amount remaining on the sieve and in the receiving pan.

The fineness of a powdered drug or chemical may be determined also by screening through the sieves in a mechanical sieve shaker, which reproduces the circular and tapping motion given to testing sieves in hand sifting but with a uniform mechanical action, following the directions provided by the manufacturer of the shaker.

Method for Determining the Particle Size

The following method-sieving-is used for the determination of particle size in herbal drug powders and topical powders. Sieving generally falls into three main

[1]US National Institute of Standards and Technology

types: manual sieving, mechanical sieving, and air-jet sieving. Manual sieving and mechanical sieving are most suitable where the majority of the particles are larger than about 75 µm. For particles smaller than 75 µm, air-jet sieving or other means of agitation may be more appropriate.

Mechanical sieving methods, using mechanical agitation, or electromagnetic agitation can induce either a vertical oscillation or a horizontal circular motion, or tapping, or a combination of both tapping and horizontal circular motion is available. Entrainment of the particles in an air stream may be used in air jet sieving.

The relative humidity of the environment in which the sieving is carried out must be controlled to prevent moisture uptake or loss by the sample. If the substance being examined is known to develop an electrostatic charge, an antistatic agent, such as colloidal silicon dioxide and/or aluminium oxide, may be added at a 0.5 per cent w/w level to minimize this effect.

1. Manual sieving

(1) SINGLE SIEVE Place a quantity of the substance being examined, as specified in the individual monograph, upon the sieve with a specified number or a nominal mesh aperture, in µm, with a close-fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction for not less than 3 minutes, and gently tap on the sieve frequently in vertical direction. Weigh accurately the amount in the receiving pan, and calculate the percentage.

(2) TWO SIEVES Place the accurately weighed content of 5 single-dose units or of 1 multiple-dose unit upon the upper sieve. Shake the sieve in a left-to-right

Number of Sieve*	Nominal Mesh Aperture Size	Preferred Average Wire Diameter	Percentage Sieving Area	US Sieve No.**
µm	mm	mm		
4000	**4.00**	**1.40**	**55**	**5**
3350	3.35			6
2800	**2.80**	**1.12**	**51**	**7**
2360	2.36	1.00	49	8
2000	**2.00**	**0.90**	**48**	**10**
1700	1.70	0.80	46	12
1400	**1.40**	**0.71**	**44**	**14**
1180	1.18	0.63	43	16
1000	**1.00**	**0.56**	**41**	**18**
µm	µm	µm		
850	850			20
710	**710**	**450**	**37**	**25**
600	600	400	36	30
500	**500**	**315**	**38**	**35**
425	425	280	36	40
355	**355**	**224**	**38**	**45**
300	300	200	36	50
250	**250**	**160**	**37**	**60**
212	212	140	36	70
180	**180**	**125**	**35**	**80**
150	150	100	36	100
125	**125**	**90**	**34**	**120**
106	106	71	36	140
90	**90**	**63**	**35**	**170**
75	75	50	36	200
63	**63**	**45**	**34**	**230**
53	53	36	35	270
45	**45**	**32**	**34**	**325**

*Entries in bold area ISO "principal sizes".

**The list of United States standard sieves is included for information purposes.

horizontal direction by gently tapping on the sieve for 3 minutes. Weigh accurately the amount remaining on the coarser sieve and passing through the finer sieve. Calculate the percentage of the fraction.

2. Mechanical sieving

Unless otherwise specified, weigh accurately the test sieves having a 200-mm diameter and the receiving pan. Place 25 to 100 g of the substance being examined, accurately weighed, depending on the bulk density of the material, upon the top or coarsest sieve whereas the bottom sieve and cover is assembled with a close-fitting receiving pan. Set the mode and the frequency of agitation, and agitate the nest of sieves for 5 minutes. Weigh accurately each sieve and the receiving pan and determine the weight of substance on each sieve.

Calculate the percentage. Repeat these steps until the weight on any of the test sieve does not change by more than 5 per cent or 100 mg of the previous weight on that sieve. If less than 5 per cent of the total sample weight is present on a given sieve, the end-point for that sieve is increased to a weight change of not more than 20 per cent of the previous weight on that sieve.

3. Air-jet sieving

A single sieve is used at a time. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Unless otherwise specified, weigh accurately the test sieve having a 200-mm diameter. Place 25 to 100 g of the substance being examined, accurately weighed, depending on the bulk density of the material, on the sieve with a cover. Set the pressure and air-jet for 5 minutes, then weigh accurately the sieve, and determine the weight of substance on it. Calculate the percentage. Repeat these steps until the weight on the test sieve does not change by more than 5 per cent or 100 mg of the previous weight on that sieve. If less than 5 per cent of the total sample weight is present on a given sieve, the end-point for that sieve is increased to change of not more than 20 per cent of the previous weight sieve.

1.16 PHARMACEUTICAL DOSAGE FORMS pp. 343-368

Replace the text with the following version:

Dosage forms are provided for most of the Pharmacopoeial drug substances, but the processes for the preparation of many of them are, in general, beyond the scope of the Pharmacopoeia. In addition to defining the dosage forms, this section presents general requirements of some of them. Besides these requirements, the pharmaceutical products should be designed to possess certain desirable properties of bioavailability and stability.

Bioavailability

Bioavailability[1] is the rate and extent of absorption of a drug from a dosage form as determined by its concentration/time curve in the systemic circulation or by its excretion in urine. A variety of factors are knownto affect absorption. They are, for example, the method of manufacture or method of compounding; the particle size and crystal form or polymorph of the drug substance; and the diluents and excipients used in formulating the dosage form, including fillers, binders, disintegrating agents, lubricants, coatings, solvents, suspending agents, and dyes. Lubricants and coatings are foremost among these. The maintenance of a demonstrably high degree of bioavailability requires particular attention to all aspects of production and quality control that may affect the nature of the finished dosage form.

Stability

Stability of a pharmaceutical dosage form[2] refers to the capability of the dosage unit, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications. The shelf-life of the dosage form is the period of time during which a product is expected, if stored under recommended conditions, to remain within the specification as determined by stability studies on a number of batches of the product. The shelf-life is used to establish the expiration date. The stability parameters of a pharmaceutical dosage form can be influenced by environmental conditions of storage (temperature, light, air, and humidity), as well as the package components. Pharmacopoeial articles should include required storage conditions on their labelling. These are the conditions under which the expiration date shall apply. The storage requirements specified in the labelling for the article must be observed throughout the distribution of the article (for example, beyond the time it leaves the manufacturer up and including its handling by the dispenser or seller of the article to the consumer).

AEROSOLS

Aerosols are presented in special containers under pressure of a gas and contain one or more active ingredients. The preparations are released from the container, upon actuation of an appropriate valve, in the form of an aerosol (dispersion of solid or liquid particles in a gas, the size of the particles being adapted to the intended use). The pressure for the release is generated by suitable propellants. The preparations consist of a solution, an emulsion or a suspension and are intended for local application to the skin or the mucous membranes of various body orifices or for inhalation. The term "aerosol" refers to the fine mist of spray that results from most pressurized systems. However, the

[1]Further information on bioavailability may be obtained from (1) "ASEAN Guidelines for the Conduct of Bioavailability and Bioequivalence Studies", issued by ASEAN Pharmaceutical Product Working Group)ASEAN PPWG), 2005; (2)" Instruction for the *In Vivo* Bioequivalence Study Protocol Development", notified by Drug Control Division, Food and Drug Administration, Ministry of Public Health, Thailand, 2006.

[2]The stability study requirements may be obtained from (1)" ASEAN Guideline on Stability Study on Drug Product ,"issued by ASEAN Pharmaceutical Product Working Group)ASEAN PPWG" (2) ;2005 ,(Guidelines for Stability Testing of Pharmaceutical Products", published by Food and Drug Administration and Department of Medical Sciences, Ministry of Public Health, Thailand, 2004.

term has been broadly misapplied to all self-contained pressurized products, some of which deliver foams or semisolid fluids. The basic components of an aerosol system are the container, the propellant, the concentrate containing the active ingredient(s), the valve, and the actuator. Suitable auxiliary substances may also be used, for example solvents, solubilizers, emulsifying agents, suspending agents and lubricants for the valve to prevent clogging.

Propellants The propellants are either gases liquefied under pressure or compressed gases or low-boiling liquids. Liquefied gases are, for example, halogenated hydrocarbons (especially chloro-fluoro-derivatives of methane and ethane) and low-molecular-mass hydrocarbons (such as propane and butane). Compressed gases are, for example, carbon dioxide, nitrogen and nitrous oxide. Mixtures of these propellants may be used to obtain optimal solution properties and desirable pressure, delivery and spray characteristics.

Valves A suitable valve keeps the container tightly closed when not in use and regulates the delivery of the contents during use. The spray characteristics are influenced by the type of spraying device, in particular by the dimensions, number and location of orifices. Some valves provide a continuous release, others ("metering dose valves") deliver a defined quantity of product upon each valve actuation. The various valve materials in contact with the contents are compatible with them.

Actuators An actuator is the fitting attached to an aerosol valve stem which, when depressed or moved, opens the valve, and directs the spray containing the drug preparation to the desired area. The actuator usually indicates the direction in which the preparation is dispensed and protects the hand or finger from the refrigerant effects of the propellant. Actuators incorporate an orifice which may vary widely in size and shape. The size of this orifice, the expansion chamber design, and the nature of the propellant and formulation influence the physical characteristics of the spray foam, or stream of solid particles dispensed. For inhalation or oral dose aerosols, an actuator capable of delivering the medication in the proper particle size range is utilized.

Production Aerosols are prepared by one of two general processes. In the "cold-fill" process, the concentrate (generally cooled to a temperature below 0°) and the refrigerated propellant are measured into open containers (usually chilled). The valve-actuator assemblyis then crimped onto the container to form a pressure-tight seal. During the interval between propellant addition and crimping, sufficient volatilization of propellant occurs to displace air from the container. In the "pressure-fill" method, the concentrate is placed in the container, and either the propellant is forced under pressure through the valve orifice after the valve is sealed, or the propellant is allowed to flow under the valve cap and then the valve assembly is sealed ("under-the-cap" filling). In both cases of the "pressure-fill" method, provision must be made for evacuation of air by means of vacuum or displacement with a small amount of propellant. Manufacturing process controls usually include monitoring or proper formulation and propellant fill weight, and pressure testing and leak testing and valve function testing of the finished aerosol. Microbiological attributes should also be controlled.

Leak testing Aerosols comply with the Leak testing under "Test for Aerosols" (Appendix 4.19).

Minimum fill Aerosols comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage The containers are tight and resistant to the internal pressure and may be made of metal, glass, plastic or combinations of these materials. They are compatible with their contents. Suitable metals include stainless steel, aluminium, and tin-plated steel. Glass containers are protected with a plastic coating. Aerosols shall be stored at a temperature not exceeding 50° and protected from frost.

Labelling The label of aerosols states (1) the method of use, and, if necessary, that the container should be shaken before use; (2) if necessary, the precautions to be taken, for example, avoid inhaling, avoid contact with the eyes and other mucous membranes; (3) that the container should not be exposed to or stored at a temperature above 50° and should not be exposed to direct sunlight; (4) for a container with a metering dose valve, the amount of active ingredient in a unit spray; (5) that the container should not be punctured or incinerated.

AROMATIC WATERS

Aromatic waters are clear saturated solutions of volatile oils or other aromatic substances in water, usually employed for their flavouring rather than their medicinal properties. Aromatic waters prepared as described below contain a small amount of Ethanol.

Production Aromatic waters are prepared by (1) dilution of a concentrated, ethanolic solution of the aromatic substance with water; (2) solution of the aromatic substance, with or without the use of a dispersing agent; or (3) distillation of the aromatic substance.

Packaging and storage Aromatic waters should be kept in tightly closed containers, protected from intense light and excessive heat.

CAPSULES

Capsules are solid dosage forms with hard or soft shells. They are of various shapes and sizes, and contain a single dose of one or more active ingredients. They are intended for oral administration, but preparations for alternative applications may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph. Starch capsules (often known as cachets) are not described in this monograph. The different categories of capsules that exist include hard, soft, and modified-release capsules. Their surfaces may bear symbols or other markings. They should be sufficiently robust to withstand handling, including packaging,

storage, and transportation, without cracking or breaking. They should be packaged and stored in a manner that protects them from microbial contamination. Capsule shells are made of gelatin or other substances, the consistency of which may be modified by the addition of substances such as glycerol and sorbitol. Preservatives may also be necessary. The shell should disintegrate in the presence of digestive fluids so that the contents are released. The contents of capsules may be solid, liquid or of a paste-like consistency. Capsule shells and contents may contain excipients such as diluents, sweeteners, colouring matters, flavouring substances, disintegrating agents, glidants, lubricants, and substances capable of modifying the behaviour of the active ingredient(s) in the gastrointestinal tract. The contents should not cause deterioration of the shell. When excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between any of the components of the dosage form.

Disintegration Capsules comply with the "Disintegration Test for Tablets and Capsules" (Appendix 4.23). For those capsules for which a dissolution requirement is included in the individual monograph, omission of the requirement for disintegration is considered justifiable and is therefore authorized.

Uniformity of dosage units Unless otherwise prescribed in the individual monographs, capsules comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Visual inspection Unpack and inspect at least 20 capsules. They should be smooth and undamaged. Evidence of physical instability is demonstrated by gross changes in physical appearance, including hardening or softening, cracking, swelling, mottling, or discoloration of the shell.

Packaging and storage Capsules should be kept in well-closed containers at a temperature not exceeding 30° and protected from light, excessive moisture, or dryness.

Hard Capsules

Hard capsules have shells consisting of two prefabricated cylindrical sections that fit together. One end of each section is rounded and closed, and the other is open. The contents of hard capsules are usually in solid form (powder or granules); in certain cases the contents may be in the form of encapsulated powders or micropellets.

Production Hard capsules are prepared by mixing the active ingredient(s) with a number of excipients. Sometimes, the physical characteristics of the mixture allow it to be directly filled into the shell, but it may occasionally be necessary to granulate before filling. Normally the granulate needs to be mixed with lubricants and/or disintegrating agents.

A uniform mass of the capsule mixture is volumetrically fed into the narrower lower section of the shell body which is then closed by slipping the larger section or cap over it. The security of the closure may be ensured by suitable means.

Soft Capsules

Soft capsules have thicker shells than hard capsules, and preservatives are usually added. The shells are of one piece and various shapes. Partial migration of the contents into the shell may occur (and vice versa) depending on the nature of the materials used.

Production Soft capsules are prepared by mixing the active ingredient(s) with a number of excipients. Soft gelatin capsules are usually formed, filled, and sealed in one operation. However, shells for extemporaneous use are sometimes prefabricated. Liquids may be incorporated directly. Solids are usually dissolved or dispersed in a suitable excipient(s) to give a solution or dispersion of thick consistency.

Modified-Release Capsules

Modified-release capsules are hard or soft capsules in which the contents or the shell or both contain additives or are prepared by special procedures such as micro-encapsulation which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastro-intestinal tract.

DELAYED-RELEASE CAPSULES (ENTERIC CAPSULES)

Delayed-release capsules are hard or soft capsules prepared in such a manner that either the shell or the contents resist the action of the gastric fluid but release the active ingredient(s) in the presence of the intestinal fluid.

All requirements for these specialized dosage forms are given in the individual monographs.

EXTENDED-RELEASE CAPSULES Extended-release capsules are designed to slow the rate of release of the active ingredient(s) in the gastro-intestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

Production Delayed-release capsules are prepared by providing hard or soft capsules with a gastro resistant shell (enteric capsules) or by filling capsules with either granules or particles covered with a gastro-resistant coating. See also under Hard Capsules or Soft Capsules.

EAR PREPARATIONS

Ear preparations are liquid, semi-solid or powder preparations usually containing one or more active ingredients in a suitable vehicle. They are intended for instillation, for spraying, for insufflation, for application to the auditory meatus or as an ear wash. Ear preparations may contain auxiliary substances, for example, to adjust tonicity or viscosity, to adjust or stabilize the pH, to increase the solubility of the active ingredients, to stabilize the preparation or to provide adequate antimicrobial properties. Such additives should not adversely affect the intended medicinal action of the preparation, nor, at the concentrations used, cause toxicity or undue local irritation.

Preparations for application to the injured ear, particularly where the ear-drum is perforated, or prior to surgery are sterile, free from antimicrobial preservatives and supplied in single-unit containers.

Unless otherwise justified and authorized, ear preparations supplied in multiple-unit containers contain a suitable antimicrobial preservative in appropriate concentration, except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservatives should be compatible with the other ingredients of the preparation and should remain effective throughout the period of use of the ear preparations. Five categories of ear preparations may be distinguished: (1) ear drops; (2) ear sprays; (3) semisolid ear preparations; (4) ear powders; (5) ear washes.

Minimum fill Ear preparations comply with the test described in the "Minimum Fill" (Appendix 4.26).

Sterility Where the ear preparations are labelled as sterile, unless otherwise directed in the individual monograph, they comply with the "Sterility Test" (Method I, Appendix 10.1).

Ear Drops

Ear drops are suspensions, emulsions or solutions of one or more active ingredients suspended, dispersed or dissolved in liquids such as water, glycols or fatty oils, suitable for application to the auditory meatus without exerting harmful pressure on the ear-drum. They may also be placed in the auditory meatus by means of a plug impregnated with the liquid. Suspended solids may separate slowly on standing but are easily redispersed on shaking. The size of the dispersed particles should be controlled. Ear drops are usually supplied in multiple-unit containers fitted with an appropriate applicator.

Containers Ear drops are supplied in containers of glass or suitable plastic that are fitted with an integral dropper or with a screw cap of suitable materials incorporating a dropper and rubber or plastic teat. Alternatively, such a cap assembly is supplied separately.

Labelling The label of ear drops states (1) the name(s) and concentration(s) of the active ingredient(s); (2) the name(s) and concentration(s) of any antimicrobial preservative(s); (3) that they are intended for external use only; (4) where appropriate, that the preparation is sterile.

Ear Sprays

(**Note** When ear sprays are supplied in aerosol containers, these comply with the appropriate requirements for Aerosols.)

Ear sprays are suspensions, emulsions or solutions of one or more active ingredients suspended, dispersed or dissolved in liquids suitable for spraying to auditory meatus without exerting harmful pressure on the eardrum. The special requirements may be necessary for the selection of propellants, for particle size for the single-dose delivered by the metering valves.

See also under Ear Drops.

Packaging and storage Ear sprays are supplied in multiple-unit containers fitted with an appropriate applicator.

Labelling When ear sprays are supplied in aerosol containers, the label shall state (1) the method of use and, if necessary, that the container should be shaken before use; (2) if necessary, the precautions to be taken, for example, avoid inhaling; (3) that the container shall not be exposed to or stored at a temperature above 50° and should not be exposed to direct sunlight; (4) for a container with a metering dose valve, the amount of active ingredient in a unit-spray; (5) that the container should not be punctured or incinerated.

See also under Ear Drops.

Semi-Solid Ear Preparations

(**Note** Semi-solid ear preparations comply with the appropriate requirements for Topical Semi-solid Preparations.)

Semi-solid ear preparations are semi-solid dosage forms such as creams, gels, or ointments, etc. Intended for application to the external auditory meatus, if necessary by means of a plug impregnated with the preparation. Semi-solid ear preparations are supplied in containers fitted with a suitable applicator.

Labelling See under Ear Drops.

Ear Powders

(**Note** Ear powders comply with the appropriate requirements for powders.)

Ear powders are fine powders intended for application or insufflation to the external auditory meatus.

Containers Ear powders are supplied in containers fitted with a suitable device for application or insufflation.

Labelling See under Ear Drops.

Ear Washes

Ear washes are solutions intended to cleanse the external auditory meatus. They are usually aqueous solutions with a pH within physiological limits.

See also under Ear Drops.

Containers Ear washes are supplied in containers fitted with a suitable applicator.

Labelling See under Ear Drops.

EXTRACTS

Extracts are preparations of liquid, solid or semisolid consistency, obtained from herbal or animal matter, which is usually dried. Extracts may be subjected to purification processes that increase the content of characterized constituents with respect to the content of dry extractable matter from that which would be expected from extraction with the stated solvent: such extracts are termed "enriched".

Three types of extract can be distinguished:

Type A Type A extracts (standardized extracts) are adjusted to a defined range of therapeutically active constituents. Standardization is achieved by adjustment of the extract with inert material or by blending extracts.

Type B Type B extracts (quantified extracts) are adjusted to a defined range of active constituents. Adjustments are made either by blending batches of extracts or by blending batches of herbal or animal matter prior to extraction.

Type C Type C extracts are essentially defined by the production process (state of the matter to be extracted, solvent, extraction conditions). Constituents considered to be relevant markers may be determined.

Production Extracts are prepared by maceration, percolation or other suitable validated methods using ethanol or other suitable solvent. The matter to be extracted may undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting. In addition, unwanted matter may be removed, if necessary, after extraction. Herbal drugs, animal matters and organic solvents used for the preparation of extracts comply with any relevant monograph of the Pharmacopoeia. For soft and dry extracts where the organic solvent is removed by evaporation, recovered or recycled solvent may be used, provided that the recovery procedures are controlled and monitored to ensure that solvents meet appropriate standards before reuse or admixture with other approved materials.

Water used for the preparation of extracts is of suitable quality. Except for the test for bacterial endotoxins, water complying with the section on Purified Water in bulk of the monograph on Purified Water is suitable. Potable water may be suitable if it complies with a defined specification that allows the consistent production of a suitable extract. Where applicable, concentration to the intended consistency is carried out using suitable methods, usually under reduced pressure, and at a temperature at which deterioration of the constituents is reduced to a minimum. Volatile oils that have been distilled during processing may be restored to the extracts at an appropriate stage in the manufacturing process. Suitable inert excipients may be added at the various stages of the manufacturing process to improve technological qualities like homogeneity, consistency or stability of active constituents. Where applicable, as a result of analysis of the herbal or animal matter used for the production of extracts, tests for microbiological quality, heavy metals, aflatoxins, and pesticide residues in the extracts have to be carried out.

Labelling For Type A, the label on the container states (1) the herbal or animal matter used; (2) whether the extract is dry, soft or liquid; (3) the composition of the extraction solvent; (4) where applicable, that fresh herbal or animal matter has been used; (5) where applicable, that the extract is "enriched"; (6) the name and amount of any excipient used. For Types B and C, the label on the container states (1) to (6) as for type A; (7) the content of constituents (markers) used for quantification and (8) the range of starting material: final extract (Drug: Extract Ratio or DER).

Liquid Extracts

Liquid extracts are liquid preparations of which, in general, one part by mass or volume is equivalent to one part by mass of the original dried herbal or animal matter. These preparations are adjusted, if necessary, so that they satisfy the requirements for content of solvent, and, where applicable, for constituents or dry residue.

Production Liquid extracts are prepared by using ethanol of suitable concentration or water to extract the stated herbal or animal matter or by dissolving a soft or dry extract (which has been produced using the same strength of extraction solvent as is used in preparing the liquid extract by direct extraction) of the stated herbal or animal matter in either ethanol of suitable concentration or water and filtering, if necessary. A slight sediment may form on standing, which is acceptable as long as the composition of the liquid extract is not changed significantly. Liquid extracts may contain suitable antimicrobial preservatives.

Relative density Where applicable, the liquid extract complies with the limits prescribed in the monograph.

Ethanol content For ethanolic liquid extracts, carry out the "Determination of Ethanol" (Appendix 6.5). The preparation complies with the limits prescribed in the monograph.

Methanol and 2-propanol Not more than 0.05 per cent v/v of methanol and not more than 0.05 per cent v/v of 2-propanol for ethanolic liquid extracts unless otherwise prescribed.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, introduce rapidly 2.0 g or 2.0 ml of the extract to be examined. Evaporate to dryness on a water-bath and dry at 105° for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage or in grams per litre.

Packaging and storage Liquid extracts should be kept in well-closed containers, protected from light.

Labelling In addition to the requirements listed above the label on the container states the final extract; (2) the concentration of any added antimicrobial preservative.

Soft Extracts

Soft extracts are semi-solid preparations obtained by evaporation of the solvent used for preparation. Soft extracts generally have a dry residue of not less than 70 per cent w/w. They may contain suitable antimicrobial preservatives.

Dry residue In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 2.0 g of the extract to be examined. Heat to dryness on a water-bath and dry at 105° for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a soft extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Soft extracts should be keptin well-closed containers, protected from light.

Labelling In addition to the requirements listed above. The label on the container states the concentration of any added antimicrobial preservative.

Dry Extracts

Dry extracts are solid preparations obtained by evaporation of the solvent used for their production.

Dry extracts generally have a dry residue of not less than 95 per cent w/w.

Loss on drying Where applicable, the dry extract complies with the limits prescribed in the monograph. In a flat-bottomed dish about 50 mm in diameter and about 30 mm in height, weigh rapidly 500 mg of the extract to be examined, finely powdered. Dry at 105° for 3 hours. Allow to cool in a desiccator over *phosphorus pentoxide desiccant* or *self-indicating silica gel* and weigh. Calculate the result as a percentage weight in weight. Where applicable, a monograph on a drug extract prescribes a limit test for the solvent used for extraction.

Packaging and storage Dry extracts should be kept in well-closed containers, protected from light.

EYE PREPARATIONS

Eye preparations are sterile liquid, semi-solid or solid preparations intended for administration upon the eyeball and/or to the conjunctiva, or for insertion in the conjunctival sac. Several categories of eye preparations may be distinguished: (1) eye drops; (2) eye lotions; (3) powders for eye drops and powders for eye lotions; (4) semi-solid eye preparations; (5) ocular systems.

Production During the development of an eye preparation, whose formulation contains an antimicrobial preservative, the necessity for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in "Efficacy of Antimicrobial Preservation" (Appendix 10.6).

Eye preparations are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro organisms; recommendations on this aspect are provided in "Sterilization and Sterility Assurance" (Appendix 12).

In the manufacture of eye preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

For multiple-unit containers of eye preparations, the period after opening the container, after which the contents must not be used, does not exceed 4 weeks.

Sterility Eye preparations comply with the "Sterility Test" (Appendix 10.1). Applicators supplied separately also comply with the test for sterility. Remove the applicator with aseptic precautions from its package and transfer it to a tube of culture medium so that it is completely immersed. Incubate and interpret the results as described in the test for sterility.

Minimum fill Eye preparations except eye strips and ocular systems comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage Eye preparations should be kept in a sterile, tightly closed, tamper-evident container.

Labelling The label of eye preparations states the name(s) of any added antimicrobial preservative(s).

Eye Drops

Eye drops are sterile aqueous or oily solutions, emulsions or suspensions of one or more active substances intended for instillation into the eye. Eye drops may contain excipients, for example, to adjust the tonicity or the viscosity of the preparation, to adjust or stabilize the pH, to increase the solubility of the active substance, or to stabilize the preparation. These substances do not adversely affect the intended medicinal action or, at the concentrations used, cause undue local irritation.

Aqueous preparations supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen must be compatible with the other ingredients of the preparation and must remain effective throughout the period of time during which eye drops are in use.

If eye drops are prescribed without antimicrobial preservatives, they are supplied wherever possible in single-dose containers. Eye drops intended for use in surgical procedures do not contain antimicrobial preservatives and are supplied in single-dose containers.

Eye drops that are solutions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

Eye drops that are suspensions may show a sediment that is readily redispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Multidose preparations are supplied in containers that allow successive drops of the preparation to be administered. The containers contain at most 10 mL of the preparation.

Limit of particle size Unless otherwise specified in the individual monograph, eye drops in the form of a suspension comply with the following test. Introduce a suitable quantity of the suspension into a counting cell or with a micropipette onto a slide, as appropriate, and scan under a microscope an area corresponding to 10 µg of the solid phase. For practical reasons, it is recommended that the whole sample be first scanned at low magnification (e.g., ×50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g., ×200 to ×500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a

maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

Labelling The label of eye drops states the storage condition.

Eye Lotions

Eye lotions are sterile aqueous solutions intended for use in rinsing or bathing the eye or for impregnating eye dressings. Eye lotions may contain excipients, for example to adjust the tonicity or the viscosity of the preparation or to adjust or stabilize the pH. These substances do not adversely affect the intended action or, at the concentrations used, cause undue local irritation. Eye lotions supplied in multidose containers contain a suitable antimicrobial preservative in appropriate concentration except when the preparation itself has adequate antimicrobial properties. The antimicrobial preservative chosen is compatible with the other ingredients of the preparation and remains effective throughout the period of time during which the eye lotions are in use.

If eye lotions are prescribed without an antimicrobial preservative, they are supplied in single-dose containers. Eye Lotions intended for use in surgical procedures or in first-aid treatment do not contain an antimicrobial preservative and are supplied in singledose containers.

Eye lotions, examined under suitable conditions of visibility, are practically clear and practically free from particles.

The containers for multidose preparations do not contain more than 200 mL of eye lotion, unless otherwise specified in the individual monograph.

Labelling See under Eye Drops.

Powders for Eye Drops and Eye Lotions

Powders for the preparation of eye drops and eye lotions are supplied in a dry, sterile form to be dissolved or suspended in an appropriate liquid vehicle at the time of administration. They may contain excipients to facilitate dissolution or dispersion, to prevent caking, to adjust the tonicity, to adjust or stabilize the pH or to stabilize the preparation.

After dissolution or suspension in the prescribed liquid, they comply with the requirements for eye drops or eye lotions, as appropriate.

Uniformity of dosage units Single-unit powders for eye-drops and eye lotions comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Semi-solid Eye Preparations

Semi-solid eye preparations are sterile ointments, creams or gels intended for application to the conjunctiva. They contain one or more active substances dissolved or dispersed in a suitable basis. They have a homogeneous appearance.

Semi-solid eye preparations comply with the requirements of the monograph on Topical Semi-solid Preparations. The basis is non-irritant to the conjunctiva.

Semi-solid eye preparations are packed in small, sterilized collapsible tubes fitted or provided with a sterilized cannula and having a content of not more than 10 g of the preparation. The tubes must be well-closed to prevent microbial contamination. Semi-solid eye preparations may also be packed in suitably designed single-dose containers. The containers, or the nozzles of tubes, are of such a shape as to facilitate administration without contamination.

Limit of particle size Semi-solid eye preparations containing dispersed solid particles comply with the following test: spread gently a quantity of the preparation corresponding to at least 10 µg of solid active substance as a thin layer. Scan under a microscope the whole area of the sample. For practical reasons, it is recommended that the whole sample is first scanned at a small magnification (e.g., ×50) and particles greater than 25 µm are identified. These larger particles can then be measured at a larger magnification (e.g., ×200 to ×500). For each 10 µg of solid active substance, not more than 20 particles have a maximum dimension greater than 25 µm, and not more than 2 of these particles have a maximum dimension greater than 50 µm. None of the particles has a maximum dimension greater than 90 µm.

Labelling See under Eye Drops.

Ocular Systems

See under Systems.

GRANULES

Granules are preparations consisting of solid, dry aggregates of powder particles sufficiently resistant to withstand handling. They are intended for oral administration. Some are swallowed as such, some are chewed and some are dissolved or dispersed in water or another suitable liquid before being administered. Granules contain one or more active ingredients with or without added substances including, where necessary, authorized colouring matter and flavouring agents. Granules are presented as single-unit or multiple-unit preparations. For single-unit preparations each dose is enclosed in an individual container, for example, a sachet, a paper packet or a vial. Each dose of a multiple-unit preparation is administered by means of a device suitable for measuring the quantity prescribed.

Several categories of granules may be distinguished: (1) uncoated granules; (2) granules for the preparation for oral liquids (see under Oral Liquids); (3) coated granules; (4) modified-release granules.

Uniformity of dosage units Unless otherwise prescribed in the individual monographs, granules comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element granules and herbal drug granules.

Particle size Topical granules and herbal drug granules comply with the following requirement.

Carry out the determination as described in the "Method for Determining the Particle Size" (Manual Sieving-Two Sieves, Appendix 1.11), use about 10 g of the granules, accurately weighed. Not more than 15 per

cent of the granules examined pass through a sieve with a nominal mesh aperture of 180 µm and none passes through a sieve with a nominal mesh aperture of 2000 µm.

Packaging and storage Unless otherwise specified the individual monograph. Granules shall be kept in tightly closed containers and stored in a dry place.

Labelling For single-unit containers the label states the name(s) and amount(s) of active ingredient(s) per container and for multiple-unit containers the label states the name(s) and amount(s) of active ingredient(s) in a suitable quantity by weight.

Uncoated Granules

Uncoated granules may be plain or effervescent granules.

EFFERVESCENT GRANULES Effervescent granules are uncoated granules generally containing acid substances and either carbonates or bicarbonates which react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Disintegration Place a single dose of the granules in a beaker containing 200 mL of *water* at 15° to 25°; numerous gas bubbles are evolved. When the evolution of gas around the individual grains has ceased, the granules have disintegrated, being either dissolved or dispersed in the water. Repeat the operation on a further five doses. The granules comply with the test if each of the six doses used in the test disintegrates within 5 minutes.

Coated Granules

Coated granules are granules covered with one or more layers of mixtures of various substances. Substances used as coatings are usually applied as a solution or suspension in conditions in which evaporation of the vehicle occurs. Coated granules are usually presented as multiple-unit preparations.

Modified-release Granules

DELAYED-RELEASE GRANULES (ENTERIC-COATED GRANULES) Delayed-release granules are intended to resist the gastric fluid and to release the active ingredient (s) in the intestinal fluid. These properties are achieved by covering the granules with a gastro-resistant material (enteric-coated granules) or by other suitable means.

EXTENDED-RELEASE GRANULES Extended-release granules are coated or uncoated granules prepared by using added substances or procedures which, separately or together, are designed to modify the rate or the place at which the active ingredient(s) are released.

HERBAL DRUG PREPARATIONS

Herbal drug preparations are obtained by subjecting herbal drugs to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal drugs, tinctures, extracts, essential oils, expressed juices and processed exudates.

Herbal teas comply with the monograph on Herbal teas.

Instant herbal teas consist of powder or granules of one or more herbal drug preparation(s) intended for the preparation of an oral solution immediately before use.

HERBAL TEAS

Herbal teas consist exclusively of one or more herbal drugs intended for oral aqueous preparations by means of decoction, infusion or maceration. The preparation is prepared immediately before use. Herbal teas are usually supplied in bulk form or in sachets. The herbal drugs used comply with the appropriate individual monographs.

Recommendations on the microbiological quality of herbal teas under the "Limits for Microbial Contamination" (Category 2 in Table 2, Appendix 10.5) taking into account the prescribed preparation method (use of boiling or non-boiling water).

Identification The identity of herbal drugs present in herbal teas is checked by botanical examinations. The proportion of herbal drugs present in herbal teas is checked by appropriate methods. Herbal teas in sachets comply with the following test:

Weight variation Determine the average weight of twenty randomly chosen units as follows: weigh a single full sachet of herbal tea, open it without losing any fragments. Empty it completely using a brush. Weigh the empty sachet and calculate the mass of the contents by subtraction. Repeat the operation on the nineteen remaining sachets. Unless otherwise justified not more than two of the twenty individual masses of the contents deviate from the average mass of the contents by more than the percentage deviation shown in the table below and none deviates by more than twice that percentage.

Average Weight	Percentage Deviation
Less than 1.5 g	15 per cent
1.5 g to 2 g	10 per cent
More than 2.0 g	7.5 per cent

Packaging and storage Herbal teas should be protected from light.

INFUSIONS

Infusions are dilute solutions that contain the readily soluble constituents of crude drugs. Fresh infusions are made by pouring boiling water onto the drug, in a suitable state of comminution, and macerating for a short time, or they are usually prepared by diluting one volume of a concentrated infusion to ten volumes with water. Concentrated infusions are usually made by maceration of the drug with Ethanol (25 Per Cent).

For dispensing purposes, Infusions should be used within 12 hours of preparation from concentrated infusions.

Packaging and storage Infusions should be kept in well-closed containers.

Labelling The label on the container states the

storage conditions.

IRRIGATION SOLUTIONS

Irrigation solutions are sterile solutions of one or more active ingredients intended for irrigation. If the solution is intended to be used for the irrigation of body cavities, for the flushing of wounds or operation cavities or for the irrigation of the urogenital system, it is sterile and a pyrogenic. Such solutions are prepared using Water for Irrigation. Irrigation solutions may contain added substances such as suitable substances to make the preparation isotonic with blood. They are supplied in containers holding sufficient of the solution for use on one occasion only. When viewed under suitable conditions of visibility, they are practically clear and practically free from particles.

Sterility Unless otherwise directed in the individual monograph, irrigation solutions comply with the "Sterility Test" (Method I, Appendix 10.1).

Packaging and storage Irrigation solutions are supplied in containers made from materials that are sufficiently transparent to permit the visual inspection of the contents and that do not cause deterioration of the preparation as a result of diffusion into or across the material of the container or by yielding foreign substances into the preparation. The containers should be readily distinguishable from containers for preparations intended for parenteral administration. The containers are tamper-evident and are sealed so as to exclude micro-organisms. Unless otherwise specified in the individual monograph, Irrigation Solutions should be stored at a temperature not exceeding 25°.

Labelling The label of irrigation solutions states (1) that the irrigation solution is sterile and, where applicable, that the irrigation solution is apyrogenic; (2) that the irrigation solution is not to be used for injection; (3) that the irrigation solution should be used on one occasion only and that any remainder should be discarded.

LOZENGES

See under Oromucosal Preparations.

MEDICATED FOAMS

Medicated foams are preparations consisting of large volumes of gas dispersed in a liquid generally containing one or more active ingredients, a surfactant ensuring their formation and various other excipients. Medicated foams are usually intended for application to the skin or mucous membranes. Medicated foams are usually formed at the time of administration from a liquid preparation in a pressurized container. The container is equipped with a device consisting of a valve and a push button suitable for the delivery of the foam. Medicated foams intended for use on severely injured skin and on large open wounds are sterile. Medicated foams supplied in pressurized containers comply with the requirements in the monograph for "Aerosols" (Appendix 1.16).

Production Sterile medicated foams are prepared using materials and methods designed to ensure sterility and to avoid the introduction of contaminants and the growth of micro-organisms.

Relative foam density Maintain the container at about 25° for at least 24 hours. Taking care not to warm the container, fit a rigid tube 70 mm to 100 mm long and about 1 mm in internal diameter onto the push button. Shake the container to homogenize the liquid phase of the contents and dispense 5 ml to 10 ml of foam to waste. Tare a flat-bottomed dish of about 60 ml volume and about 35 mm high. Place the end of the rigid tube attached to the push button in the corner of the dish, press the push button and fill the dish uniformly, using a circular motion. After the foam has completely expanded, level off by removing the excess foam with a slide. Weigh. Determine the weight of the same volume of *water* by filling the same dish with *water*. The relative foam density is equivalent to the ratio:

$$\frac{m}{e},$$

where m is the weight of test sample of foam in g and e is the weight of same volume of water in g. Carry out three measurements. None of the individual values deviate by more than 20 per cent from the mean value.

Duration of expansion The apparatus (Fig. 1) consists of a 50-mL burette, 15 mm in internal diameter, with 0.1-mL graduations and fitted with a 4-mm single bore stopcock. The graduation corresponding to 30 mL is at least 210 mm from the axis of the stopcock. The lower part of the burette is connected by means of a plastic tube not longer than 50 mm and 4 mm in internal diameter to the foam-generating container equipped with a push button fitted to this connection. Maintain the container at about 25° for at least 24 hours. Shake the container, taken care not to warm it, to homogenize the liquid phase of the contents and dispense 5 mL to 10 mL of the foam to waste.

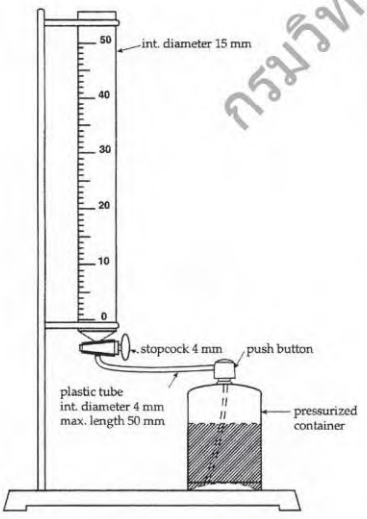


Fig. 1 The Apparatus for Duration of Expansion

Connect the push button to the outlet of the burette. Press the button and introduce about 30 mL of foam in a single delivery. Close the stopcock and at the same time start the chronometer and read the volume of foam in the burette. Every 10 seconds read the growing

volume until the maximum volume is reached. Carry out three measurements. None of the times needed to obtain the maximum volume is more than 5 minutes.

Sterility Comply with the "Sterility Test" (Appendix 10.1), when the label indicates that the preparation is sterile.

Labelling The label on the container states (1) that the Medicated Foam is intended for external use only and (2) the storage conditions.

MEDICATED TAMPONS

Medicated tampons are solid, single-dose preparations intended to be inserted into the body cavities for a limited period of time. They consist of a suitable material such as cellulose, collagen or silicone impregnated with one or more active ingredients.

Production In manufacturing, packaging, storage and distribution of medicated tampons, suitable means are taken to ensure their microbial quality.

Microbial limit Comply with the "Limits for Microbial Contamination" (Appendix 10.5).

NASAL PREPARATIONS

Nasal preparations are liquid, semi-solid or solid preparations containing one or more active ingredients. They are intended for administration to the nasal cavities (nostrils) for local or systemic effects. Nasal preparations should as far as possible be non-irritating and should not adversely affect the functions of the nasal mucosa and its cilia. Aqueous Nasal preparations are usually isotonic. Nasal preparations are supplied in multiple-unit or single-unit containers provided, if necessary, with a suitable administration device.

Unless otherwise justified and authorized, aqueous nasal preparations supplied in multiple-unit containers contain a suitable antimicrobial preservative in appropriate concentration, except when the preparation itself has adequate antimicrobial properties. Five categories of nasal preparations may be distinguished: (1) nasal drops; (2) liquid nasal sprays; (3) nasal powders; (4) semi-solid nasal preparations; (5) nasal washes.

Minimum fill Nasal preparations comply with the test described in the "Minimum Fill" (Appendix 4.26).

Labelling The label of nasal preparations states the instructions for use.

Nasal Drops

Nasal drops are solutions, emulsions or suspensions intended for instillation into the nostrils. Emulsions should not show evidence of phase separation; they have a uniform appearance after shaking. Suspensions may show a sediment which is readily redispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Production Single doses of nasal drops intended for systemic absorption of active ingredients through the nasal mucosa are tested for uniformity of volume, content or weight.

Containers Nasal drops are usually supplied in glass or plastic containers provided with a suitable applicator.

Uniformity of weight Nasal drops that are solutions comply with the following test: weigh individually the contents of ten containers emptied as completely as possible, and determine the average mass. Not more than two of the individual masses deviate by more than 10 per cent from the average mass and none deviates by more than 20 per cent.

Uniformity of content Nasal drops that are suspensions comply with the "Content Uniformity" (Appendix 4.28).

Liquid Nasal Sprays

(**Note** Where liquid nasal sprays are supplied in aerosol containers, these comply with the appropriate requirements for Aerosols.)

Liquid nasal sprays are solutions, emulsions or suspensions intended for spraying into the nostrils.

See also under Nasal Drops.

Production For liquid nasal sprays that are suspensions, the size of the dispersed particles of the spray should be such as to localize their deposition in the nostril.

Single doses of liquid nasal sprays intended for systemic absorption of active ingredients through the nasal mucosa are tested for uniformity of volume, content or weight.

Packaging and storage Liquid nasal sprays are supplied in glass or plastic containers with atomizing devices or in aerosol containers fitted with a suitable adapter and with or without a metering dose valve. They may also be administered by means of suitable inhalers. Aerosol preparations shall be stored at a temperature not exceeding 50° and protected from frost.

Uniformity of weight Metered dose nasal sprays that are solutions comply with the following test: discharge once to waste. Wait for not less than 5 seconds and discharge again to waste. Repeat this procedure for a further three actuations. Weigh the mass of the container, discharge once to waste and weigh the remaining mass of the container. Calculate the difference between the two masses. Repeat the procedure for a further nine containers. They comply with the test if not more than two of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose Metered dose nasal sprays that are suspensions or emulsions comply with the following test: use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomising device.

Shake a container for 5 seconds and discharge once to waste. Wait for not less than 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further three actuations. After 2 seconds, fire one dose of the metered dose nasal spray into the collecting vessel by actuating the atomizing device.

Collect the contents of the collecting vessel by successive rinses. Determine the content of active ingredient in the combined rinses.

Repeat the procedure for a further nine containers. Unless otherwise justified and authorized, the preparation complies with the test if not more than one of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent and 135 per cent of the average content. If two or three individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for twenty more containers. The preparation complies with the test if not more than three individual contents of the thirty individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

Nasal Powders

(**Note** Nasal powders comply with the appropriate requirements for Powders.)

Nasal powders are powders intended for insufflation into the nostrils by means of a suitable device.

Production The size of the particles should be such as to localize their deposition in the nostril. Particle size should be verified by adequate methods of particle-size determination.

Containers See under Nasal Drops. **Semi-solid Nasal Preparations**

(**Note** Semi-solid nasal preparations comply with the appropriate requirements for Topical Semi-solid Preparations.)

Semi-solid nasal preparations are semi-solid dosage forms such as creams, gels, or ointments, etc. intended for application into the nostrils.

Containers The containers for semi-solid nasal preparations should be adapted to deliver the product to the site for application.

Nasal Washes

Nasal washes are generally aqueous solutions intended for irrigation of the nostrils. Nasal washes intended for application to injured parts or prior to a surgical operation are sterile.

Sterility Where the nasal washes are labelled as sterile, unless otherwise directed in the individual monograph, they comply with the "Sterility Test" (Method I, Appendix 10.1).

Containers See under Nasal Drops. **Labelling** The label of nasal washes shall state,

where applicable, a statement that the preparations are sterile.

ORAL LIQUIDS

Oral liquids usually consist of solutions, suspensions or emulsions of one or more active ingredients in a suitable vehicle; some oral liquids may consist of liquid active ingredients as such. They are intended to be swallowed either undiluted or after dilution. Oral liquids may contain suitable antimicrobial preservatives, antioxidants and other auxiliary substances such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilizing, stabilizing, flavouring, sweetening agents, and authorized colouring agents. The vehicle for any particular oral liquids should be chosen having regard to the nature of the active ingredient(s) and providing organoleptic characteristics appropriate to the intended use of the preparation.

Oral liquids other than oral emulsions may be supplied as liquids or prepared just before issue for use by diluting concentrated liquid preparations or dissolving or dispersing granules or powder in the liquid stated on the label.

Suspensions may show a sediment that is readily dispersible on shaking. Emulsions may show evidence of phase separation but are easily reformed on shaking. The preparation remains sufficiently stable to enable a homogeneous dose to be withdrawn.

Several categories of oral liquids may be distinguished: (1) elixirs; (2) linctuses; (3) mixtures; (4) oral drops; (5) oral emulsions; (6) oral solutions; (7) oral suspensions; (8) preparations for oral liquids; (9) syrups.

Deliverable volume Oral liquids comply with the test described in the "Deliverable Volume" (Appendix 4.21).

Uniformity of dosage units Oral liquids that are suspensions or solids in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Packaging and storage Oral liquids should be kept in well-closed containers. They are supplied in multiple-unit or single-unit containers. They are administered either in volumes such as 5 mL, or multiples of 5 mL, or in small volumes (drops). Each dose of a multiple-unit preparation is administered by means of a device suitable for measuring the prescribed volume.

Oral liquids are supplied in containers that comply with the appropriate requirements given in "Containers" (Appendix 11).

Labelling The label of oral liquids states for Oral Emulsions, Oral Suspensions and, where appropriate, for Mixtures, that the bottle should be shaken before use.

Elixirs

Elixirs are clear, flavoured oral liquids containing one or more active ingredients dissolved in a vehicle that usually contains a high proportion of Sucrose or a suitable polyhydric alcohol or alcohols and may also contain Ethanol (95 Per Cent) or Dilute Ethanols.

Linctuses

Linctuses are viscous oral liquids that may contain one or more active ingredients in solution. The vehicle usually contains a high proportion of Sucrose, other sugars or a suitable polyhydric alcohol or alcohols. Linctuses are intended for use in the treatment or relief of cough, and are sipped and swallowed slowly without the addition of water.

Mixtures

Mixtures are oral liquids containing one or more active ingredients dissolved, suspended or dispersed in a suitable vehicle. Suspended solids may separate slowly on standing but are easily redispersed on shaking.

Oral Drops

Oral drops are oral liquids that are intended to be administered in small volumes with the aid of a suitable measuring device (Appendix 1.15).

Oral Emulsions

Oral emulsions are oral liquids containing one or more active ingredients. They are stabilized oil-in-water dispersions, either or both phases of which may contain dissolved solids. Solids may also be suspended in oral emulsions.

Containers When issued for use, oral emulsions should be supplied in wide-mouthed bottles.

Oral Solutions

Oral solutions are oral liquids containing one or more active ingredients dissolved in a suitable vehicle.

Oral Suspensions

Oral suspensions are oral liquids containing one or more active ingredients suspended in a suitable vehicle. Suspended solids may slowly separate on standing but are easily redispersed.

Preparations for Oral Liquids

Preparations for oral liquids are solids or mixtures of solids intended for the preparations of solutions or suspensions by dissolving or dispersing them in a suitable vehicle. They may contain auxiliary substances in particular to facilitate dispersion or dissolution and to prevent caking. They are designated "for Oral Solution" or "for Oral Suspension" (e.g., Ampicillin for Oral Suspension).

Packaging and storage Preparations for oral liquids shall be kept in tightly closed containers.

Labelling The label of preparations for oral liquids states (1) the directions for preparing the oral liquids including the nature and quantity of liquid to be used; (2) the storage condition; (3) the conditions and the duration of storage after constitution.

Syrups

Syrups are concentrated aqueous solutions of sucrose, other sugars or sweetening agents, to which small quantities of suitable polyhydric alcohols may be added to retard crystallization or to increase the solubility of the other ingredients. Syrups usually contain aromatic or other flavouring materials and may also contain active ingredient(s). They should be recently prepared unless they contain suitable antimicrobial preservatives.

Packaging and storage Syrups should be kept in well-closed containers and stored at temperatures not exceeding 30°.

OROMUCOSAL PREPARATIONS

Oromucosal preparations are solid, semi-solid or liquid preparations, containing one or more active substances intended for administration to the oral cavity and/or the throat to obtain a local or systemic effect. Preparations intended for a local effect may be designed for application to a specific site within the oral cavity such as the gums (gingival preparations) or the throat (oropharyngeal preparations). Preparations intended for a systemic effect are designed to be absorbed primarily at one or more sites on the oral mucosa (e.g., sublingual preparations). Mucoadhesive preparations are intended to be retained in the oral cavity by adhesion to the mucosal epithelium and may modify systemic drug absorption at the site of application. For many oromucosal preparations, it is likely that some proportion of the active substance(s) will be swallowed and may be absorbed via the gastrointestinal tract.

Oromucosal preparations may contain suitable antimicrobial preservatives and other excipients such as dispersing, suspending, thickening, emulsifying, buffering, wetting, solubilizing, stabilizing, flavouring and sweetening agents. Solid preparations may in addition contain glidants, lubricants and excipients capable of modifying the release of the active substance (s).

Several categories of preparations for oromucosal use may be distinguished: (1) gargles; (2) mouthwashes; (3) gingival solutions; (4) oromucosal solutions and oromucosal suspensions; (5) semi-solid oromucosal preparations (including for example gingival gel, gingival paste, oromucosal gel, oromucosal paste); (6) oromucosal drops, oromucosal sprays, sublingual drops, and sublingual sprays (including oropharyngeal sprays); (7) lozenges and pastilles; (8) compressed lozenges; (9) sublingual tablets and buccal tablets; (10) oromucosal capsules; (11) mucoadhesive preparations.

Production During the development of an oromucosal preparation containing an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with the criteria for judging the preservative properties of the formulation are provided under "Efficacy of Antimicrobial Preservation" (Appendix 10.6). In the manufacture, packaging, storage and distribution of oromucosal preparations, suitable means are taken to ensure their microbiological quality; recommendations on this aspect are provided under "Limit for Microbial Contamination" (Appendix 10.5). In the manufacture of semi-solid and liquid oromcosal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Uniformity of dosage units Single-unit oromucosal preparations comply with the "Uniformity of dosage units" (Appendix 4.28).

Gargles

Gargles are aqueous solutions intended for gargling to obtain a local effect. They are not to be

swallowed. They are supplied as ready-to-use solutions or concentrated solutions to be diluted. They may also be prepared from powders or tablets to be dissolved in water before use. Gargles may contain excipients to adjust the pH which, as far as possible, is neutral.

Mouthwashes

See under Topical Preparations.

Gingival Solutions

Gingival solutions are intended for administration to the gingivae by means of a suitable applicator.

Oromucosal Solutions and Oromucosal Suspensions

Oromucosal solutions and oromucosal suspensions are liquid preparations intended for administration to the oral cavity by means of a suitable applicator.

Oromucosal suspensions may show a sediment which is readily dispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Semi-solid Oromucosal Preparations

Semi-solid oromucosal preparations are hydrophilic gels or pastes intended for administration to the oral cavity or to a specific part of the oral cavity such as the gingivae (gingival gel, gingival paste). They may be provided as single-dose preparations. Semi-solid oromucosal preparations comply with the requirements of the monograph for "Topical semisolid Preparations" (Appendix 1.16).

Oromucosal Drops, Oromucosal Sprays, Sublingual Drops and Sublingual Sprays

Oromucosal drops, oromucosal sprays, sublingual drops and sublingual sprays are solutions, emulsions or suspensions intended for local or systemic effect. They are applied by instillation or spraying into the oral cavity or onto a specific part of the oral cavity such as instilling or spraying under the tongue (sublingual drops or sprays) or into the throat (oropharyngeal sprays).

Emulsions may show evidence of phase separation but are readily redispersed on shaking. Suspensions may show a sediment which is readily dispersed on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Liquid oromucosal sprays are supplied in containers with atomizing devices or in pressurized containers having a suitable adaptor, with or without a metering dose valve, which comply with the requirements of the monograph for "Aerosols" (Appendix 1.6).

The size of the droplets of the spray is such as to localize their deposition in the oral cavity or the throat as intended.

Unless otherwise prescribed or justified and authorized, oromucosal drops supplied in single-dose containers, single doses of metered-dose oromucosal sprays and sublingual sprays, all intended for systemic action, comply with the following requirement

Oromucosal Drops in Single-dose Containers

Uniformity of dosage units Oromucosal drops in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Metered-dose Oromucosal Sprays and Sublingual Sprays

Uniformity of dosage units Metered-dose oromucosal sprays and sublingual sprays comply with the "Uniformity of Dosage Units" (Appendix 4.28) or, where justified and authorized, with the test for uniformity of weight or the test for uniformity of delivered dose shown below.

IN THE CASE OF METERED-DOSE OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS THAT ARE SOLUTIONS, PROCEED AS FOLLOWS Discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 weights. Repeat the procedure for a further 9 containers. Determine the weight variation (Appendix 4.28).

IN THE CASE OF METERED-DOSE OROMUCOSAL SPRAYS AND SUBLINGUAL SPRAYS THAT ARE SUSPENSIONS OR EMULSIONS PROCEED AS FOLLOWS Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomizing device. Shake the container for 5 seconds and discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 seconds, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomizing device. Collect the contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers. Determine the content uniformity (Appendix 4.28).

Weight variation Metered-dose oromucosal sprays and sublingual sprays that are solutions comply with the following test. Discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. Weigh the container, discharge once to waste and weigh the container again. Calculate the difference between the 2 weights. Repeat the procedure for a further 9 containers. The preparation complies with the test if maximum 2 of the individual values deviate by more than 25 per cent from the average value and none deviates by more than 35 per cent.

Uniformity of delivered dose Metered-dose oromucosal sprays and sublingual sprays that are suspensions or emulsions comply with the following test. Use an apparatus capable of quantitatively retaining the dose leaving the actuator of the atomizing device. Shake the container for 5 seconds and discharge once to waste. Wait for a minimum of 5 seconds, shake for 5 seconds and discharge again to waste. Repeat this procedure for a further 3 actuations. After 2 seconds, fire 1 dose of the metered-dose spray into the collecting vessel by actuating the atomizing device. Collect the

contents of the collecting vessel by successive rinses. Determine the content of active substance in the combined rinses. Repeat the procedure for a further 9 containers. Unless otherwise justified and authorized, the preparation complies with the test if maximum 1 of the individual contents is outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content. If 2 or maximum 3 individual contents are outside the limits of 75 per cent to 125 per cent but within the limits of 65 per cent to 135 per cent, repeat the test for 20 more containers. The preparation complies with the test if maximum 3 individual contents of the 30 individual contents are outside the limits of 75 per cent to 125 per cent and none is outside the limits of 65 per cent to 135 per cent of the average content.

Lozenges and Pastilles

Lozenges and pastilles are solid, single-dose preparations intended to be sucked to obtain, usually, a local effect in the oral cavity and the throat. They contain one or more active substances, usually in a flavoured and sweetened base, and are intended to dissolve or disintegrate slowly in the mouth when sucked. Lozenges are hard preparations prepared by moulding. Pastilles are soft, flexible preparations prepared by moulding of mixtures containing natural or synthetic polymers or gums and sweeteners.

Compressed Lozenges

Compressed lozenges are solid, single-dose preparations intended to be sucked to obtain a local or systemic effect. They are prepared by compression and are often rhomboid in shape. Compressed lozenges conform with the general definition of tablets.

Production In the manufacture of compressed lozenges, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablet" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution For compressed lozenges intended for a systemic effect, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Sublingual Tablets and Buccal Tablets

Sublingual tablets and buccal tablets are solid, single-dose preparations to be applied under the tongue or to the buccal cavity, respectively, to obtain a systemic effect. They are prepared by compression of mixtures of powders or granulations into tablets with a shape suited for the intended use. Sublingual tablets and buccal tablets conform to the general definition of tablets.

Production In the manufacture of sublingual tablets and buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablets" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution Unless otherwise justified and authorized, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

Oromucosal Capsules Oromucosal capsules are soft capsules to be chewed or sucked.

Mucoadhesive Preparations

Mucoadhesive preparations contain one or more active substances intended for systemic absorption through the buccal mucosa over a prolonged period of time. They may be supplied as mucoadhesive buccal tablets or as other mucoadhesive solid or semi-solid preparations. Mucoadhesive buccal tablets are prepared by compression of mono- or multi-layered tablets. They usually contain hydrophilic polymers, which on wetting with the saliva produce a flexible hydrogel that adheres to the buccal mucosa.

Production In the manufacture of mucoadhesive buccal tablets, measures are taken to ensure that they possess suitable mechanical strength to resist handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablet" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31).

Dissolution Unless otherwise justified and authorized, a suitable test is carried out to demonstrate the appropriate release of the active substance(s).

PARENTERAL PREPARATIONS

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the body. There are five main forms of these preparations defined as follows: (1) medicaments or solutions or emulsions thereof suitable for injection, bearing titles of the form, ____ Injection; (2) dry solids or liquid concentrates containing active ingredient(s) with or without buffer(s), diluent(s) or other added substances, and which, upon the addition of suitable solvents, yield solutions conforming in all respects to the requirements for Injections, and which are distinguished by titles of the form, ____ for Injection; (3) solids which are suspended in a suitable fluid medium and which are not to be injected intravenously or into the spinal canal, distinguished by titles of the form, Sterile ____ Suspension; (4) dry solids which, upon the addition of suitable vehicles, yield preparations conforming in all respects to the requirements for sterile suspensions; and which are distinguished by titles of the form, Sterile ____ for Suspension; and (5) sterile solid preparations, of which size and shape are suitable for implantation into body tissues, distinguished by titles of the form, ____ Implant. The term "Injections" may be used for preparations (1) to (4).

Where used in this Pharmacopoeia, the designation Large-volume intravenous solution applies to a single-dose injection that is intended for intravenous use and is packaged in containers labelled as containing more than 100 mL. The designation Small-volume Injection applies to an injection that is packaged in containers labelled as containing 100 mL or less.

Production Parenteral preparations are prepared

by methods designed to ensure their sterility and to avoid the introduction of contaminants, the presence of pyrogens and the growth of micro-organisms. Water used in the manufacture of injections complies with the requirements for Water for Injections in bulk.

Aqueous vehicles The vehicles for aqueous parenteral preparations comply with the "Pyrogen Test" (Appendix 8.2) or the "Test for Bacterial Endotoxins" (Appendix 8.5), whichever is specified. Water for Injection generally is used as the vehicle, unless otherwise specified in the individual monograph. Sodium Chloride may be added in amounts sufficient to render the resulting solution isotonic; Sodium Chloride Injection, or Ringer's Injection, may be used in whole or in part instead of Water for Injection unless otherwise specified in the individual monograph. For conditions applying to other adjuvants, see Added substances, in this appendix.

Other vehicles Fixed oils used as vehicles for nonaqueous injections are of vegetable origin, are odourless or nearly so, and have no odour or taste suggesting rancidity. They comply with the test for solid paraffin under Liquid Paraffin, the cooling bath being maintained at 10°, have a saponification value of between 185 and 200 (Appendix 5.7), have an iodine value of between 79 and 128 (Iodine Bromide Method, Appendix 5.6), and comply with the requirements of the following tests.

UNSAPONIFIABLE MATTER Reflux on a water-bath 10 mL of the oil with 15 mL of a 16.7 per cent w/v solution of sodium hydroxide and 30 ml of ethanol, with occasional shaking until the mixture becomes clear. Transfer the solution to a shallow dish, evaporate the ethanol on a water-bath, and mix the residue with 100 mL of water: a clear solution results.

ACID VALUE Not more than 0.22 (Appendix 5.4); using 0.020 M potassium hydroxide as titrant.

Synthetic mono- or di-glycerides of fatty acids may be used as vehicles, provided they are liquid and remain clear when cooled to 10° and have an iodine value of not more than 140 (Iodine Bromide Method, Appendix 5.6).

These and other nonaqueous vehicles may be used, provided they are safe in the volume of parenteral preparations administered, and also provided they do not interfere with the therapeutic efficacy of the preparation or with its response to prescribed assays and tests.

Added substances Suitable substances may be added to parenteral preparations to increase stability or usefulness, unless prescribed in the individual monograph, provided they are harmless in the amounts administered and do not interfere with the therapeutic efficacy or with the responses to the specified assays and tests. No colouring agent may be added, solely for the purpose of colouring the finished preparation, to a solution intended for parenteral administration [see also Added Substances, under General Notices, and "Efficacy of Antimicrobial Preservation" (Appendix 10.6)].

Observe special care in the choice and use of added substances in injections that are administered in a volume exceeding 5 mL. The following maximum limits prevail unless otherwise directed: for agents containing mercury and the cationic, surface-active compounds, 0.01 per cent; for those of the types of chlorobutanol, cresol, and phenol, 0.5 per cent; and for sulfur dioxide, or an equivalent amount of the sulfite, bisulfite, or metabisulfite of potassium or sodium, 0.2 per cent.

A suitable substance or mixture of substances to prevent the growth of micro-organisms must be added to preparations intended for injection that are packaged in multiple-dose containers, regardless of the method of sterilization employed, unless otherwise specified in the individual monograph, or unless the active ingredients are themselves antimicrobial. Such substances are used in concentrations that will prevent the growth of or kill micro-organisms in the preparations. Such substances also comply with the "Efficacy of Antimicrobial Preservation" (Appendix 10.6) and "Content of Antimicrobial Agents" (Appendix 6.22). Sterilization processes are employed even though such substances are used [see also Added Substances, under General Notices, and "Sterilization and Sterility Assurance" (Appendix 12)]. The air in the container may be evacuated or be displaced by a chemically inert gas. If the injection is oxygen-sensitive, that information must appear in the labelling.

Containers for parenteral preparations Containers, including the closures, for parenteral preparations do not interact physically or chemically with the preparations in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling shipment, storage, sale, and use. The container is made of material that permits inspection of the contents. Parenteral preparations are supplied in glass ampoules, bottles or vials or in other containers such as plastic bottles or bags and in prefilled syringes the integrity of which is ensured by suitable means. The type of glass preferable for each parenteral preparation is usually stated in the individual monograph.

For definitions of single-dose and multiple-dose containers, see Containers under General Notices, Containers comply with the "Containers" (Appendix 11). Containers are closed by fusion, or by application of suitable closures, in such manner as to prevent contamination or loss of contents. Closures for multipledose containers permit the withdrawal of the contents without removal or destruction of the closure. The closure permits penetration by a needle, and, upon withdrawal of the needle, at once recloses the container against contamination.

Containers for sterile solids Containers, including the closures, for dry solids intended for parenteral use do not interact physically or chemically with the preparation in any manner to alter the strength, quality, or purity beyond the official requirements under the ordinary or customary conditions of handling, shipment, storage, sale and use.

A container for a sterile solid permits the addition of a suitable solvent and withdrawal of portions of the resulting solution or suspension in such manner that the sterility of the product is maintained.

Where the Assay in a monograph provides a procedure for Assay preparation in which the total withdraw able contents are to be withdrawn from a single-dose container with a hypodermic needle and syringe, the contents are to be withdrawn as completely as possible into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be withdrawn and fitted with a 21-gauge needle not less than 2.5 cm in length, care being taken to expel any air bubbles, and discharged into a container for dilution and assay.

Volume in containers Each container of an injection is filled with a volume in slight excess of the labelled size or the volume which is to be withdrawn. The excess volumes recommended in the accompanying table are usually sufficient to permit withdrawal and administration of the labelled volumes.

DETERMINATION OF VOLUME OF INJECTIONS IN CONTAINERS Select one or more containers if the volume is 10 mL or more, three or more if the volume is more than 3 mL and less than 10 mL, or five or more if the volume is 3 mL or less. Take up individually the contents of each container selected into a dry hypodermic syringe of a rated capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2 cm (1 inch) in length. Expel any air bubbles from the syringe and needle, and then discharge the contents of the syringe, without emptying the needle, into a standardized, dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40 per cent of its rated volume. Alternatively, the contents of the syringe may be discharged into a dry, tared beaker, the volume, in mL, being calculated as the weight, in g, of injections taken divided by its density. The contents of two or three 1-mL or 2-mL containers may be pooled for the measurement, provided that a separate, dry syringe assembly is used for each container. The content of containers holding 10 mL or more may be determined by means of opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the labelled volume in the case of containers examined individually or, in the case of 1-mL and 2-mL containers, is not less than the sum of the labelled volumes of the containers taken collectively.

For injections in multiple-dose containers labelled to yield a specific number of doses of a stated volume, proceed as directed in the foregoing, using the same number of separate syringes as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

For injections containing oil, warm the containers, if necessary, and thoroughly shake them immediately before removing the contents. Cool to 25° before measuring the volume.

Constituted solutions Sterile dosage forms from which constituted solutions are prepared for injection bearing titles of the form, for Injection comply with the "Constituted Solutions" (Appendix 4.20).

Particulate matter All large-volume injections for single-dose infusion, and those small-volume injections for which the monographs specify such requirements, are subject to the particulate matter limits set forth in the "Particulate Matter in Injections" (Appendix 4.27). An article packaged as both a large-volume and a small volume injection meets the requirements set forth for "Small-volume Injections" where the container is labelled as containing 100 mL or less if the individual monograph includes a test for "Particulate matter"; it meets the requirements set forth for "Large-volume Injections for Single-dose Infusion" where the container is labelled as containing more than 100 mL. Injections packaged and labelled for use as irrigating solutions are exempt from requirements for "Particulate matter".

Pyrogen test; Bacterial endotoxins test Parenteral preparations comply with the "Pyrogen Test" (Appendix 8.2) or the "Test for Bacterial Endotoxins" (Appendix 8.5) as specified in the individual monograph.

Sterility Parenteral preparations comply with the "Sterility Test" (Appendix 10.1). Unless otherwise specified in the individual monograph, carry out the test using Method I.

Uniformity of dosage units Parenteral preparations that are packaged in single-unit containers comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is required for sterile solids containing a unit weight equal to or less than 40 mg but not required for multivitamin and trace element parenteral preparations.

Packaging and storage The volume of the parenteral preparations in single-dose containers provides the amount specified for parenteral administration at one time and in no case is more than sufficient to permit the withdrawal and administration of 1 litre.

Preparations intended for intraspinal, intracisternal, or peridural administration are packaged only in single dose containers.

Unless otherwise specified in the individual monograph, no multiple-dose container contains a volume of parenteral preparation more than sufficient to permit the withdrawal of 30 mL.

Injections packaged for use as irrigation solutions or for hemofiltration or dialysis or for parenteral nutrition are exempt from the 1-litre restriction of the foregoing requirements relating to packaging. Containers for injections packaged for use as irrigation solutions or for hemofiltration may be designed to empty rapidly and may contain a volume of more than 1 litre.

Labelling The label of injection complies with the

Labelling, under General Notices. The label also states the name of any added substances and the storage conditions.

The container is so labelled that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents. In case the area of the container is not sufficient to be labelled as mentioned above, unless otherwise specified in the individual monograph, the label shall state (1) the name of the drug product and (2) the batch or lot number assigned by the manufacturer.

The label of a single-dose parenteral preparation states that any portion of the contents remaining should be discarded.

In the case of a dry preparation or other preparation to which a diluent is intended to be added before use, the label includes the following information: the amount of each ingredient, the composition of recommended diluent(s) [the name(s) alone, if the formula is specified in the individual monograph], the amount to be used to attain a specific concentration of active ingredient and the final volume of solution so obtained, a brief description of the physical appearance of the constituted solution, directions for proper storage of the constituted solution, and an expiration date limiting the period during which the constituted solution may be expected to have the required or labelled potency if it has been stored as directed.

POWDERS

Powders are preparations consisting of solid, loose, dry particles of varying degrees of fineness that contain one or more active ingredients with or without added substances including, where necessary, flavouring agents and authorized colouring matter. Two categories of powders may be distinguished: (1) oral powders; (2) topical powders.

Minimum fill Powders comply with the test described in the "Minimum Fill" (Appendix 4.26).

Uniformity of dosage units Powders comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element powders and herbal drug powders.

Particle size Topical powders and herbal drug powders comply with the following requirement.

Carry out the determination as described in the "Method for Determining the Particle Size" (Manual Sieving-Single Sieve, Appendix 1.11), use about 10 g of the powder, accurately weighed. Not less than 95 per cent of the powder examined pass through a sieve with a nominal mesh aperture of 125 µm for chemical drugs and the sieve with a nominal mesh aperture of 150 µm for herbal drugs.

Packaging and storage Powders should be kept in tightly closed containers.

Oral Powders

Oral powders are generally administered in or with water or another suitable liquid. They may also be swallowed directly.

Oral powders are presented as single-unit or multiple-unit preparations. For single-unit powders each dose is enclosed in a separate container, for example, a sachet, a paper packet or a vial. Multiple-unit powders require the provision of a measuring device capable of delivering the quantity prescribed.

Effervescent oral powders are presented as single unit or multiple-unit powders and generally contain acid substances and either carbonates or bicarbonates that react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Labelling For single-unit containers the label states the name(s) and quantity(ies) of active ingredien(s) per container. For multiple-unit containers the label states the name(s) and quantity(ies) of active ingredien(s) in a suitable amount by weight.

Topical Powders

Topical powders are free from grittiness. They are presented as single-unit or multiple-unit preparations. If a topical powder is specifically intended for use on large open wounds or on severely injured skin, it must be sterile.

Multiple-unit topical powders should preferably be dispensed in sifter-top containers or in aerosol containers.

Dusting powders are topical powders consisting of finely divided powders that are intended to be applied to the skin for therapeutic, prophylactic or lubricant purposes. In general, they should be passed through at least a No. 150 sieve to assure freedom from grit that could irritate traumatized areas.

Labelling The label of topical powders states, where applicable, that the topical powder is sterile.

PREPARATIONS FOR INHALATION

Preparations for inhalation are solid or liquid preparations that contain one or more active ingredients. They are intended for administration to the lower respiratory tract for local or systemic effect.

Preparations for inhalation should not adversely affect the functions of the mucosa of the respiratory tract and its cilia. Preparations for inhalation are supplied in multiple-unit or single-unit containers provided with a suitable administration device, if necessary.

Four categories of preparations for inhalation may be distinguished: (1) inhalations; (2) powders for inhalation; (3) inhalation aerosols; (4) inhalants.

Preparations for inhalation that are converted into an aerosol are generally administered by nebulizers, by pressurized metered-dose inhalers or by dry-powder inhalers.

Production During the development of a preparation for inhalation which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable method of test together with the criteria for judging the preservative properties of the formulation are described in the "Efficacy of Antimicrobial Preservation" (Appendix 10.6). The size of particles to be inhaled should be adjusted so as to localize their deposition in the lower respiratory tract and verified by suitable methods of particle-size determination.

For preparations for inhalation that are supplied in single-unit containers provided with a suitable administration device, the single doses released by the administration device are tested for uniformity of volume, weight or content. The content of a unit spray of suspensions for inhalation dispensed in multiple-unit aerosol containers is verified using a suitable number of unit sprays for quantification.

Minimum fill Preparations for inhalation comply with the test described in the "Minimum Fill" (Appendix 4.26).

Packaging and storage Preparations for inhalation should be kept in well-closed containers. Aerosol preparations shall be stored at a temperature not exceeding 50° and should be protected from frost.

Labelling The label of preparations for inhalation states the name(s) and quantity(ies) of any antimicrobial preservative(s).

Inhalations

Inhalations are solutions or suspensions or emulsions of one or more active ingredients administered by the nasal or oral respiratory route for local or systemic effect.

As far as possible, liquids not dispensed in aerosol containers are aqueous, isotonic liquids. The pH of the liquid should be not lower than 3 and not higher than 8.5. Co-solvents or suitable solubilizers may be used to increase the solubility of the active ingredients. Aqueous inhalations supplied in multiple-unit containers should contain a suitable antimicrobial preservative at a suitable concentration except where the preparation itself has adequate antimicrobial properties.

Suspensions are readily dispersible on shaking and the suspension remains sufficiently stable to enable the correct dose to be delivered by the device. Suitable suspension stabilizers may be added.

Inhalations are usually supplied in glass or plastic containers. If inhalations are provided in concentrated form, they should be diluted in the nebulizers to the prescribed volume with the prescribed liquid before use.

Powders for Inhalation

Powders for inhalation are powders containing medicinal substances usually diluted with suitable diluents. These powders are normally dispensed in hard gelatin capsules. They may also be administered by mechanical devices that require manually produced pressure or a deep inhalation by the patient, e.g., Cromoglycate Sodium for Inhalation.

Uniformity of dosage units Powders for inhalation comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Labelling The label on the container states that the capsules are intended for use in an inhaler and are not to be swallowed.

Inhalation Aerosols

(**Note** Inhalation aerosols comply with the appropriate requirements for aerosols.)

Inhalation aerosols are metered-dose preparations. They are drugs or solutions or suspensions or emulsions intended to be inhaled in controlled amounts and are delivered by the actuation of an appropriate metering valve. They contain multiple doses, often exceeding several hundreds. The most common single-dose volumes delivered are from 25 to 100 µL (also expressed as mg) per actuation.

Production The formulation of inhalation aerosols and the components of the delivery device (i.e., the aerosol container with its integral metering valve and the actuator) should be designed and, where appropriate, the particle size of the active ingredients should be controlled so that, when the inhalation aerosols are used in accordance with the manufacturer's recommendations, an adequate proportion of the active ingredients is made available for inhalation. A proportion of the active ingredient is deposited on the inner surface of the actuator; the amount available for inhalation is therefore less than the amount released by actuation of the valve. Inhalation aerosols should be manufactured in conditions designed to minimize microbial and particulate contaminations.

Packaging and storage Inhalation aerosols are supplied in suitable containers fitted with an appropriate metering valve that forms an integral part of the container. The containers are usually supplied with an appropriate actuator. Inhalation aerosols should be protected from extremes of temperature and from undue fluctuations in temperature.

Labelling The label of inhalation aerosols states (1) the quantity(ies) of active ingredient(s) delivered by each actuation of the valve; (2) the instructions for using the inhalation aerosols; (3) any special precautions associated with the use of the inhalation aerosols.

Inhalants

Inhalants are drugs or combination of drugs which may contain an inert, suspended, diffusing agent. They are intended to release volatile constituents for inhalation either when placed on a pad or when added to hot, but not boiling, water.

Labelling The label on the container states that the inhalants are not to be taken by mouth.

RECTAL PREPARATIONS

Rectal preparations are intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes. Several categories of rectal preparations may be distinguished: (1) rectal suppositories; (2) rectal capsules; (3) rectal solutions and suspensions (enemas); (4) powders and tablets for rectal solutions and suspensions; (5) rectal creams; (6) rectal gels; (7) rectal ointments; (8) rectal foams; and (9) rectal tampons.

Production During the development of a rectal preparation, the formulation for which contains an antimicrobial preservative, the effectiveness of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable method of testing together with criteria for judging the preservative of the formulation is provided under "Efficacy of Antimicrobial Preservation" (Appendix 10.6).

In the manufacture, packaging, storage and distribution of rectal preparations, suitable means are taken to ensure their microbial quality; recommendations on this aspect are provided under "Limit for Microbial Contamination" (Appendix 10.5).

In the manufacture of semi-solid and liquid rectal preparations containing dispersed particles, measures are taken to ensure a suitable and controlled particle size with regard to the intended use.

Dissolution A suitable test may be required to demonstrate the appropriate release of the active ingredient(s) from solid, single dose preparations, for example the dissolution test for suppositories and soft capsules (Appendix 4.24). Where a dissolution test is prescribed, a disintegration test may not be required.

Uniformity of dosage units Rectal preparations comply with the "Uniformity of Dosage Units" (Appendix 4.28).

Rectal Suppositories

Rectal suppositories are solid, single-dose preparations. The shape, volume and consistence of suppositories are suitable for rectal administration.

They contain one or more active ingredients dispersed or dissolved in a simple or compound excipient which may be soluble or dispersible in water or may melt at body temperature. Excipients such as diluents, adsorbents, surface-active agents, lubricants, antimicrobial preservatives and colouring matter, authorized by the competent authority, may be added if necessary.

Production Rectal suppositories are prepared by compression or moulding. If necessary, the active ingredient(s) are previously ground and sieved through a suitable sieve. When prepared by moulding, the medicated mass, sufficiently liquified by heating, is poured into suitable moulds. The suppository solidifies on cooling. Various excipients are available for this process, such as hard fat, macrogols, cocoa butter, and various gelatinous mixtures consisting, for example, of gelatin, water and glycerol.

A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from suppositories intended for modified release or for prolonged local action.

Disintegration Unless intended for modified release or for prolonged local action, rectal suppositories comply with the "Disintegration Test for Suppositories and Pessaries" (Appendix 4.22). For suppositories with a fatty base, examine after 30 minutes and for suppositories with a water-soluble base after 60 minutes, unless otherwise justified and authorized.

Packaging and storage Rectal suppositories shall be kept in well-closed containers.

Labelling The label on the container states (1) that they are intended for external use only; (2) the storage conditions.

Rectal Capsules

Rectal capsules (shell suppositories) are solid, single-dose preparations generally similar to soft capsules as defined in capsules except that they may have lubricating coatings. They are of elongated shape, are smooth and have a uniform external appearance.

Production A suitable test is carried out to demonstrate the appropriate release of the active ingredient(s) from rectal capsules intended for modified release or prolonged local action.

Disintegration Unless intended for modified release or for prolonged local action, rectal capsules comply with the "Disintegration Test for Suppositories" (Appendix 4.22). Examine the state of the capsules after 30 minutes, unless otherwise justified and authorized.

Rectal Solutions and Suspensions (Enemas)

Rectal solutions and suspensions (enemas) are liquid preparations intended for rectal use in order to obtain a systemic or local effect, or they may be intended for diagnostic purposes.

They are single-dose preparations containing one or more active ingredients dissolved or dispersed in water, glycerol, macrogols or other suitable solvents. Suspensions may show a sediment which is readily dispersible on shaking to give a suspension which remains sufficiently stable to enable the correct dose to be delivered.

Rectal solutions and suspensions may contain excipients intended, for example, to adjust the viscosity of the preparation, to adjust or stabilize pH, to increase the solubility of the active ingredient(s) or to stabilize the preparation. These substances do not adversely affect the intended medical action or, at the concentrations used, cause undue local irritation.

Rectal solutions and suspensions are supplied in containers containing a volume in the range of 2.5 mL to 2000 mL. The container is adapted to deliver the preparation to the rectum or it is accompanied by

a suitable applicator.

Powders and Tablets for Rectal Solutions and Suspensions

Powders and tablets intended for the preparation of rectal solutions or suspensions are single-dose preparations which are dissolved or dispersed in water at the time of administration. They may contain excipients to facilitate dissolution or dispersion or to prevent aggregation of the particles.

After dissolution or suspension, they comply with the requirements for rectal solutions or rectal suspensions, as appropriate.

Disintegration Tablets for rectal solutions or suspensions disintegrate within 3 minutes when tested as described under "Disintegration Test for Suppositories and Pessaries" (Appendix 4.22), but using water at 15° to 25°.

Labelling The label on the container states (1) the directions for preparing the rectal solution or suspension; (2) the storage conditions; (3) the period during which the constituted rectal solutions or suspensions may be expected to remain satisfactory for use when prepared and stored in accordance with the manufacturer's recommendations.

Rectal Creams

See under Topical Semi-solid Preparations.

Rectal Gels

See under Topical Semi-solid Preparations.

Rectal Ointments

See under Topical Semi-solid Preparations.

Rectal Foams

See under Medicated Foams.

Rectal Tampons

Rectal tampons are solid, single-dose preparations intended to be inserted into the lower part of the rectum for a limited time.

See also under Medicated Tampons.

SPIRITS

Spirits are ethanolic or hydroethandic solutions of volatile substances usually prepared by simple solution or by admixture of the ingredients. Some spirits serve as flavouring agents while others have medicinal value. Reduction of the high ethanolic content of spirits by admixture with aqueous preparations often causes turbidity.

Packaging and storage Spirits shall be kept in tightly closed containers, protected from light to prevent loss by evaporation and to limit oxidative changes.

SYSTEMS

Systems are preparations that allow for the uniform release or targeting of drugs to the body. These preparations are commonly called drug delivery systems or delivery systems. The most widely used of these are transdermal systems.

Transdermal Systems

Transdermal drug delivery systems are self-contained, discrete dosage forms that, when applied to intact skin, are designed to deliver the drug(s) through the skin to the systemic circulation. Systems typically comprise an outer covering (barrier), a drug reservoir, which may have a rate controlling membrane, a contact adhesive applied to some or all parts of the system and the system/skin interface, and a protective liner that is removed before applying the system. The activity of these systems is defined in terms of the release rate of the drug(s) from the system. The total duration of drug release from the system and the system surface area may also be stated.

Labelling The label of transdermal systems states (1) the dose released per unit time; (2) the storage conditions.

Ocular Systems

Ocular systems are sterile, solid or semi-solid preparations of suitable size and shape, designed to be inserted in the conjunctival cul-de-sac, to produce an ocular effect. They generally consist of a reservoir of active ingredient(s) embedded in a matrix or bounded by a rate-controlling membrane. The active ingredient, which is more or less soluble in physiological fluids, is released over a predetermined period of time. Ocular systems are individually distributed into sterile containers.

Sterility Unless otherwise specified in the individual monograph, ocular systems comply with the "Sterility Test" (Method I, Appendix 10.1).

Drug release pattern Ocular systems comply with the test for drug release pattern in the individual monograph.

Labelling The label of ocular systems states (1) the dose released per unit time; (2) the storage conditions.

Intrauterine Systems

An intrauterine system, based on a similar principle but intended for release of drug over a much longer period of time, i.e. one year, is also available.

TABLETS

Tablets are solid dosage forms containing one or more active ingredients. They are obtained by single or multiple compression (in certain cases they are moulded) and may be uncoated or coated. They are usually intended for oral administration.

The different categories of tablets that exist include soluble tablets, effervescent tablets, tablets for use in the mouth, and modified-release tablets. Tablets are normally circular in shape and their surfaces are flat or convex. Tablets may have lines or break-marks, symbols, or other markings. They should be sufficiently hard to withstand handling, including packaging, storage, and transportation, without crumbling or

breaking.

Tablets may contain excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behaviour of the dosage forms and the active ingredient(s) in the gastrointestinal tract, colouring matter, and flavouring substances. When such excipients are used, it is necessary to ensure that they do not adversely affect the stability, dissolution rate, bioavailability, safety, or efficacy of the active ingredient(s); there must be no incompatibility between and of the components of the dosage form.

Preparations for alternative applications, such as solution-tablets for injections, irrigations, or for external use, etc., are also available in this presentation. These preparations may require a special formulation, method of manufacture, or form of presentation, appropriate to their particular use. For this reason they may not comply with certain sections of this monograph.

Production The following information is intended to provide very broad guidelines concerning the main steps to be followed during production. Tablets may be prepared by three general methods: wet granulation, dry granulation (roll compaction or slugging), and direct compression. The purpose of both wet and dry granulation is to improve flow of the mixture and/or to enhance its compressibility. Tablets are compressed by the application of high pressures, utilizing steel punches and dies, to powders or granulations. However, throughout manufacturing, certain procedures should be validated and monitored by carrying out appropriate inprocess controls.

For modified-release tablets, a suitable test is carried out to demonstrate the appropriate release of the active ingredient(s).

Delayed-release tablets are prepared by covering tablets with a gastro-intestinal coating or from granules or particles already covered with a gastro-resistant coating.

In the manufacture of tablet cores, means are taken to ensure that they possess a suitable mechanical strength to withstand handling without crumbling or breaking. This may be demonstrated by examining the "Friability of Uncoated Tablets" (Appendix 4.30) and the "Resistance to Crushing of Tablets" (Appendix 4.31). Chewable tablets are prepared to ensure that they are easily crushed by chewing.

Disintegration Comply with the "Disintegration Test for Tablets and Capsules" (Appendix 4.23). For those tablets for which a dissolution requirement is included in the individual monograph, omission of the requirement for disintegration is considered justifiable and is therefore authorized.

Dissolution Comply with the "Dissolution Test" (Appendix 4.24). Where a dissolution test is specified in the individual monograph, a disintegration test may not be required.

Uniformity of dosage units Comply with the "Uniformity of Dosage Units" (Appendix 4.28). The test for Content Uniformity is not required for multivitamin and trace element tablets.

Visual inspection Unpack and inspect at least 20 tablets. They should be undamaged, smooth and usually of uniform colour. Evidence of physical instability is demonstrated by: (1) presence of excessive powder and/or pieces of tablets at the bottom of the container (from abraded, crushed, or broken tablets); (2) cracks or capping, chipping in the tablet surfaces or coating, swelling, mottling, discoloration, fusion between tablets; (3) the appearance of crystals on the container walls or on the tablets.

Packaging and storage Tablets should be kept in well-closed containers at a temperature not exceeding 30° and protected from crushing and mechanical shock.

Moisture-sensitive forms such as effervescent tablets should be kept in tightly closed containers or moisture proof packs and may require the use of separate packages containing water-adsorbent agents, such as silica gel.

Uncoated Tablets

The majority of uncoated tablets are made in such a way that the release of active ingredients is unmodified. A broken section, when examined under a lens, shows either a relatively uniform texture (single-layer tablets) or a stratified texture (multi-layer tablets), but no signs of coating.

TABLETS FOR SOLUTIONS (SOLUBLE TABLETS) Tablets for solutions are uncoated tablets that dissolve in water to give a clear solution.

EFFERVESCENT TABLETS Effervescent tablets are uncoated tablets generally containing acid substances and carbonates or hydrogen carbonates that react rapidly in the presence of water to release carbon dioxide. They are intended to be dissolved or dispersed in water before administration.

Labelling The label should state "Not to be swallowed directly".

DISPERSIBLE TABLETS Dispersible tablets are uncoated tablets that produce a uniform dispersion in water.

Uniformity of dispersion Place two tablets in 100 mL of water and stir until completely dispersed. A smooth dispersion is produced which passes through a sieve screen with a nominal mesh aperture of 710 µm.

TABLETS FOR USE IN THE MOUTH Tablets for use in the mouth are usually uncoated tablets. They are formulated to effect a slow release and local action of the active ingredient (for example, compressed lozenges) or the release and systemic absorption of the active ingredient under the tongue (sublingual tablets) or in the other parts of the mouth. (Certain tablets for use in the mouth may be referred to as "Lozenges".)

Chewable tablets Chewable tablets are tablets intended to be chewed, producing a pleasant tasting residue in the oral cavity that is easily swallowed and does not leave a bitter or unpleasant after-taste. These

tablets have been used in tablet formulations for children, especially multivitamin formulations, and for the administration of antacids and selected antibiotics. Chewable tablets are prepared by compression, usually utilizing mannitol, sorbitol, or sucrose as binders and fillers, and containing colours and flavours to enhance their appearance and taste.

Buccal and sublingual tablets Buccal tablets are tablets intended to be inserted in the buccal pouch, and sublingual tablets are intended to be inserted beneath the tongue, where the active ingredient is absorbed directly through the oral mucosa. Few drugs are readily absorbed in this way, but for those that are (such as nitroglycerin and certain steroid hormones), a number of advantages may result.

Coated Tablets

Coated tablets are tablets covered with one or more layers of mixtures of substances such as natural or synthetic resins, polymers, gums, fillers, sugars, plasticizers, polyhydric alcohols, waxes, colouring matters, flavouring substances, and sometimes also active ingredients. A broken section, when examined under a lens, shows a core which is surrounded by a continuous layer of a different texture.

The tablets may be coated for a variety of reasons such as protection of the active ingredients from air, moisture, or light, masking of unpleasant tastes and odours, or improvement of appearance. The substance used for coating is usually applied as a solution or suspension.

SUGAR-COATED TABLETS Sugar-coated tablets are tablets containing a sugar coating.

FILM-COATED TABLETS Film-coated tablets are tablets covered with a thin layer of resins, polymers, and/or plasticizers capable of forming a film.

Modified-release Tablets

Modified-release tablets are coated, uncoated, or matrix tablets containing excipients or prepared by procedures which, separately or together, are designed to modify the rate of release of the active ingredient(s) in the gastro-intestinal tract.

DELAYED-RELEASE TABLETS Delayed-release tablets are tablets intended to resist gastric fluid but to release the active ingredient(s) in the intestinal fluid. This is achieved by using coating substances such as cellacefate (cellulose acetate phthalate) and anionic copolymers of methacrylic acid and its esters. It is sometimes necessary to apply more than one layer.

All requirements for these specialized dosage forms are given in the individual monographs.

EXTENDED-RELEASE TABLETS Extended-release tablets are tablets designed to slow the rate of release of the active ingredient(s) in the gastro-intestinal tract.

All requirements for these specialized dosage forms are given in the individual monographs.

TINCTURES

Tinctures are ethanolic or hydroethanolic solutions usually obtained from dried vegetable or animal matter or from chemical substances. They are usually obtained either using 1 part of drug and 10 parts of extraction solvent or 1 part of drug and 5 parts of extraction solvent. Tinctures are usually clear; a slight sediment may be produced on standing provided that the composition is not changed significantly.

Production For some preparations, the matter to be extracted must undergo a preliminary treatment, for example, inactivation of enzymes, grinding or defatting.

Tinctures are prepared by maceration, percolation or other suitable, justified methods, using Ethanol of suitable concentration. Tinctures may also be obtained by dissolving or diluting extracts in Ethanol of suitable concentration.

PRODUCTION BY PERCOLATION If necessary, the drug is reduced to pieces of suitable size, mixed thoroughly with a portion of extraction solvent and allowed to stand for an appropriate time. The mixture is transferred to a percolator and the percolate allowed to flow slowly ensuring that the drug is always covered with the remaining extraction solvent. The drug residue may be pressed out and the expressed fluid combined with the percolate.

PRODUCTION BY MACERATION Unless otherwise prescribed, the drug is reduced to pieces of suitable size, mixed thoroughly with the prescribed extraction solvent and allowed to stand in a closed container for anappropriate time. The drug residue is separated from the extraction solvent and, if necessary, pressed out. In the latter case, the two liquids obtained are combined.

PRODUCTION FROM EXTRACTS The tincture is prepared by dissolving or diluting an extract, using Ethanol of appropriate concentration. The content of solvent and constituents or, where applicable, the contents of solvent and of dry residue correspond to that of tinctures obtained by maceration or percolation.

When the content of constituents has to be adjusted, such adjustment may be carried out, if necessary, either by adding the extraction solvent of suitable concentration or by adding another tincture of the vegetable or animal matter used for the preparation.

Ethanol Comply with the limits prescribed in the monograph (Appendix 6.5).

Methanol and 2-propanol Not more than 0.05 per cent v/v of methanol or 2-propanol (Appendix 5.15), unless otherwise prescribed in the monograph.

Total solids Comply with the limits prescribed in the monograph (Appendix 7.21).

Weight per millilitre Comply with the limits prescribed in the monograph (Appendix 4.9).

Packaging and storage Tinctures should be kept in tightly closed containers, protected from light, in a

cool place.

Labelling The label on the container states (1) the vegetable, animal matter or chemical substances used; (2) where applicable, that fresh vegetable or animal matter was used; (3) the name and concentration of the solvent used for the preparation; (4) the concentration of ethanol in the final tincture; (5) the content of active principle and/or the ratio of starting material to extraction fluid and of starting material to final tincture.

TOPICAL PREPARATIONS

Topical preparations are drugs intended for topical application in a wide variety of dosage forms.

Minimum fill Topical preparations except plasters comply with the test described in the "Minimum Fill" (Appendix 4.26).

Applications Applications are liquid or semi-liquid preparations containing one or more active ingredients intended for application to the skin. They may contain suitable antimicrobial preservatives, unless the active ingredients or vehicles have sufficient intrinsic antibacterial and antifungal activity. They may contain other suitable auxiliary substances such as stabilizers. They have traditionally been used to administer antiparasitic medications.

Collodions

Collodions are liquid preparations, usually containing pyroxylin in a mixture of ether and ethanol, that are intended for application to the skin. When they are allowed to dry, a flexible film is formed at the site of application.

Packaging and storage Collodions should be kept in well-closed containers, at a temperature not exceeding 25° and remote from fire.

Jellies

See hydrophilic gels under Topical Semi-solid Preparations.

Liniments

Liniments are liquid or semi-liquid preparations containing one or more active ingredients in suitable vehicles. They are intended to be applied to the unbroken skin with friction. They may contain suitable antimicrobial preservatives.

Packaging and storage Liniments should be kept in well-closed containers. Certain plastic containers, such as those made from polystyrene, are unsuitable for liniments.

Lotions

Lotions are liquid or semi-liquid preparations containing one or more active ingredients in suitable vehicles. They are usually intended to be applied to the unbroken skin without friction. Lotions may contain suitable antimicrobial preservatives, unless the active ingredients or vehicles have sufficient intrinsic antibacterial and antifungal activity. They may contain other suitable added substances such as stabilizers. The term "lotion" is applied to solutions or suspensions or emulsions applied topically.

TOPICAL SOLUTIONS Topical solutions are solutions, usually aqueous but often containing other solvents, such as alcohol and polyols, intended for topical application to the skin, or to the oral mucosal surface.

TOPICAL SUSPENSIONS Topical suspensions are liquid preparations containing solid particles dispersed in a liquid vehicle, intended for application to the skin.

TOPICAL EMULSIONS Topical emulsions are liquid preparations in which one liquid is dispersed throughout another liquid in the form of small droplets.

Packaging and storage Lotions should be kept in well-closed containers.

Mouthwashes

Mouthwashes are aqueous solutions containing one or more active ingredients. They are intended for use in contact with the mucous membranes of the oral cavity, usually after dilution with warm water. They may contain added substances such as suitable antimicrobial preservatives.

Packaging and storage Mouthwashes should be kept in well-closed containers.

Labelling The label of mouthwashes states the directions for the dilution of the mouthwash for use, if appropriate.

Paints

Paints are solutions or dispersions of one or more active ingredients. They are intended for application to the skin or, in some cases, mucous membranes.

Packaging and storage Paints should be kept in tightly closed containers.

Plasters

Plasters are usually solid at ordinary temperature, and are intended for external application. They are used by spreading on cloth, paper, or plastic film and adhering to the skin.

Medicated plaster, long used for local or regional drug delivery, are the prototypical transdermal system.

Topical Emulsions

See under Lotions.

Topical Powders

See under Powders.

Topical Semi-solid Preparations

Topical semi-solid preparations are intended to be applied to the skin or to certain mucous surfaces for local action or percutaneous penetration of medicaments, or for their emollient or protective action. They are of homogeneous appearance. Topical semi-solid preparations consist of a simple or compound base in which, usually, one or more active substances are

dissolved or dispersed. According to its composition, the base may influence the action of the preparation and the release of the active substance(s).

The bases may consist of natural or synthetic substances and may be single-phase or multi-phase systems. According to the nature of the base the preparation may have hydrophilic or hydrophobic (lipophilic) properties; it may contain suitable additives such as antimicrobial preservatives, antioxidants, stabilizers, emulsifiers and thickeners.

If a preparation is specifically intended for use on large open wounds or on severely injured skin, it should be sterile. Preparations required to be sterile must comply with the test for sterility.

If the particle size of the ingredients is of importance for the therapeutic purpose of a topical semi-solid preparation, the test to be applied should be specified. Topical semi-solid preparations can be distinguished into four categories: (1) creams (hydrophobic or hydrophilic); (2) gels (hydrophobic or hydrophilic); (3) ointments (hydrophobic, water-emulsifying or hydrophilic); (4) pastes.

Sterility Where the preparation is labelled as sterile and unless otherwise directed in the individual monograph, it complies with the "Sterility Test" (Method I, Appendix 10.1).

Packaging and storage Topical semi-solid preparations should be stored in well-closed containers or, if the preparation contains water or other volatile constituents, in a tightly closed container. The containers are preferably collapsible metal tubes from which the preparation may be readily extruded. Other types of container may be used. Containers for preparations for nasal, aural, vaginal, or rectal use should be adapted to deliver the product to the site of application or should be accompanied by a suitable applicator. They should be stored at a temperature not exceeding 30° unless otherwise prescribed. For creams and gels, they shall not be frozen.

Labelling The label of topical semi-solid preparations states (1) the name and concentration of any added antimicrobial preservative(s); (2) where applicable, that the preparation is sterile.

CREAMS Creams are homogeneous, semi-solid preparations consisting of opaque emulsion systems. They are multiphase preparations composed of a lipophilic phase and an aqueous phase.

Hydrophobic creams Hydrophobic creams have the lipophilic phase as the continuouss phase. They contain water-in-oil emulsifying agents such as wool fat, sorbitan esters and monoglycerides.

Hydrophilic creams Hydrophilic creams have the aqueous phase as the continuous phase. They contain oil -in-water emulsifying agents such as sodium or triethanolamine soaps, sulfated fatty alcohols and polysorbates, combined, if necessary, with water-in-oil emulsifying agents.

GELS Gels are usually homogeneous, clear, semisolid preparations consisting of a liquid phase within a three-dimensional polymeric matrix with physical or sometimes chemical cross-linkage by means of suitable gelling agents.

Hydrophobic gels Hydrophobic gel (oleogel) bases usually consist of liquid paraffin with polyethylene or fatty oils gelled with colloidal silica or aluminium or zinc soaps.

Hydrophilic gels Hydrophilic gel (hydrogel) bases usually consist of water, glycerol, or propylene glycol gelled with suitable agents such as tragacanth, starch, cellulose derivatives, carboxyvinyl polymers and magnesium aluminium silicates.

OINTMENTS Ointments are homogeneous, semi-solid preparations intended for external application to the skin or mucous membranes. They are formulated using hydrophobic, hydrophilic, or water-emulsifying bases to provide preparations that are immiscible, miscible, or emulsifiable with skin secretions. They can also be derived from hydrocarbon (fatty), absorption, water-removable, or water-soluble bases.

Hydrophobic ointments Hydrophobic (lipophilic) ointments are usually anhydrous and can absorb only small amounts of water. Typical bases used for their formulation are water-insoluble hydrocarbons such as hard, soft, and liquid paraffin, vegetable oil, animal fats, waxes, synthetic glycerides, and polyalkylsiloxanes.

Water-emulsifying ointments Water-emulsifying ointments can absorb large amounts of water. They typically consist of a hydrophobic fatty base in which a water-in-oil agent, such as wool fat, wool alcohols, sorbitan esters, monoglycerides, or fatty alcohols can be incorporated to render them hydrophilic. They may also be water-in-oil emulsions that allow additional quantities of aqueous solutions to be incorporated. Such ointments are used especially when formulating aqueous liquids or solutions.

Hydrophilic ointments Hydrophilic ointment bases are either water-removable or water-soluble bases. They are oil-in-water emulsions such as hydrophilic ointment or greaseless ointment bases comprised of water soluble constituents such as mixtures of liquid and solid polyethyleneglycols (macrogols).

PASTES Pastes are homogeneous, semi-solid preprations usually containing high concentrations of insoluble powdered substances (commonly not less than 20 per cent) dispersed in a suitable base. One class is made from a single phase aqueous gel (e.g., zinc oxide gelatin paste). The other class, the fatty pastes (e.g., zinc oxide paste) consists of thick, stiff ointments that do not ordinarily flow at body temperature. The pastes should adhere well to the skin. In many cases they form a protective film that controls the evaporation of water.

Topical Solutions

See under Lotions.

Topical Suspensions

See under Lotions.

VAGINAL PREPARATIONS

Vaginal preparations are liquid, semi-solid preparations intended for administration to the vagina usually in order to obtain a local effect. They contain one or more active ingredients in a suitable basis. Several categories of vaginal preparations may be distinguished: (1) moulded pessaries; (2) vaginal tablets; (3) vaginal capsules; (4) vaginal foams; and (5) vaginal tampons.

Production In the manufacturing, packaging, storage and distribution of vaginal preparations,

GENERAL NOTICES

กรมวิทยาศาสตร์การแพทย์

The main objective of the Thai Pharmacopoeia is to establish the Thai National Standards of selected pharmaceutical substances and preparations. Apart from their standards and requirements set forth in the monographs, the non-mandatory information given includes their main classes of actions and applications (categories) and their safety (contra-indications, warnings, etc.). Subjected to other pertinent laws and ministerial regulations and notifications promulgated by Thai Minister of Public Health, the various standards of articles set up in this book, together with their pharmacopoeial requirements, are legally recognized by the Royal Thai Government.

The General Notices and general requirements appearing in appendices provide the basic guidelines for the interpretation and application of the standards, tests, assays, and other specifications of the Pharmacopoeia. Where exceptions to the General Notices or Appendices are made, the expressions such as "unless otherwise specified", "unless otherwise directed", or "unless other- wise indicated" are employed.

Title

The full title of this book, including its supplements, is the Pharmacopoeia of the Kingdom of Thailand, second edition. This title may be abbreviated to Thai Pharmacopoeia, second edition, or to TP II. Where the term TP is used, without further qualification, during the period in which this Pharmacopoeia is officially effective, it refers to TP II.

"Official", "Official Name" and "Official Standards"

The word "official", as used in this Pharmacopoeia or with reference hereto, is synonymous with "Pharmacopoeial", with "TP", and with "compendial".

The "official name" implies the "main title" of each Pharmacopoeial substance or preparation which is given in English and printed with capital letters. Any "other name" or "subsidiary title", where included, has the same status as the main title. It is mostly derived from the main title of that substance or preparation as it was used in the National List of Essential Medicine and the other recognized pharmacopoeiae such as the International Pharmacopoeia, the British Pharmacopoeia, and the United States Pharmacopeia. The spelling "sulph-" may be substituted for "sulf-" in the main title. Similarly, the spelling "aluminum" may also be substituted for "aluminium".

In a monograph of pharmaceutical preparations, "other name" or "subsidiary title" is nevertheless excluded.

The terminology "article" is an item for which a monograph is provided, whether a substance or a preparation.

The requirements stated in the monographs of the Pharmacopoeia apply to articles that are intended only for medicinal use. An article intended or labelled for medicinal use that is described by means of an official title must comply with the requirements of the relevant monograph. A formulated preparation must comply throughout its assigned shelf-life (period of validity). The subject of any other monograph must comply throughout its period of use.

Unless otherwise indicated in the General Notices or in the monographs, statements in monographs constitute mandatory requirements. The chemical formula and solubility statements are presented for informative purposes only and are not to be considered in the same category as the standards or tests for purity. The tests or methods in general appendices become mandatory when referred to in a monograph, unless such reference is made in a way to indicate that it is not the intention to make the text referred to mandatory but rather to cite it for information.

An article is not of pharmacopoeial quality unless it complies with all of the requirements stated. The general notice on Assays and Tests indicates that analytical methods other than those described in the Pharmacopoeia may be employed for routine purposes.

Where a monograph on a biological substance or preparation refers to a strain, a test, a method, a substance, etc., using the qualifications "suitable" or "appropriate" without further definition in the text, the choice of such strain, test, method, substance, etc., is made in accordance with any international agreements or national regulations affecting the subject concerned.

The designation "TP" in conjunction with the official title or elsewhere on the label of article indicates that a monograph is included in the Thai Pharmacopoeia and that the article must comply with all applicable TP standards.

Printing Types

In the text, words which refer to reagents, which conform to the require- ments specified in the appendices of other parts of the book, and the systematic names of plants, animals and micro-organisms are usually printed in italics or other types to distinguish them from the other words in that portion of the text.

Graphic Formulae, Molecular Formulae, Molecular Weights, CAS Registry Numbers, and Chemical Names

When the chemical composition of a pharmaceutical substance is known or generally accepted, the graphic and molecular formulae, the molecular weight, the Chemical Abstracts Service (CAS) registry number and the chemical name are normally given at the beginning of the monograph for the purpose of information. This information refers to chemically pure substances and is not to be regarded as an indication of the purity of the official drugs. Elsewhere, in statements of standards of purity and strength and in descriptions of processes of assay, it is evident from the context that the formulae denote chemically pure substances.

Consonant with the employment of Chemical Abstracts (CA) nomenclature, and also in the interest of uniformity of style, the orientation of ring systems and the depiction of stereoisomeric features in graphic formulae are generally consistent with CAS practices.

The molecular formulae and the molecular weights also generally conform to CAS practices.

The chemical names given in the monographs are those Index names used by the CAS of the American Chemical Society. It is the inverted form of the systemic chemical name developed by CAS, in general accordance with the rules established over the years by the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB), and employed in the current issues of CA.

Monographs carrying chemical names generally carry also CAS registry numbers. These italicized numbers function, independently of nomenclature, as invariant numerical designators of unique unambiguous chemical substances in the CAS registry and thus find wide, convenient use.

Atomic Weights

The atomic weights used in computing molecular weights and factors in the assays and elsewhere are those recommended in 2005 by the International Commission on Atomic Weights.

Molecular Weights

The molecular weights of compounds (or where appropriate, atomic weights) are given at the beginning of the monographs. These molecular weights are calculated by adding together the atomic weights, or multiples thereof, using all the figures of the International Table of Atomic Weights; the total is then rounded off to two decimal places as follows:

If this first digit to be dropped is less than 5, the last digit retained is left unchanged.

If the first digit to be dropped is greater than 5 or is 5 followed by digits more than zero, the last digit retained is increased by one.

If the first digit to be dropped is 5 followed by zeros, the last digit retained is left unchanged if even and is increased by one if odd.

In applying the foregoing rounding operations, all digits to be dropped are dropped in one operation.

The molecular weights shown in the monographs are theoretical values and neither these nor the molecular and graphic formulae constitute analytical standards for the substances described.

Significant Figures and Tolerances

Where limits are expressed numerically herein, the upper and lower limits of a range include the two values themselves and all intermediate values, but no values outside the limits. The limits expressed in monograph definitions and tests, regardless of whether the values are expressed as percentages or as absolute numbers, are considered significant to the last digit shown.

EQUIVALENCE STATEMENTS IN TITRIMETRIC PROCEDURES In the equivalence statement of titrimetric procedures, the number of significant figures in the concentration of the titrant corresponds to the number of significant figures in the weight of the analyte.

TOLERANCES The limits specified in the monographs for Pharmacopoeial articles are established with a view to the use of these articles as drugs, except where it is indicated otherwise. The use of the molecular formula for the active ingredient(s) named in defining the required strength of a Pharmacopoeial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 per cent) purity.

A dosage form shall be formulated with the intent to provide 100 per cent of the quantity of each ingredient declared on the label. The tolerances and limits stated in the Definitions in the monographs for Pharmacopoeial articles allow for analytical error, for unavoidable variations in manufacturing and com- pounding, and for deterioration to an extent considered acceptable under practical conditions. No further tolerances should be applied to the values obtained in an assay to determine whether the product being examined complies with the requirements of monograph.

The specified tolerances are based upon such attributes of quality as might be expected to characterize an article produced from suitable raw materials under recognized principles of good manufacturing practice.

INTERPRETATION OF REQUIREMENTS Analytical results in the laboratory (observed or calculated from experimental measurements) are compared with stated limits to determine whether there is conformance with compendial assay or test requirements. The observed or calculated values usually will contain more significant figures than there are in the stated limit, and a reportable result is to be rounded off to the number of places that is in agreement with the limit expression by the following procedure. Intermediate calculations (e.g., slope for linearity in validation of compendial method) may be rounded for reporting purposes, but the original value (not rounded) should be used for any additional required calculations. Rounding off should not be done until the final calculations for the reportable value have been completed. (**Note** Limits, which are fixed numbers, are not rounded off.) When rounding off is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to 5 or more than 5, it is eliminated and the preceding digit is increased by one.

Illustration of Rounding Numerical Values for Comparison with Requirements

Compendial Requirement	Unrounded Value	Rounded Result	Conforms
Assay limit ≥ 99.0 per cent	98.97 per cent	99.0 per cent	Yes
	98.93 per cent	98.9 per cent	No
	98.95 per cent	99.0 per cent	Yes
Assay limit ≤ 102.5 per cent	102.55 per cent	102.6 per cent	No
	102.54 per cent	102.5 per cent	Yes
	102.56 per cent	102.6 per cent	No
Limit test ≤ 0.03 per cent	0.035 per cent	0.04 per cent	No
	0.034 per cent	0.03 per cent	Yes
	0.037 per cent	0.04 per cent	No
Limit test ≤ 2 per cent	2.4 per cent	2 per cent	Yes
	2.5 per cent	3 per cent	No
	2.6 per cent	3 per cent	No

Limit of Content

When limits of content are prescribed in a monograph, they are determined by the method described under "Assay".

When the result of a test or assay is referred to the "dried", "anhydrous" or "ignited" substance, the determination of loss on drying, water content or loss on ignition is carried out by the method prescribed under the heading "Loss on drying", "Water" or "Loss on ignition", respectively, in the monograph concerned.

Units of Potency of Antibiotics

The "Unit" referred to in an individual monograph is the specific antimicrobial activity contained in such an amount of the respective antibiotic Reference Substance as the appropriate international or national organization may from time to time indicate. (The necessary information is usually provided with the samples of the Reference Substance).

Unless otherwise specified in the individual monographs, the activity assigned to each "Unit" or "µg" is equivalent to an "International Unit (IU)", "Unit" or "µg" used in other recognized pharmacopoeia.

Freshly and Recently Prepared

The direction that a preparation must be freshly prepared indicates that it must be made not more than 24 hours before it is issued for use. The direction that a preparation should be recently prepared indicates that deterioration is likely if the preparation is stored for longer than about 4 weeks at 15° to 25°.

Added Substances

The word "added substances" as used in this Pharmacopoeia or with reference hereto is synonymous with "auxiliary substances".

An official substance, as distinguished from an official preparation, contains no added substances except where specifically permitted in the individual monograph. Where such addition is permitted, the label indicates the name(s) and amount(s) of any added substance(s).

Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, suitable substances such as antimicrobial agents, bases, carriers, coatings, colours, flavours, preservatives, stabilizers, and vehicles may be added to an official preparation to enhance its stability, usefulness, or elegance or to facilitate its preparation. Such substances are regarded as unsuitable and are prohibited unless (a) they are harmless in the amounts used, (b) they do not exceed the minimum quantity required to provide their intended effect, (c) their presence does not impair the bioavailability or the therapeutic efficacy or safety of the official preparation, and (d) they do not interfere with the assays and tests prescribed for determining compliance with the Pharmacopoeial standards.

Colouring Agents

Added substances employed solely to impart colour may be incorporated into official preparations, except those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colouring agents issued by the Thai Food and Drug Administration provided such added substances are otherwise appropriate in all respects.

Description

Information on the "description" pertaining to an article, which is relatively general in nature, is provided for those who use, prepare, and dispense drugs, solely to indicate the properties of an article complying with monograph standards. The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article.

In the case of crude drugs, the macroscopical description of a drug includes those features which can be seen by the unaided eye or by the use of a hand lens. The diagnostic characters given under a powdered crude drug are to be read in conjunction with the microscopical description given under the whole drug.

Statements of the distinctive microscopic structural elements in powdered crude drugs may be included in the individual monograph as a means of determining identity, quality, or purity.

Solubility

Statements given in the monographs under "Solubility" are intended as information on the approximate solubility only and are not regarded as part of the standard for a substance. Where no temperature is given, statements of solubility apply at ordinary room temperature.

Statements given under "Solubility test" express exact requirements and constitute part of the

standards for the substances under which they occur.

When the term "parts" is used in expressing the solubility of a substance, it means parts by weight (grams) of a solid in parts by volume (millilitres) of the solvent, or parts by volume (millilitres) of a liquid in parts by volume (millilitres) of the solvent. The following table indicates the meanings of such terms:

Descriptive Term	Part of Solvent Required for 1 Part of Solute
Very soluble	less than 1
Freely soluble	from 1 to 10
Soluble	from 10 to 30
Sparing solution	from 30 to 100
Slightly soluble	from 100 to 1000
Very slightly soluble	from 1000 to 10,000
Practically insoluble or insoluble	more than 10,000

Safety of Drugs

Important information on drug hazards is only partially and concisely included in the safety sections of this Pharmacopoeia. This part of a monograph does not constitute standards. Selection of information is based on what is considered practical and clinically significant in the effective and safe use of a drug. It is intended to aid the professional in minimizing the risks and enhanc- ing the benefits of its use. It should also be noted that there are still many other potential adverse reactions which have been omitted. Additional relevant information should therefore be obtained from other sources.

The risks of certain drug used during pregnancy are well recognized and the general principle is to give a drug only when the benefit to the individual mother outweighs the risk to the fetus. Caution should always be exercised in administering drugs during the first trimester of pregnancy. Unless the life of the patient is actually threatened by her morbidity, the desirability of postpon- ing treatment until after delivery should be considered.

Where there is a clear risk, this is noted under the heading "Warning" or "Precaution", but safety should not be inferred from the absence of this statement for any drugs.

Although the results of certain studies, both *in vitro* and in certain experimental animals, have demonstrated that a drug does not produce any teratogenic, carcinogenic, nor mutagenic effect, it does not always imply that it could also be safely used without producing such adverse effects in patients. Cautions and care should be observed in this respect, especially during pregnancy, and should also be extended to the case of administering a drug to nursing mothers, where the potential risk of the presence of the drug in the mother's milk could arise.

An increasing number of drugs that may produce hypersensitivity in some patients have been recognized, and it is well established that a person with known hypersensitivity to one or more particular drugs should inform the physician and avoid using them. It is also well established that the hypersensitivity to the drug limits its use; it is, therefore, not stated under the heading "Contra-indication". When there is no other alternative to using a drug, the use of such a drug should be attempted with due careful consideration of its potential risk against its actual benefit. The possible risk of hypersensitivity is emphasized under the heading "Warning" or "Precaution" in certain drug monographs, but, in its absence, it should not be assumed that a drug does not carry such a potential hazard.

Category

A statement of Category usually is provided for each pharmacopoeial substance. This indicates the class of its main pharmacological action and its application. Where there are several main actions or applications, usually not more than the two most important are selected, with due consideration to the prevailing medical situation. No attempt has been made to describe in detail

the actions and uses of a drug nor to specify its indications. The statement of Category is thus not intended to limit in any way the choice or use of the drug nor to indicate that it has no other activity or utility.

Strength(s) Available

Statements under the side-headings Strength or Strengths Available are included as a guide and are not necessarily comprehensive. For solid dosage forms such as Capsules and Tablets, the strength is given as the amount of active ingredient in each unit. For liquid dosage forms such as Injections and semi-solid dosage forms such as Creams, the strength is given as a concentra- tion. For Powders for Injections, the strength is given as the amount of active ingredient in each sealed container. Unless otherwise stated, the strength is given in terms of the weight or concentration of the official medicinal substance used in making the formulation.

Dose

The statements given under "Dose" in the monographs of the Pharmacopoeia are primarily intended to serve only as a guide to physicians or pharmacists who may vary it in the best interests of patients and in accordance with the variables that affect the action of the drug.

The specific terms used to classify the age of the patients are as follows:

- Adults: over 12 years of age
- Adolescents: 12 to 15 years of age
- Children: 1 to 12 years of age
- Infants: 5 weeks to 1 year of age
- Neonates: from newborn to 1 month of age

Unless otherwise specified, the route of administration is oral.

The statements of dosage in the case of Capsules and Tablets are expressed in terms of the content of active ingredient and seldom represent the total weight of the capsule contents or of the tablets.

In some instances, the dosage may be stated in terms of the pharmacologically active portion (moiety) of the molecule in order to permit the prescriber or dispenser to correlate the weight equivalent for salts, esters, or other chemical forms of the same drug moiety. However, it is not to be inferred that all chemical forms in which the active moiety may be presented are therapeutically equivalent.

Where the body surface area is stated as a basis for the specified dose, the "Table of Body Surface Area from Height and Weight (m^2)" in Appendix 1.17 may be employed to simplify the calculation of square metres of body surface.

The dose given in each monograph is that which may ordinarily be expected to produce in the patients with normal renal/hepatic function, following administration in the manner indicated, at such time intervals as may be stated, the diagnostic, therapeutic or prophylactic effect for which the mono- graph is recognized.

Contra-indication

This section specifies those conditions in which the drug should NOT be used.

Warning and Precaution

Warnings of the possible risk of certain hazards from the use of a drug are to be observed and taken care of before prescribing or administering it to a patient. Caution and careful consideration on the risk-benefit ratio of the drug should therefore be contemplated on an individual basis prior to the decision to use it.

On the other hand, important notes to be observed and carefully followed during and after the administration of a drug are described under the heading "Precaution".

Only the more important warnings and precautions are selected and included under the headings "Warning" and "Precaution" on the basis of their common or usual clinical significance to the population as a whole and it should not be assumed that the omission of a warning or a precaution in any particular monograph means that warning or precaution may not be of clinical significance for a specific patient.

Additional Information

The specific route of administration to be used for a particular drug, any pertinent personal observation or care on the part of the patient, and other special relevant information concerning an individual drug are to be grouped under the heading "Additional information".

Packaging and Storage

The substances and preparations described in the Pharmacopoeia are stored in such a way as to prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat, and light are indicated, where appropriate, in the monographs.

CONTAINERS

The container is the device that holds the substance, either in the form of the raw material or of the finished dosage form. The closure of the container, including the stopper, the cap, the attached dropper, etc., is considered as a part of the container.

The *immediate container* is the one which is in direct contact with the substance.

The container should be cleaned before use, and no extraneous matter should be introduced into it or into the substance placed in it. It must, likewise, not interact physically or chemically with the substance which it holds so as to alter the latter's quality, purity, or therapeutic potency to a level below its Pharmacopoeial requirements.

Well-closed container

A well-closed container must protect the contents from extraneous matter or from loss of the substance under ordinary or customary conditions of handling, shipment, storage, or sale.

Tightly closed container

A tightly closed container must protect the contents from contamination by extraneous matter or moisture, from loss of the substance, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, or sale, and shall be capable of tight reclosure. Where a tightly closed container is specified, it may be replaced by a hermetically closed container for a single-dose of the substance.

Hermetically closed container

A hermetically closed container must be impervious to air or any other gas under the ordinary or customary conditions of handling, shipment, storage, or sale.

Light-resistant container

A light-resistant container is the one which prevents transmission of light, such as an opaque container or a bottle of black, dark red or dark brown glass.

Single-unit container

A single-unit container is one that is designed to hold a quantity of drug product intended for administration as a single dose or a single finished device intended for use promptly after the container is opened. Preferably, the immediate container and/or the outer container or protective packaging shall be so designed as to show evidence of any tampering with the contents. Each single-unit container shall be labelled to indicate the identity, quantity and/or strength, name of the manufacturer, lot number, and expiration date of the article.

Single-dose container

A single-dose container is a single-unit container for articles intended for parenteral administration only a single-dose container is labelled as such.

Examples of single-dose containers include pre-filled syringes, cartridges, fusion-sealed containers, and closure-sealed containers when so labelled.

Unit-dose container

A unit-dose container is a single-unit container for articles intended for administration by other than the parenteral route as a single dose, direct from the container.

Multiple-unit container

A multiple-unit container is a container that permits withdrawal of successive portions of the

contents without changing the strength, quality, or purity of the remaining portion.

Multiple-dose container

A multiple-dose container is a multiple-unit container for articles in- tended for parenteral administration only.

Tamper-evident container

A tamper-evident container is a closed container fitted with a device that reveals irreversibly whether the container has been opened.

STORAGE

The following expressions are used in monographs under Packaging and storage with the meaning shown.

Protected from light means that the product is to be stored either in a light-resistant container or in a container enclosed in an outer cover that provides such protection or stored in a place from which all such light is excluded.

Protected from moisture means that the product is to be stored in a tightly closed container. Care is to be taken when the container is opened in a damp atmosphere. A low moisture content may be maintained, if necessary, by the use of a desiccant in the container provided that direct contact with the product is avoided.

Protected from freezing Where, in addition to the risk of breakage of the container, freezing subjects an article to loss of strength or potency, or to destructive alteration of its characteristics, the container label bears an appropriate instruction to protect the article from freezing.

STORAGE TEMPERATURES

When special conditions of storage are necessary, including limits of temperature, they are prescribed in the monograph. Where, in a monograph, the storage conditions are mentioned using the general expressions "at room temperature", "in a cold place", and the like, these terms are generally defined as follows.

Freezing temperature Any temperature not higher than –10°. An article for which storage at freezing temperature is directed may, alternatively, be stored in a freezer, unless otherwise specified in the individual monograph.

Very cold temperature Any temperature above –10° but not higher than 8°.

A *refrigerator* is a very cold place in which the temperature is maintained thermostatically between 2° and 8°.

Cold temperature Any temperature above 8° but not higher than 16°. **Cool temperature** Any temperature above 16° but not higher than 23°. **Room temperature** Any temperature above 23° but not higher than 35°.

Controlled room temperature A temperature maintained thermostatically (30°±2°) that encompasses the usual and customary working environment of 23° to 35°; that results in a mean kinetic temperature calculated to be 30°.

Hot temperature Any temperature above 35° but not higher than 40°.

Very hot temperature or Excessive heat Any temperature higher than 40°.

STORAGE UNDER NONSPECIFIC CONDITIONS

For articles, regardless of quantity, where no specific storage directions or limitations are provided in the individual monograph, it is to be understood that conditions of storage and distribution include protection from moisture, freezing and excessive heat.

Labelling

Every article in this Pharmacopoeia shall bear one or more labels indicating its identity and certain pertinent characteristics. This label(s) shall be affixed or firmly fastened to, or indelibly written on, the article's immediate container and, if any, its package. The label shall comply, in addition to the requirements prescribed in the Pharmacopoeia, with those labelling requirements currently promulgated by the Thai Ministry of Public Health. In cases where contradiction might arise between these requirements the latter bears more legal authority.

A *shipping* container, or a shipping package, unless such container or package is also essentially the immediate container or the outside of the consumer package, is exempt from the labelling requirements of this Pharmacopoeia.

Labelling requirements, also, do not necessarily apply when the article is supplied in compliance with a medical prescription.

The label on the container and the label on the package of every official article state:

(1) the name (commercial and/or generic) of the article, together with its dosage form;

(2) for herbal drugs and herbal drug preparations, where applicable, the Latin binomial and, following the official name, the part of the plant source from which the article was derived;

(3) the name and address of the manufacturer;

(4) amount(s) of active ingredient(s) per dosage unit (capsule, tablet, or other unit dosage form), per gram (or 100 grams), per millilitre (or 100 millilitres), or per other measurement, as the case may be;

(5) total amount of the article in the immediate container;

(6) batch or lot, or quality control, number or code of the product;

(7) the expiration date;

(8) where applicable, the name(s) of any antimicrobial preservative(s);

(9) for herbal drug preparations, "Consult a physician or pharmacist prior to use if you are pregnant or breastfeeding";

(10) other statements specifically required under "Labelling" in that article's monograph.

Where an expiration date is required to be stated on the label, this date identifies the time during which the article may be expected to meet the re- quirements of the Pharmacopoeial monograph, provided it is kept under the prescribed storage conditions. The expiration date limits the time during which the product may be dispensed or used. Where an expiration date is stated only in terms of the month and the year, it is a representation that the intended expiration date is the last day of the stated month. For articles requiring constitution prior to use, a suitable beyond-use date for the constituted product shall be identified on the label. The beyond-use date placed on the label shall not be later than the expiration date on the manufacturer's container.

Identification

Identification tests are provided for the verification of the identity of the substance described in the monograph. A judgment by the analyst is needed as to the extent of testing required, taking into account the available instrumentation.

It is generally recognized that the infrared spectrum provides the best method of identification because of the uniqueness of a well-developed "finger print" region of the spectrum for a given drug substance.

Wherever possible, infrared spectrum characteristics are used as the pri- mary test of identification. Usually this can stand by itself without any additional test. However, where the article is a salt, it is necessary to include a "specific ion test". Further identification tests provided in an individual mono- graph, taken together, are intended to provide verification of identity, should the use of an infrared spectrophotometer be precluded. Such identification tests, however specific, are not necessarily sufficient to establish proof of identity; but failure of an article taken from a labelled container to meet the requirements of a prescribed identification test indicates that the article may be mislabelled. Other tests and specifications in the monograph often contribute to establishing or confirming the identity of the article under examination.

It should further be noted that whenever a melting temperature is provided under the heading "Identification", an approximate value is usually given, since no exact reproduction of the quoted temperature is necessary.

Arsenic and Heavy Metals

With regard to vegetable drugs, the toxic elements which may be present in sufficient quantity to pose potential risk vary from plant to plant. The amount of these elements depends on the location, the quality of the soil, or environmental pollution. Because of their toxic natures, arsenic and heavy metals are of major concern.

Although not specifically required in the monograph, it is suggested that the maximum amounts of the toxic elements, based on the acceptable daily intake (ADI) values, in final dosage forms of plant materials be as follows:

Arsenic	4	ppm
Cadmium	0.3	ppm

Lead	10	ppm
Mercury	0.5	ppm

Unless otherwise indicated, the test procedures are provided in the "Limit Tests for Heavy Metals in Herbal Drugs and Herbal Drug Preparations" (Appendix 5.2).

Microbial Contamination

Although not specifically required in the monographs, possible microbial contamination should be controlled to such an extent that the preparations derived from them meet the requirements as described in the "Limits for Microbial Contamination" (Appendix 10.5).

Water and Loss on Drying

Where the water of hydration or adsorbed water of a Pharmacopoeial article is determined by the titrimetric method, the test is generally given under the heading "Water". Where the determination is made by drying under specified conditions, the test is generally given under the heading "Loss on drying".

However, "Loss on drying" is most often given as the heading where the loss in weight is known to represent residual volatile constituents including organic solvents as well as water.

Impurities

Inorganic, organic, biochemical, isomeric, or polymeric components can all be considered impurities.

Related substances Related substances are known impurities which may be identified or unidentified. These substances are structurally related to a drug substance. They include intermediates and by-products from a synthetically produced organic substance, co-extracted substances from a natural product and degradation products of the substance. The residual organic solvents, water, inorganic impurities, residues from cells and micro-organisms or culture media used in a fermentation process are excluded from this definition.

Ordinary impurities Ordinary impurities are innocuous because they have no significant, undesirable biological activity in the amounts present. They may arise from the synthesis, preparation, or degradation of the substance.

Tests for *related substances* or *chromatographic purity* may control the presence of ordinary impurities.

Tests for the presence of impurities are provided to limit such impurities to amounts that are unobjectionable under conditions in which the medicinal agent is customarily employed. The quantity is expressed in parts per million (ppm) weight in weight where the quantity is not greater than 100 ppm, and as a percentage in the case of quantities greater than 100 ppm. These quantities are approximations for comparative tests. Acceptance or rejection is deter- mined on the basis of compliance or non-compliance with the stated test.

Assays and Tests

The Assays and Tests described are the official methods upon which the standards of the Pharmacopoeia depend. Assay and test procedures are pro- vided for determining compliance with the Pharmacopoeial standards of identity, strength, quality and purity. The analysts are not precluded from employing alternative methods, including methods of micro-analysis, in any Assay or Test if they are satisfied that the method which they use will give the results of equivalent accuracy. In the event of doubt or dispute, the methods of analysis described in the Pharmacopoeia alone are authoritative.

When the solvent used for a solution is not named, the solvent is Purified Water.

The use of a proprietary designation to identify a material used in an Assay or Test does not imply that another equally suitable material may not be used.

APPARATUS

A specification for a definite size or type of container or apparatus in a test or assay is given solely as a recommendation. Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed. Where low-actinic or light-resistant containers are specified, clear containers that have been rendered

opaque by application of a suitable coating or wrapping may be used.

Where an instrument for physical measurement, such as a spectrophotometer, is specified in a test or assay by its distinctive name, another instrument of equivalent or greater sensitivity and accuracy may be used. In order to obtain solutions having concentrations that are adaptable to the working range of the instrument being used, solutions of proportionately higher or lower concentrations may be prepared according to the solvents and proportions thereof that are specified for the procedure. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification. Items capable of equal or better performance may be used if these characteristics have been validated.

Where the use of a centrifuge is indicated, unless otherwise specified, the directions are predicated upon the use of apparatus having an effective radius of about 20 cm (8 inches) and driven at a speed sufficient to clarify the supernatant layer within 15 minutes.

Unless otherwise specified, for chromatographic tubes and columns the diameter specified refers to internal diameter; for other types of tubes and tubing the diameter specified refers to outside diameter.

While one of the primary objectives of the Pharmacopoeia is to assure the user of official articles of their identity, strength, quality, and purity, it is mani- festly impossible to include in each monograph a test for every impurity, contaminant, or adulterant that might be present, including microbial contami- nation. These may arise from a change in the source of material or from a change in the processing, or may be introduced from extraneous sources. Tests suitable for detecting such occurrences, the presence of which is inconsistent with applicable good manufacturing practice or good pharmaceutical practice, should be employed in addition to the tests provided in the individual mono- graph.

PROCEDURE

In performing the assay or test procedures in this Pharmacopoeia, it is expected that safe laboratory practices will be followed. This includes the use of precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Prior to undertaking any assay or procedure described in this Pharmacopoeia, the individual should be aware of the hazards associated with the chemicals and the procedures and means of protecting against them. This Pharmacopoeia is not designed to describe such hazards or protective measures.

Automated procedures employing the same basic chemistry as those assay and test procedures given in the monograph are recognized as being equivalent in their suitability for determining compliance. Conversely, where an auto- mated procedure is given in the monograph, manual procedures employing the same basic chemistry are recognized as being equivalent in their suitability for determining compliance.

In the performance of assay or test procedures, not less than the specified number of dosage units should be taken for analysis. Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy and provided that any subsequent steps, such as dilutions, are adjusted accordingly to yield concentrations equivalent to those specified and are made in such manner as to provide at least equivalent accuracy. To minimize environmental impact or contact with hazardous materials, apparatus and chemicals specified in Pharmacopoeial procedures also may be proportionally changed.

Where it is directed in an assay or a test that a certain quantity of substance or a counted number of dosage units is to be examined, the specified quantity or number is a minimal figure (the singlet determination) chosen only for convenience of analytical manipulation; it is not intended to restrict the total quantity of substance or number of units that may be subjected to the assay or test or that should be tested in accordance with good manufacturing practices.

Where it is directed in the assay of Tablets to "weigh and finely powder not less than" a given number, usually 20, of the Tablets, it is intended that a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered tablets taken for assay is representative of the whole Tablets and is, in turn, weighed accurately. The result of the assay is then related to the amount of active ingredient per Tablet by multiplying this result by the average Tablet weight and dividing by the weight of the portion taken for the assay.

Similarly, where it is directed in the assay of Capsules to remove, as com- pletely as possible, the contents of not less than a given number, usually 20, of the Capsules, it is intended that a counted number of Capsules should be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken for the assay is representative of the contents of the Capsules and is, in turn, weighed accurately. The result of the assay is then related to the amount of active ingredient per Capsule by multiplying this result by the average weight of Capsule content and dividing by the weight of the portion taken for the assay.

Where the definition in a monograph states the tolerances as being "calculated on the dried (or anhydrous or ignited) basis," the directions for drying or igniting the sample prior to assaying are generally omitted from the *Assay* procedure. Assay and test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for Loss on drying, or Water, or Loss on ignition, respectively, is given in the monograph. Results are calculated on an "as-is" basis unless otherwise specified in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

Throughout a monograph that includes a test for Loss on drying or Water, the expression "previously dried" without qualification signifies that the substance is to be dried as directed under Loss on drying or Water (gravimetric determination).

In stating the appropriate quantities to be taken for assays and tests, the use of the word "about" indicates a quantity within 10 per cent of the specified weight or volume. However, the weight or volume taken is accurately determined, and the calculated result is based upon the exact amount taken. The same tolerance applies to specified dimensions.

Where the use of a pipette is directed for measuring a specimen or an aliquot in conducting a test or an assay, the pipette conforms to the standards set forth under "Volumetric Apparatus" (Appendix 1.9), and is to be used in such manner that the error does not exceed the limit stated for a pipette of its size. Where a pipette is specified, a suitable burette, conforming to the standards set forth under "Volumetric Apparatus" (Appendix 1.9), may be substituted. Where a "to contain" pipette is specified, a suitable volumetric flask may be substituted.

Expressions such as "25.0 ml" and "25.0 mg," used with respect to volume- tric or gravimetric measurements, indicate that the quantity is to be "accurately measured" or "accurately weighed" within the limits stated under "Volumetric Apparatus" (Appendix 1.9) or under "Weights and Balances" (Appendix 1.10).

The term "transfer" is used generally to specify a quantitative manipulation.

The term "concomitantly," used in such expressions as "concomitantly determine" or "concomitantly measured," in directions for assays and tests, is intended to denote that the determinations or measurements are to be performed in immediate succession.

Blank Determination Where it is directed that "any necessary correction" be made by a blank determination, the determination is to be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

Desiccator The expression "in a desiccator" specifies the use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of self-indicating silica gel or other suitable desiccant.

Drying to Constant Weight The specification "dried to constant weight" means that the drying shall be continued until two consecutive weighings do not differ by more than 0.5 mg per g of substance taken, (2.5 mg per g in case of crude drug), the second weighing following an additional hour of drying at the prescribed conditions.

Ethanol The term "ethanol" used without other indication means ethanol 95 per cent v/v. Where other strengths are intended, the term "ethanol" is used followed by the statement of the strength.

Filtration Where it is directed to "filter", without further qualification, the intent is that the liquid be filtered through suitable filter paper or equivalent device until the filtrate is clear.

Ignition to Constant Weight The specification "ignite to constant weight" means that the ignition shall be continued until two consecutive weighings do not differ by more than 0.5 mg per g of substance taken, the second weighing following an additional 15-minute ignition period.

Indicators Unless otherwise directed, where starch is specified in a test or an assay as an indicator, 3 ml of *starch TS* shall be used; in cases of the other test solutions, approximately 0.2 mL, or

4 drops, of the specified solution shall be added.

Logarithms Logarithms used in this Pharmacopoeia are to the base 10 and are denoted by the abbreviation "log". Natural logarithms, if used, are denoted by the abbreviation "ln".

Negligible This term indicates a quantity not exceeding 0.5 mg.

Odour A suitable quantity is left open to the air in an evaporating dish for 15 minutes, after which any odour is defined. An odour designation is descriptive only and is not to be regarded as a standard of purity for a particular lot of an article, except in those cases where a particular odour is specifically prohibited in the individual monograph.

Percentage Expressions Percentage concentrations are expressed as follows:

Per cent weight in weight (w/w) expresses the number of g of a constituent in 100 g of solution or mixture.

Per cent weight in volume (w/v) expresses the number of g of a constituent in 100 mL of solution, and is used regardless of whether water or another liquid is the solvent.

Per cent volume in volume (v/v) expresses the number of mL of a constituent in 100 mL of solution.

The term "per cent" used without qualification means, for mixtures of solids and semisolids, per cent weight in weight; for solutions or suspensions of solids in liquids, per cent weight in volume; for solutions of liquids in liquids, per cent volume in volume; and for solutions of gases in liquids, per cent weight in volume. For example, a 1 per cent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

Pressure Pressure is expressed in kPa.

1 kPa is 7.5006 Torr.

1 Torr is the pressure exerted by a column 1 mm of mercury.

Reagents The proper conduct of the analytical procedures described in the Pharmacopoeia and the reliability of the results depend, in part, upon the quality of the reagents used. The reagents, including the indicators and solutions required for the Assay and Tests of the Pharmacopoeia, are defined in the Appendices.

Reference Standards Reference Standards are authentic specimens that have been verified for suitability for use as comparison standards in Pharmacopoeial tests and assays. (See "Reference Substances", Appendix 1.8).

Where a Reference Substance is referred to in an assay or a test in this pharmacopoeia, the words "Reference Substance" are abbreviated to "RS".

Where a test or an assay calls for the use of a Pharmacopoeial article rather than a Reference Substance, as a material standard of reference, a substance meeting all of the requirements of the monograph for that article is to be used.

Any information necessary for proper use of the reference substance or reference preparation is given on the label or in the accompanying leaflet or brochure. Where no drying conditions are stated in the leaflet or on the label, the substance is to be used as received. The stability of the contents of opened containers cannot be guaranteed.

Solutions Unless otherwise specified in the individual monograph, all solutions called for in tests and assays are prepared with *water*.

An expression such as "(1 in 10)" means that 1 part *by volume* of a liquid is to be diluted with, or 1 part *by weight* of a solid is to be dissolved in, sufficient amount of the diluent or solvent to make the volume of the finished solution 10 parts *by volume*.

An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids are to be mixed, unless otherwise indicated.

Steam-Bath Where the use of a steam-bath is directed, exposure to actively flowing steam or to another form of regulated heat, corresponding in temperature to that of flowing steam, may be used.

Temperatures Unless otherwise specified, all temperatures in this Pharmacopoeia are expressed in Celsius degrees, and all measurements are made at 25°. Where "room temperature" is stated, a temperature from 20° to 30° is intended. Absolute temperatures are expressed in Kelvin degrees; the absolute tempera- ture is related to a Celsius temperature by the equation °K = °C + 273.16.

Vacuum The term "in vacuum" denotes exposure to a pressure not exceeding 0.7 kPa (about 5 Torr), unless otherwise indicated.

Water Where the use of *water* is specified or implied in identifications, tests and assays, for the

preparation of reagents, or as a diluent in any of these circumstances, water complying with the requirements of the monograph on Purified Water is used. The term "distilled water" indicates purified water prepared by distillation.

Water-Bath Where the use of a water-bath is directed without qualification with respect to temperature, a bath of vigorously boiling water is intended.

Weights and Measures The metric system of weights and measures is employed in the Pharmacopoeia; the International System of Units (SI) has been adopted wherever practicable. Unless otherwise stated, all measurements involved in the analytical operations of the Pharmacopoeia are intended to be made at 25°.

Crude Drugs

Crude drugs in the monographs include medicinal parts obtained from plants or animals, their cell inclusions, secretions, and extracts, or minerals.

MONOGRAPHS

PHARMACEUTICAL SUBSTANCES AND PREPARATIONS

ALOES

Aloe

Thai name ยาดำ (YA DAM)

Category Purgative.

Aloes is the solid residue obtained by evaporating the juice which drains from the leaves cut from various species of *Aloe* (Family Liliaceae).

BARBADOS ALOES

Curaçao Aloes

Barbados Aloes is the aloes obtained from *Aloe vera* (L.) Burm. f. (*A. barbadensis* Miller). It contains not less than 16.0 per cent of aloin ($C_{21}H_{22}O_9$), calculated on the dried basis.

Constituents Barbados Aloes contains anthraquinone glycosides, the principal one of which is aloin (aloe-emodin anthrone C-10 glucoside). It also contains chromones such as aloeresin A and aloesin, and anthraquinones.

Description Dark brown masses, slightly shiny or opaque with a conchoidal fracture, or brown powder. Odour, strong and characteristic; taste, nauseous and bitter.

Solubility Partly soluble in boiling *water*; soluble in hot *ethanol*.

Packaging and storage Barbados Aloes shall be kept in tightly closed containers, and stored at a temperature not exceeding 25°.

Identification

A. Mix 1 g, in *fine powder*, with 25 mL of cold *water*. Shake the mixture occasionally for 2 hours, filter, and wash the filter and residue with sufficient cold *water* to make 100.0 mL: a dark orange is observed when viewed in a bulb of a 100-mL volumetric flask.

B. To 5 mL of the filtrate obtained in the test for Identification A, add 2 mL of *nitric acid* and mix: a reddish orange colour is produced.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution A solution containing 1.0 mg per mL of Aloin RS in *methanol*.

Test solution Dissolve 500 mg, in *fine powder*, in 10 mL of *methanol*. Sonicate for 15 minutes, centrifuge or filter, and use the supernatant or the filtrate.

Adsorbent *Silica gel GF254* (HPTLC plate)

Mobile phase *Ethyl acetate*, *methanol*, and *water* (100:17:13)

Application Apply 2 µL of *Standard solution* and 5 µL of *Test solution* as 8-mm bands.

Development and drying Allow the solvent front to ascend 6 cm above the line of application. Dry the developed plate in air.

Detection Spray the plate with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* (prepared in an ice-bath), heat at 110° for 5 minutes and observe the result. Examine the plate under visible light and ultraviolet light (366 nm).

Results When examined under visible light, the test solution shows a brown band due to aloin at about the middle of the chromatogram, corresponding in colour and R_f to the band shown by the standard solution. The test solution shows a violet band due to 7-hydroxyaloin just below the band due to aloin. When examined under ultraviolet light (366 nm), the test solution shows a yellow fluorescent band due to aloin at about the middle of the chromatogram, corresponding in colour and R_f to the band shown by the standard solution and a light blue fluorescent band due to aloesin at about the upper third of the chromatogram.

Loss on drying Not more than 12.0 per cent w/w, determined on 2 to 5 g, in *No. 425 powder*, by drying at 105° for 5 hours (Appendix 4.15).

Total ash Not more than 4.0 per cent w/w (Appendix 7.7).

Ethanol-insoluble substance Not more than 10.0 per cent w/w. Boil about 1 g of powdered Barbados Aloes, accurately weighed, with 50 mL of *ethanol* on a water-bath for 30 minutes under a reflux condenser. Filter through a small dried and tared filter paper or a suitable dried and tared filtering crucible, and wash the residue on the filter with *ethanol* until the last washing is colourless. Dry the residue at 105° to constant weight and weigh.

Water-soluble extractive Not less than 50 per cent w/w (Appendix 7.12).

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation A solution containing 0.1 mg per mL of Aloin RS in a mixture of equal volumes of *methanol* and *water*. (**Note** The standard preparation is stable for 8 hours at a temperature of 25°.)

Assay preparation: Transfer about 100 mg, in *fine powder*, accurately weighed, to a 100-mL volumetric flask, and add 75 mL of *methanol*. Sonicate for 30 minutes, cool to room temperature, adjust with *methanol* to volume, and mix. Filter. (**Note** The assay preparation is stable for 8 hours at a temperature of 25°.)

Mobile phase: *Acetonitrile* and *water* (3:7).

Chromatographic System

DETECTOR Ultraviolet light (295 nm)

COLUMN A stainless steel column (25 cm × 4.6

mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm), end-capped.

COLUMN TEMPERATURE 43°±1°

FLOW RATE 1.0 mL per minute

System Suitability

SAMPLE *Standard preparation*

Suitability requirements

COLUMN EFFICIENCY Not less than 2000 theoretical plates for the aloin peak.

SYMMETRY FACTOR Not more than 2.0 for the aloin peak.

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent determined from the aloin peak.

Procedure Separately inject equal volumes (about 20 µL) of *Standard preparation* and *Assay preparation* into the chromatograph and measure the responses for the major peaks.

Calculation Calculate the content of $C_{21}H_{22}O_9$ in the Barbados Aloes taken, using the declared content of $C_{21}H_{22}O_9$ in Aloin RS.

CAPE ALOES

Cape Aloes is the aloes obtained from *Aloe ferox* Miller, and hybrids of this species with *A. africana* Miller or *A. spicata* Baker. It contains not less than 6.0 per cent of aloin ($C_{21}H_{22}O_9$), calculated on the dried basis.

Constituents Cape Aloes contains anthraquinone glycosides, the principal one of which is aloin (aloe-emodin anthrone C-10 glucoside). It also contains chromones such as aloeresin A, aloesin.

Description Dark brown masses, tinged with green, with a shiny conchoidal fracture, or a greenish brown powder. Odour, strong and characteristic; taste, nauseous and bitter.

Solubility Partly soluble in boiling *water*; soluble in hot *ethanol*.

Packaging and storage Cape Aloes shall be kept in tightly closed containers, and stored at a temperature not exceeding 25°.

Identification

A. Mix 1 g, in *fine powder*, with 25 mL of cold *water*. Shake the mixture occasionally for 2 hours, filter, and wash the filter and residue with sufficient cold *water* to make 100.0 mL: a greenish yellow is observed when viewed in a bulb of a 100-mL volumetric flask.

B. To 5 mL of the filtrate obtained in the test for Identification A, add 2 mL of *nitric acid*: a reddish brown colour that changes rapidly to green is produced.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution, Adsorbent, Mobile phase, Application, Development and drying, and **Detection** Proceed as directed in the Identification under *Barbados Aloes*.

Test solution Dissolve 500 mg, in *fine powder*, in 10 mL of *methanol*. Sonicate for 15 minutes, centrifuge or filter, and use the supernatant or the filtrate.

Results When examined under visible light, the test solution shows a brown band due to aloin at about the middle of the chromatogram, corresponding in colour and R_f to the band shown by the standard solution. The test solution shows no violet bands due to 7-hydroxyaloin just below the band due to aloin.

When examined under ultraviolet light (366 nm), the solution shows a yellow fluorescent band due to aloin at about the middle of the chromatogram, corresponding in colour and R_f to the band exhibited by the standard solution and a light blue fluorescent band due to aloesin at about the upper third of the chromatogram.

Loss on drying Not more than 10.0 per cent w/w, determined on 2 to 5 g, in *No. 425 powder*, by drying at 105° for 5 hours (Appendix 4.15).

Total ash Not more than 4.0 per cent w/w (Appendix 7.7).

Ethanol-insoluble substance Not more than 10.0 per cent w/w. Boil about 1 g of powdered Cape Aloes, accurately weighed, with 50 mL of *ethanol* on a water-bath for 30 minutes under a reflux condenser. Filter through a small dried and tared filter paper or a suitable dried and tared filtering crucible, and wash the residue on the filter with *ethanol* until the last washing is colourless. Dry the residue at 105° to constant weight and weigh.

Water-soluble extractive Not less than 50.0 per cent w/w (Appendix 7.12).

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation, Mobile phase, Chromatographic System, System Suitability, Suitability requirements, Procedure, and **Calculation** Proceed as directed in the Assay under *Barbados Aloes*.

Assay preparation Transfer about 200 mg, in *fine powder*, accurately weighed, to a 100-mL volumetric flask, and add 75 mL of *methanol*. Sonicate for 30 minutes, cool to room temperature, adjust with *methanol* to volume, and mix. Filter.

ALOES, POWDERED

Powdered Aloe

Thai name ยาดำผง (YA DAM PHONG)

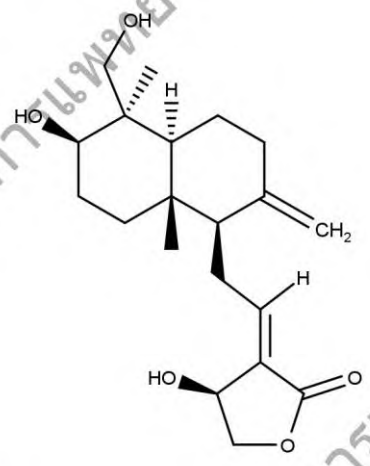
Complies with the requirements for content of aloin, Identification, Loss on drying, Total ash, Ethanol-insoluble substance, and Water-soluble extractive stated under Barbados Aloes or Cape Aloes, and with the following requirements.

Description Yellowish brown to dark reddish brown powder. Odour, strong and characteristic; taste, nauseous and bitter. When mounted in *lactophenol* and examined under a microscope, it is seen to consist of yellowish to reddish brown angular or irregular fragments, which are composed of masses of small acicular crystals (Barbados Aloes) or either amorphous and structureless (Cape Aloes); the powder ultimately dissolves in the mountant.

Solubility Almost entirely soluble in *ethanol (60 per cent)*.

Packaging and storage Powdered Aloes shall be kept in tightly closed containers, and stored at a temperature not exceeding 25°.

ANDROGRAPHOLIDE



Andrographis

$C_{20}H_{30}O_5$ 350.45 *5508-58-7*

(3*E*,4*S*)-3-{2-[(1*R*,4a*S*,5*R*,6*R*,8a*S*)-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1*H*-naphthalen-1-yl]ethylidene}-4-hydroxyoxolan-2-one.

Category Anti-inflammatory in laryngitis, antipyretic, antidiarrheal.

Andrographolide is the diterpene lactone extracted from the dried aerial parts of *Andrographis paniculata* (Burm. f.) Nees (Family Acanthaceae). It contains not less than 97.0 per cent w/w of $C_{20}H_{30}O_5$.

Description White to off-white powder.

Solubility Insoluble in *water*; very slightly soluble in *chloroform*; slightly soluble in *ethanol* and in *methanol*.

Other relevant information High storage temperatures, i.e., 70° to 90° result in degradation of andrographolide to other diterpene lactones, particularly 14-deoxy-11,12-didehydroandrographolide, which may decrease blood pressure when consumed in a large amount.

Packaging and storage Andrographolide shall be kept in well-closed containers, protected from light and stored at a temperature of 2° to 8°.

Identification The infrared absorption spectrum corresponds to the spectrum obtained from Andrographolide RS (Fig. 1).

Melting temperature 230° to 231° (Appendix 4.3).

Solubility test A solution of 250 mg in 25 mL of a mixture of *chloroform* and *methanol* (1:1) is clear.

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5). (**Note** Filter *Standard preparations (a)* and *(b)*, *Assay preparation* and *Mobile phases* through a membrane having a 0.22-µm porosity or finer before subjecting into the chromatograph.)

Standard stock preparation A solution containing 250 µg per mL of Andrographolide RS in a mixture of equal volumes of *methanol* and *acetonitrile*.

Standard preparation (a) Prepare a solution containing 125 µg per mL of andrographolide by quantitatively diluting *Standard stock preparation* with a 0.050 per cent v/v solution of *formic acid*.

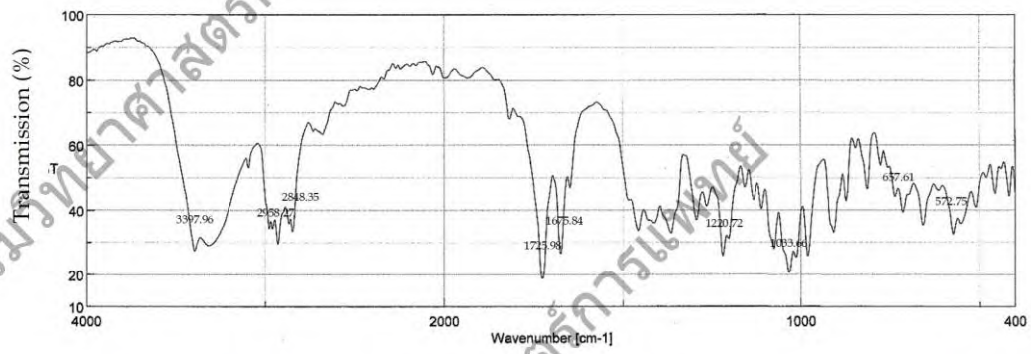
Standard preparation (b) Weigh accurately a quantity of Powdered Andrographis Extract RS equivalent to about 25 mg of diterpene lactones. Dissolve in 25 mL of a mixture of equal volumes of *methanol* and *acetonitrile*. Sonicate or heat gently for 15 to 20 minutes. Dilute further with the same solvent to obtain a solution having a known concentration of about 240 µg per mL of diterpene lactones.

Assay preparation Weigh accurately a suitable quantity of Andrographolide, dissolve in and dilute with a mixture of equal volumes of *methanol* and *acetonitrile* to obtain a solution containing 250 µg per mL of andrographolide. Dilute further with a 0.050 per cent v/v solution of *formic acid* to have a solution of a known concentration of about 125 µg per mL of andrographolide.

Mobile Phase:

MOBILE PHASE A A 0.050 per cent v/v solution of *formic acid*.

MOBILE PHASE B A 0.025 per cent v/v solution of *formic acid* in *acetonitrile for chromatography*.



Frequency (cm^{-1})	Band and Vibration Mode	Functional Group
3397.96	OH stretching	Hydroxyl group
2958.27	=CH stretching	Alkenes
2848.35	=CH stretching	Alkenes
1725.98	C=O stretching	Lactones
1675.84	C=C stretching	Alkenes
1220.72	C–O stretching	Ethers
1033.66	C–C	Alkanes
657.61	C–C=C	Allyl
572.76	C=O	Carbonyl

Fig. 1 Infrared Spectrum of Andrographolide and Its Characteristic Peaks
(Fourier Transform Using Diffuse Reflectance Technique with Potassium Bromide as Window Material)

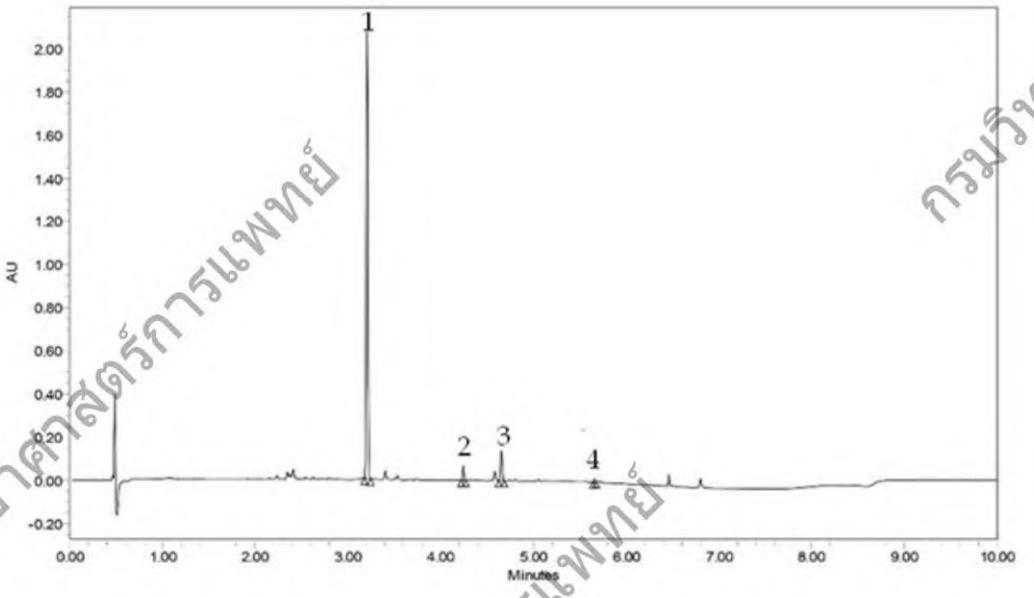


Fig. 2 Chromatogram of Powdered Andrographis Extract RS
1. Andrographolide 2. Neoandrographolide
3. 14-Deoxy-11,12-didehydroandrographolide 4. Andrograpanin

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0	90	10
4.5	55	45
6.25	20	80
7.00	20	80
8.00	90	10
10.00	90	10

Chromatographic system

DETECTOR Ultraviolet light (223 nm)

COLUMN A stainless steel column (10 cm × 2.1 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (1.7 µm). (Waters -Acquity® or equivalent is suitable)

COLUMN TEMPERATURE 40°

FLOW RATE 0.5 mL per minute

System suitability

SAMPLE *Standard preparation (a)* and *Standard preparation (b)*.

Suitability requirements The chromatogram of the standard preparation (b) is concordant with the reference chromatogram (Fig. 2). The approximate relative retention times of andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and andrograpanin are 1.00, 1.32, 1.45, and 1.75, respectively, with reference to the andrographolide peak.

COLUMN EFFICIENCY Not less than 5000 theoretical plates for the andrographolide peak in the chromatogram of the standard preparation (a).

SYMMETRY FACTOR Not more than 1.5 for the andrographolide peak in the chromatogram of the standard preparation (a).

RESOLUTION Not less than 5 between the neoandrographolide and 14-deoxy-11,12-didehydroandrographolide peaks in the chromatogram of the standard preparation (b).

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for andrographolide in the chromatogram of the standard preparation (a).

Procedure Separately inject equal volumes (about 2 µL) of *Standard preparation (a)* and *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the major peak.

Calculation Calculate the content of $C_{20}H_{30}O_5$ in the portion of the Andrographolide taken, using the declared content of $C_{20}H_{30}O_5$ in Andrographolide RS.

ANISE

Aniseed; Sweet Cummin; Sweet Cumin

Thai name เทียนสัตตบุษย์ (THIAN SATTABUT)

Category Carminative.

Anise is the dried whole cremocarp of *Pimpinella anisum* L. (*Anisum vulgare* Gaertn.) (Family Apiaceae). It contains not less than 2 per cent v/w of volatile oil, calculated on the anhydrous basis.

Origin of plant Anise is native to Egypt and eastern Mediterranean region, western Asia and Europe.

Constituents Anise contains volatile oil, of which *trans*-anethole is its major component with *g*-himachalene and estragole. It also contains coumarins, flavonoids, fixed oils, etc.

Description Odour, reminiscent of that of anethole; taste, sweet and aromatic.

Macroscopical Cremocarp ovoid or pyriform, 3 to 6 mm long, 1 to 3 mm wide, slightly compressed on the lateral sides, yellowish green or greenish grey. Mericarps frequently separated; each in crescent-shaped and slightly pubescent; dorsal side convex with 5 primary longitudinal ridges which are non-prominent and lighter in colour, and a short conical stylopodium with 2 short, reflexed stylar points; commissural side almost flat with persisting carpophore and pedicel.

Microscopical Transverse section of the mericarp through the cotyledon shows epicarp, mesocarp, endocarp, spermoderm and endosperm, and cotyledon. Epicarp comprises epidermal cells with numerous papillae and frequently unicellular trichomes, and covered with striated cuticle. Mesocarp consists of elongated parenchyma cells, small lignified vascular bundle under the ridge, and elliptic and brown vittae surrounded by small epithelial secretory cells; small vittae forming a line along the dorsal side and 2 large vittae surrounded by elongated cells in the commissural side. Endocarp characterized by elongated thin-wall cells. Spermoderm attached to the cremocarp and consisting of elongated cells with some collapsed cells. Endosperm composed of thick-walled polygonal cells containing oil globules and aleurone grains with small cluster crystals of calcium oxalate. Cotyledon with thin-walled cells containing oil globules and aleurone grains.

Packaging and storage Anise shall be kept in tightly closed containers, protected from light and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 3 µL of *anethole* and 40 µL of *olive oil* in 1 mL of *toluene*.

Test solution Shake 100 mg of the powdered drug with 2 mL of *dichloromethane* for 15 minutes. Filter and carefully evaporate the filtrate to dryness on a water-bath at 60°. Dissolve the residue in 0.5 mL of *toluene*.

Adsorbent *Silica gel GF254*

Mobile phase *Toluene*

Application Apply separately to the plate, 1 µL, 2 µL and 3 µL of *Standard solution* and 2 µL and 3 µL of *Test solution*, as spots.

Development and drying Allow the solvent front to ascend 10 cm above the line of application. Dry the developed plate in air.

Detection Examine the plate under ultraviolet light (254 nm). Spray the plate with a freshly prepared 20 per cent w/v solution of *phosphomolybdic acid* in *ethanol*, heat at 120° for 5 minutes and observe the result. Examine the plate under visible light.

Results When examined under ultraviolet light (254 nm), the test solution shows a quenching spot due to anethole at about the middle of the chromatogram, corresponding in R_f to the spot shown by the standard solution.

When examined under visible light, the test solution shows a blue spot due to anethole against a yellow background; the spot is intermediate in size between the corresponding spots obtained with 1 µL and 3 µL of the standard solution; a blue spot due to triglycerides of olive oil is also obtained at about the lower third of each of the chromatograms obtained with the test solution and the standard solution.

Water Not more than 9.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12); use 20.0 g, in *coarse powder*, freshly prepared and accurately weighed.

Foreign matter Not more than 2 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 2.5 per cent w/w (Appendix 7.6).

Total ash Not more than 12.0 per cent w/w (Appendix 7.7).

Assay Carry out the "Determination of Volatile Oil" (Appendix 7.3). Use 10 g, in *coarse powder*, freshly prepared and accurately weighed. Use 100 mL of *water* as the distillation liquid and a 250-mL round-bottomed flask. Distil at a rate of 2.5 to 3.5 mL per minute for 2 hours. Use 0.5 mL of *xylene* in the graduated tube. Calculate the content of volatile oil, in mL, in the portion of the Anise taken.

ANISE, POWDERED

Powdered Aniseed

Thai name เทียนสัตตบุษย์ผง (THIAN SATTABUT PHONG)

Complies with the requirements for content of anise oil, Packaging and storage, Identification, Water, Acid-insoluble ash, Total ash and Foreign matter stated under *Anise* and with the following requirement.

Description Greenish yellow or brownish green; odour, reminiscent of anethole; taste, sweet and aromatic. Diagnostic structures show whole or broken trichomes, mostly unicellular, sometimes curved with blunt apex and papillae; fragments of epidermis with striated cuticle, occasional *anomocytic* stomata, fragments of numerous narrow vittae, fragments of endosperm containing aleurone grains and small cluster crystals of calcium oxalate; oblong sclereids from the commissural zone and bundles of short sclerenchymatous fibres from the carpophore and pedicel.

ANISE WATER, CONCENTRATED

Category Carminative; pharmaceutic aid (flavouring agent).

Anise Oil	20 mL
Ethanol (90 Per Cent)	700 mL
Purified Water sufficient to produce	1000 mL

Dissolve the Anise Oil in the Ethanol (90 Per Cent) and add gradually, with vigorous shaking after each addition, sufficient Purified Water to produce 1000 mL. Add 50 g of previously sterilized Purified Talc, or other suitable filtering aid, allow to stand for a few hours, shaking occasionally, and filter.

Dose 0.3 to 1 mL.

Weight per millilitre 0.898 to 0.908 g (Appendix 4.9).

Ethanol content 60 to 64 per cent v/v (Method II, Appendix 6.5).

Other requirements Complies with the requirements described under "Aromatic Waters" (Appendix 1.16).

ANISE OIL

Aniseed Oil

Category Carminative; pharmaceutic aid (flavouring agent).

Anise Oil is the volatile oil distilled with steam from anise, the dried ripe fruits of *Pimpinella anisum* L. (Family Apiaceae).

Constituents Anise oil contains *trans*-anethole as the predominant compound. It also contains g-himachalene, estragole and *cis*-anethole.

Description Colourless, or pale yellow, highly refractive liquid; odour, that of the crushed fruit; taste, sweet and aromatic. Crystallizes on cooling.

Identification

A. The infrared absorption spectrum corresponds to the spectrum obtained from *anise oil* as shown below.

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 µL of *linalool*, 30 µL of *anisaldehyde* and 200 µL of *anethole* in *toluene* and dilute to 15 mL with the same solvent. Dilute 1 mL of this solution to 5 mL with *toluene*.

Test solution Dissolve 1 g of the sample in *toluene* and dilute to 10 mL with the same solvent.

Adsorbent *Silica gel F254*

Mobile phase *Ethyl acetate* and *toluene* (7: 93)

Application Apply 5 µL of each solution, as 10-mm bands.

Development and drying Develop the plate and dry in air.

Detection Examine the plate under ultraviolet light (254 nm). Spray the plate with *methyl 4-acetylbenzoate TS*, heat at 105° for 10 minutes and observe the result. Examine the still hot plate under visible light within 5 minutes.

Results When examined under ultraviolet light (254 nm), the test solution shows a quenching band due to anisaldehyde at the lower third and a very strong quenching band due to anethole at the upper third of chromatogram, corresponding in R_f to the bands shown by the standard solution.

When examined under visible light, the test solution shows a grey band due to linalool at the lower third; a yellow band due to anisaldehyde at the middle; a brown band due to anethole at the upper third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution; a violet-brown band may be present just below the solvent front.

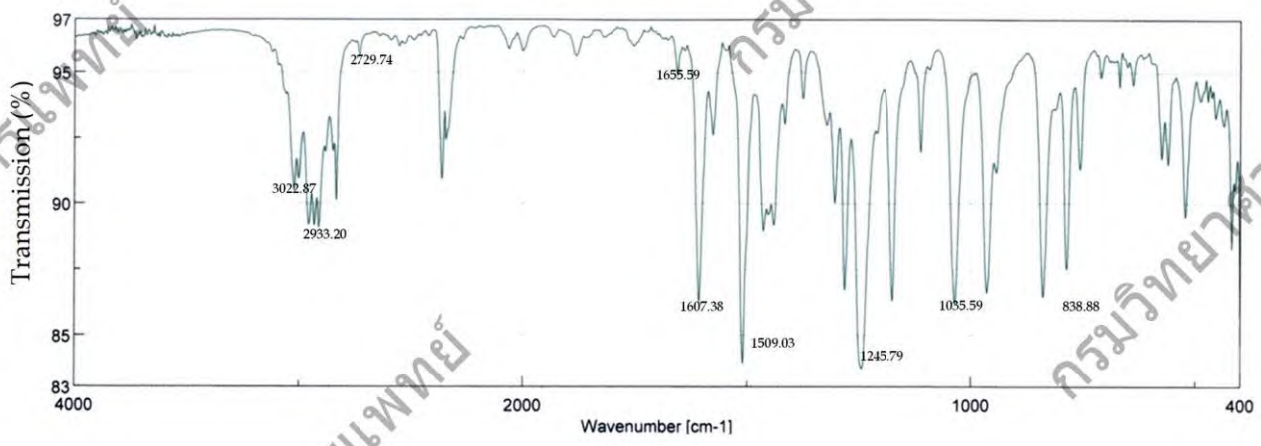
Packaging and storage Anise Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°. If the oil has crystallized, it should be melted and mixed before use.

Freezing temperature Not below 15° (Appendix 4.4).

Optical rotation −2° to +1° (Appendix 4.8).

Refractive index 1.553 to 1.560, at 20° (Appendix 4.7).

Instrument: Fourier Transform Window Material: Potassium Bromide



Infrared Absorption Peaks and Their Related Functional Groups for Anise Oil

Frequency (cm^{-1})	Band and Vibration Mode	Functional Group
3022.87	=CH stretching	aromatic ring
2933.20	OCH_3 stretching	ether
1655.59	C=C stretching	alkene
1607.38	C=C stretching	aryl C=C
1245.79	C-O stretching	alkyl aryl ether

Specific gravity 0.978 to 0.988 (Appendix 4.9).

Limit of phenols A 1 in 3 solution of recently distilled Anise Oil in *ethanol (90 per cent)* is neutral to moistened *litmus paper*, and the mixture develops no blue or brownish colour upon the addition of 1 drop of *iron(III) chloride TS* to 5 mL of the solution.

ASAFOETIDA

Asafetida; Gum Asafetida; Food of the Gods

Thai name มหาหิงคุ์ (MAHAA HINGU)

Category Carminative; antispasmodic.

Asafoetida is the oleo-gum-resin obtained by incising or cutting the living rhizome and roots of *Ferula assa-foetida* L.; *F. foetida* (Bunge) Regel; *F. rubricaulis* Boiss., and probably of other species of *Ferula* (Family Apiaceae).

Constituents Asafoetida contains about 10 to 17 per cent of volatile oil, 40 to 64 per cent of resin and about 25 per cent of gum. The main constituent of the oil is (*E*)-1-propenyl sec-butyl disulfide with a number of related organic disulfides and some terpenes. The resin consists of asaresinotannol, both free and combined with ferulic acid. Umbelliferone and sesquiterpene coumarins including umbelliprenin and galbanic acid (asacoumarin B) are also present.

Description Asafoetida occurs as a soft mass, sometimes almost semi-liquid, or as irregular, more or less pliable masses composed of agglutinated tears embedded in a weak brown to moderate yellowish brown matrix, or as loose ovoid tears, from 0.5 to 4 cm in diameter, with a few vegetable fragments. It becomes hard and occasionally brittle on drying. The surface of the freshly fractured tears is white to moderate yellowish brown, changing gradually on exposure to air or light to a strong pink and finally to a moderate yellowish brown or alternatively remaining nearly white. When moistened with water, the tears become moderate orange to weak yellow. Odour, powerful, persistent and fetid; taste bitter, alliaceous and acrid.

Other relevant information

1. The plant yielding asafoetida is native to central Asia, particularly eastern Iran and Afghanistan.
2. Pure asafoetida tear usually contains 65 to 75 per cent of substances soluble in ethanol (90 per cent) and yields about 3 to 5 per cent of ash.

Packaging and storage Asafoetida shall be kept in tightly closed containers and stored at a temperature not exceeding 25°.

Identification

A. Triturate the sample with *water*: a yellowish orange emulsion is obtained. Upon the addition of *ammonia TS*, the emulsion becomes greenish yellow.

B. Heat a fragment of the sample with *sulfuric acid*: a reddish brown solution is formed. Subsequently dilute the solution with *water*, filter and render it alkaline with *ammonia TS*: the solution acquires a purplish blue fluorescence.

C. To 10 ml of the ethanolic extract of 2 g of the sample, add a few drops each of *phloroglucinol TS* and of *hydrochloric acid*: a pink colour is produced in the mixture.

D. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution A solution containing 100 µg per mL of *ferulic acid* in *methanol*.

Test solution Extract about 25 g of the sample, in powder, with 100 mL of *methanol*, shake and filter. Evaporate the filtrate to obtain a brown extract. Transfer the extract to a 25-mL volumetric flask. Add 10 mL of *methanol*, sonicate for 10 minutes, allow to cool to room temperature, dilute to volume, and mix.

Adsorbent *Silica gel 60F254* (HPTLC plate)

Mobile phase *Toluene*, *ethyl acetate* and *formic acid* (60:40:10)

Application volume Apply 10 µL each of *Standard solution* and *Test solution*.

Development Allow the solvent front to ascend 8 cm above the line of application. Dry the developed plate in air.

Detection Examine the plate under ultraviolet light (254 nm). Spray the plate with *anisaldehyde TS*, heat at 105° for 5 to 10 minutes and examine under visible light. Observe the result.

Results When examined under ultraviolet light (254 nm), the test solution shows a quenching band due to ferulic acid in the middle of the chromatogram, corresponding in R_f to the band shown by the standard solution.

When examined under visible light, the test solution shows a dark purple band due to ferulic acid, corresponding in colour and R_f to the band obtained from the standard solution.

Acid-insoluble ash Not more than 3 per cent w/w (Appendix 7.6).

Total ash Not more than 15 per cent w/w (Appendix 7.7).

Tests for Adulterants

Ammoniac Triturate 500 mg of Asafoetida with 12 mL of *water* until an emulsion is formed, mix 2 mL of this emulsion with 5 mL of *water* and add 5 mL of *sodium hypobromite TS* to form a separate layer: no yellowish orange to red colour is produced in the mixture upon

adding.

Galbanum Add to 10 mL of the ethanolic extract of 2 g of Asafoetida enough *hydrochloric acid* to produce a faint turbidity: the mixture has a bluish green colour which fades as the mixture stands.

Rosin Triturate 1 g of Asafoetida with 10 mL of *petroleum ether (boiling range, 40° to 60°)* for 2 minutes, filter into a test-tube, and add to the filtrate 10 mL of a freshly prepared 0.5 per cent w/v solution of *copper(II) acetate*; shake well and allow the liquids to separate: the ethereal layer should not show a green colour.

Ethanol-insoluble substance Not more than 50 per cent w/w. Place about 5 g, accurately weighed, in a small beaker furnished with a glass rod and tared. Add 50 mL of *ethanol (90 per cent)* and boil gently. Filter the hot solution through a tared filter paper and boil the residue with further quantities of *ethanol (90 per cent)* until all soluble substances are removed, using the glass rod to disintegrate the insoluble substances. Wash the filter paper with hot *ethanol (90 per cent)*, transfer the paper to the beaker, dry to constant weight at 100°, and weigh.

ASAFOETIDA TINCTURE

Category Carminative, antispasmodic.

Asafoetida, bruised	200 g
Ethanol (70 Per Cent) sufficient to produce	1000 mL

Macerate Asafoetida in a closed vessel with 750 mL of Ethanol (70 Per Cent) for 7 days, shaking occasionally, filter, and pass sufficient Ethanol (70 Per Cent) through the filter to produce the required volume.

Dose *Topical*, 1 mL.

Other relevant information Large doses should be avoided in very young infants.

Weight per milliliter 0.915 g to 0.995 g (Appendix 4.9).

Ethanol content 60 to 65 per cent v/v (Appendix 6.5).

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

BELLADONNA LEAF

Belladonna Herb

Category Anticholinergic.

Belladonna Leaf consists of the dried leaf or the dried leaf and flowering, and occasionally fruit-bearing, tops of *Atropa belladonna* L. (Family Solanaceae). It contains not less than 0.35 per cent of the alkaloids of Belladonna Leaf.

Origin of plant Belladonna leaf is native to Eurasia, Mediterranean, Central and Southern Europe, including Britain, Southern and Eastern North Africa and Iran.

Constituents Belladonna Leaf contains the main alkaloid, (*S*)-hyoscyamine, which undergoes rapid racemization to atropine or *R,S*-hyoscyamine. It also contains small quantities of scopolamine or hyoscine, apoatropine and flavonoids.

Description Odour, slightly nauseous; taste, slightly bitter.

Macroscopical Entire or broken leaves green to greenish brown, often crumpled, rolled or partly matted together; when whole, lamina, elliptic to ovate, 5 to 25 cm long, 3 to 12 cm wide, apex acute to acuminate, base often decurrent, margin entire, young leaves pubescent, older leaves slightly pubescent along the vein; petiole 0.5 to 4 cm long; under a lens microsphenoidal crystal cells visible between veins as dark points by transmitted light or bright points by reflected light. Flowering tops: stems, flattened and hollow, bearing leaves in pair of unequal sizes at each node, flowers or occasionally fruits occur in the leaf axils; flowers: campanulate corolla about 2 cm long, about 1.5 cm wide, purple or yellow-brown lobes 5, short, reflexed; stamens 5, epipetalous; ovary superior, 2-lobed, containing numerous ovules; fruits, if found, occur as berries, globular, up to 12 mm in diameter, green, dark yellow, yellowish brown, to dusky red or brownish black, sometimes subtended by persistent calyx and containing numerous flattened, reniform seeds.

Microscopical

Leaf Epidermal cells with sinuous anticlinal walls and distinctly striated cuticle. Stomata anisocytic, more frequent on the lower epidermis. Non-glandular trichomes, uniseriate, up to 6-celled. Short club-shaped glandular trichomes with a one-celled stalk and multicellular head and long glandular trichomes with a uniseriate stalk and unicellular head are found on both upper and lower epidermises. Mesophyll, a layer of palisade parenchyma underlying with spongy parenchyma containing microcrystals. Midrib, an arc of bicollateral bundles, collenchyma and scattered parenchyma cells with microcrystals.

Stem Epidermis with striated cuticle and few trichomes. Endodermis distinct. Pericycle fibres, long, thin-walled, slightly lignified. A circle of bicollateral bundles presents. Parenchyma of cortex and pith contains scattered crystal cells.

Flower Calyx with numerous glandular trichomes with uniseriate stalks and 1- to 3-celled glandular heads. Corolla possesses papillose inner epidermis and outer epidermis with glandular trichomes similar to those of the calyx. Pollen grains mounted in chloral hydrate solution occurs in spherical shape, 40 µm in diameter, tricolpate, bearing 3 germinal furrows and rows of pits

between the ridges on the exine.

Fruit If found, epicarp consists of polygonal epidermal cells with striated cuticle and stomata. Mesocarp composed of large pulp cells some of which contain rosette aggregate crystals.

Seed If found, large wavy-walled epidermal cells with prominent ridges over the anticlinal walls.

Packaging and storage Belladonna Leaf shall be kept in well-closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification

A. Shake 1 g of the powdered drug with 10 mL of 0.05 M *sulfuric acid* for 2 minutes, filter, and add 1 mL of *strong ammonia solution* and 5 mL of *water* to the filtrate. Extract cautiously with 15 mL of *ether* to avoid the formation of an emulsion, dry the ether layer over *anhydrous sodium sulfate*, filter, and evaporate the ether in a porcelain dish. Add 0.5 mL of *fuming nitric acid*, evaporate to dryness over a small flame, add 10 mL of *acetone* and, dropwise, a 3 per cent w/v solution of *potassium hydroxide* in *ethanol*: a deep violet colour develops.

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 50 mg of *hyoscyamine sulfate* in 9 mL of *methanol*. Dissolve 15 mg of *hyoscine hydrobromide* in 10 mL of *methanol*. Mix 1.8 mL of the hyoscine hydrobromide solution and 8 mL of the hyoscyamine sulfate solution.

Test solution To 600 mg, in *fine powder*, add 15 mL of *dilute sulfuric acid*, shake for 15 minutes and filter. Wash the filter with *dilute sulfuric acid* until 20 mL of the filtrate is obtained. To the filtrate add 1 mL of *strong ammonia solution* and shake with two 10-mL portions of *peroxide-free ether*. If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulfate*, filter, and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 mL of *methanol*.

Adsorbent *Silica gel G*

Mobile phase *Strong ammonia solution*, *water* and *acetone* (3:7:90)

Application Apply 10 µL and 20 µL of each solution, as 20-mm bands.

Development and drying Allow the solvent fronts to ascend 10 cm above the line of application. Dry the developed plate at 105° for 15 minutes and allow to cool.

Detection A Spray the plate with *potassium iodobismuthate TS*, until orange or brown bands become visible against a yellow background, and observe the result. Examine the plate under visible light.

Results A The test solution shows a band due to hyoscyamine in the lower third and a band due to hyoscine at the upper third of the chromatogram, corresponding in colour, size and R_f to the band shown by the standard solution of the same volume. Faint secondary bands may appear, particularly in the middle of chromatogram obtained with 20 µL of the test solution or near the point of application in the chromatogram obtained with 10 µL of the test solution.

Detection B Spray with a 10 per cent w/v solution of *sodium nitrite* until the coating is transparent and examine after 15 minutes. Examine the plate under visible light.

Results B The bands due to hyoscyamine in the chromatograms obtained with the test solution and the standard solution change from brown to reddish brown but not to greyish blue (atropine), and any secondary bands disappear.

Foreign matter Not more than 3 per cent of stems with a diameter exceeding 5 mm (Appendix 7.2).

Acid-insoluble ash Not more than 4.0 per cent w/w (Method II, Appendix 7.6).

Total ash Not more than 16.0 per cent w/w (Appendix 7.7).

Assay Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

pH 9.5 Phosphate buffer Dissolve 34.8 g of *dipotassium hydrogenphosphate* in 900 mL of *water*, and adjust to a pH of 9.5, by the addition of 3 M *hydrochloric acid* or *sodium hydroxide*, with mixing.

Diluent Diluted *sulfuric acid* (1 in 350)

Internal standard solution Dissolve about 40 mg of Homatropine Hydrobromide RS, accurately weighed, in about 25 mL of *Diluent* in a 50-mL volumetric flask, add *Diluent* to volume, and mix. (**Note** Prepare freshly on the day of use.)

Standard preparation Dissolve about 10 mg of Scopolamine Hydrobromide RS, accurately weighed, in about 5 mL of *Diluent* in a 10-mL volumetric flask, add *Diluent* to volume, and mix (Solution A). Dissolve about 20 mg of Atropine Sulfate RS, accurately weighed, in about 25 mL of *Diluent* in a 50-mL volumetric flask, add 2.0 mL of Solution A, and mix. Add *Diluent* to volume, and mix. (**Note** Prepare freshly on the day of use.)

Standard curve Prepare three standard solutions as follows. Pipette into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard preparation*, and add 9.0, 8.0, and 7.0 mL, respectively, of *Diluent*. Proceed as directed under *Assay preparation*, beginning with "add 1.0 mL of *Internal standard solution*".

Assay preparation Moisten 10 g, in *moderately coarse powder*, with a mixture of 8 mL of *strong ammonia*

solution, 10 mL of *ethanol*, and 20 mL of *ether*, and extract the alkaloids by either Method I or Method II as follows. If necessary, reduce the volume of the extract to 100 mL by evaporation on a water-bath.

METHOD I Place the moistened drug in a continuous-extraction thimble, and allow maceration to proceed overnight, then extract with *ether* for 3 hours, or longer if necessary, to effect complete extraction.

METHOD II Place the moistened drug in a small percolator, and allow maceration to proceed overnight. Percolate slowly with a mixture of three volumes of *ether* and one volume of *chloroform*. Continue the percolation until the residue from 3 to 4 mL of percolate last passed, when dissolved in diluted *sulfuric acid* (1 in 70) and treated with *mercury(II) iodide TS*, shows not more than a faint turbidity.

Transfer the extract from Method I or Method II to a separator with the aid of *ether*. Extract with five 15-mL portions of diluted *sulfuric acid* (1 in 70), filtering each portion drawn off into a 100-mL volumetric flask. Wash the filter with diluted *sulfuric acid* (1 in 70), and collect the washings in the flask. Add diluted *sulfuric acid* (1 in 70) to volume, and mix. Dilute 20.0 mL of the resulting solution with the same dilute acid to 100.0 mL. Pipette 10 mL of this solution into a 60-mL separator. Add 1.0 mL of *Internal standard solution*, then add 15 mL of *chloroform*, shake vigorously, allow the layers to separate, and discard the chloroform layer. (**Note** If emulsions are formed, a mixture of 10 volumes of *chloroform* and 3 volumes of *2-propanol* may be substituted for chloroform throughout the extraction procedure.)

Add another 15 mL of *chloroform*, and extract again, discarding the chloroform phase. Add 15 mL of *Phosphate buffer* and sufficient 1 M *sodium hydroxide* to yield a final pH between 9.0 and 9.5. Add 15 mL of *chloroform*, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of *anhydrous sodium sulfate*, previously washed with *chloroform* and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of *chloroform*, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of *chloroform*. Evaporate the combined organic phases under reduced pressure at a temperature below 45°, add 1 mL of *chloroform*, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

Extraction blank Place 10 mL of *Diluent* in a 60-mL separator. Prepare as directed under *Assay preparation*, beginning with "then add 15 mL of *chloroform*". The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.

Chromatographic system

DETECTOR Flame ionization

COLUMN A glass column (1.2m × 4 mm) packed with 3 per cent of 50 per cent phenyl-50 per cent methylpolysioxane on *acid-washed and alkali-washed diatomaceous support* (100- to 120-mesh)

TEMPERATURES

Column	215°
Injection port	240°
Detector	240°

CARRIER GAS *Helium*

FLOW RATE 65 mL per minute

System suitability

SAMPLE *Standard preparation*

Suitability requirements

RESOLUTION Not less than 3.0 between atropine and homatropine peaks.

SYMMETRY FACTOR Not more than 2.0 for the atropine peak.

Relative standard deviation Chromatograph six to ten injections of *Assay preparation*, and record peak areas. The analytical system is suitable for conducting this Assay if the relative standard deviation for the ratio, R_A, is not more than 2.0 per cent for the atropine peak.

Procedure Inject a portion (about 5 mL) of each standard solution described under *Standard preparations* into the chromatograph. Measure the areas, a_A, a_H, and a_S, of the atropine, homatropine and scopolamine peaks, respectively, in each chromatogram, and calculate the area ratios R_A, and R_S by the formulae:

$$a_A/a_H \text{ and } a_S/a_H, \text{ respectively,}$$

Plot the standard curves of the values of R_A and R_S against the amounts in mg, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of *Assay preparation* into the chromatograph, measure the peak areas, and calculate the area ratios, as with the standard solutions. Record from the Standard curve the quantities, in mg, of atropine and scopolamine in the weight of sample taken. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids in the portion of Belladonna Leaf taken.

BELLADONNA LEAF EXTRACT

Category Anticholinergic.

Belladonna Leaf Extract contains, in each 100 g, not less than 1.15 g and not more than 1.35 g of the alkaloids of belladonna leaf.

Pilular Belladonna Leaf Extract

Prepare the extract by percolating 1000 g of Belladonna Leaf, using a mixture of 3 volumes of Ethanol (95 Per Cent) and 1 volume of Purified Water as the menstruum. Macerate the drug for 16 hours, and then percolate it at a moderate rate. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a pilular consistency, and adjust the remaining extract, after assaying, by dilution with Liquid Glucose so that the finished Extract will contain, in each 100 g, 1.25 g of the alkaloids of belladonna leaf.

Powdered Belladonna Leaf Extract

Prepare the extract by percolating 1000 g of Belladonna Leaf, using Ethanol (95 Per Cent) as the menstruum. Macerate the drug for 16 hours, and then percolate it slowly. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a soft extract, add 50 g of dry *starches* and continue the evaporation, at the same temperature, until the product is dry. Powder the residue. The extract may be deprived of its fat by treating either the soft extract first obtained, or the dry and powdered extract, as directed under "Extracts" (Appendix 1.16). Assay the powdered residue, and add sufficient Starches, previously dried at 100°, to obtain a finished Extract containing 1.25 g of the alkaloids of belladonna leaf in each 100 g. Mix the powders, and pass the Extract through a fine sieve.

Packaging and storage Belladonna Leaf Extract shall be kept in tightly closed containers and stored at a temperature not exceeding 30°.

Assay Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

pH 9.5 phosphate buffer, Diluent, Internal standard preparation, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and **System suitability** Proceed as directed in the Assay under *Belladonna Leaf*.

Assay preparation Weigh accurately about 500 mg of Belladonna leaf Extract, transfer to a 125-mL conical flask, and add 40 mL of *Diluent*. Heat to a temperature not above 45°, and stir to hasten solution. Filter the solution through a filter paper into a 100-mL volumetric flask. Wash the flask and the filter with two 20-mL portions of warmed *Diluent*, and collect the washings in the 100-mL volumetric flask. Add *Diluent* to volume, and mix.

Pipette 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of *chloroform*, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a mixed solvent consisting of 10 volumes of *chloroform* and 3 volumes of *2-propanol* may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of *chloroform*, and extract again, discarding the chloroform phase. Add 15 mL of *pH 9.5 Phosphate buffer* and sufficient 1 M *sodium hydroxide* to yield a final pH between 9.0 and 9.5. Add 15 mL of *chloroform*, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of *anhydrous sodium sulfate*, previously washed with *chloroform* and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of *chloroform*, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of *chloroform*. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of *chloroform*, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

Procedure Proceed as directed for Procedure in the Assay under *Belladonna Leaf*. Record from the Standard curve the quantities, in mg, of atropine and scopolamine in the volume of sample taken. Add the quantity, in mg, of atropine and scopolamine, and multiply by 10 to obtain the weight, in mg, of alkaloids in the portion of the Extract taken.

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16).

BELLADONNA LEAF EXTRACT TABLETS

Category Anticholinergic.

Belladonna Leaf Extract Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the labelled amount of the alkaloids of belladonna leaf.

Strength available 15 mg.

Dose 15 mg (equivalent to approximately 0.2 mg of atropine) three or four times a day, 30 minutes to 1 hour before meals and at bedtime, the dosage being adjusted as needed and tolerated.

Other relevant information

It may impair mental and/or physical abilities required for the performance of potentially hazardous tasks, patients should observe caution while driving or performing other tasks requiring alertness.

Transient stinging, dryness of mouth, blurred vision, photophobia with or without corneal staining, tachycardia, headaches, allergic reactions, and somnolence may appear during uses.

Packaging and storage Belladonna Leaf Extract Tablets shall be kept in tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Macerate a quantity of powdered Tablets,

equivalent to about 5 mg of the alkaloids of belladonna extract, with 20 mL of *water*, and transfer to separator. Render the solution alkaline with *ammonia TS*, and extract the alkaloids with 50 mL of *chloroform*. Filter the chloroform layer, divide it into two equal portions, and evaporate to dryness: the residue complies with the following test.

A. To one portion of the dry residue add 2 drops of *nitric acid*, evaporate on a water-bath to dryness, and add a few drops of *ethanolic potassium hydroxide TS*: a violet colour is produced.

B. Dissolve the other portion of the residue in 1 mL of diluted *hydrochloric acid* (1 in 120), and add *gold chloride TS*, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool: a lustreless precipitate is produced.

Assay Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

pH 9.5 phosphate buffer, Diluent, Internal standard preparation, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and **System suitability** Proceed as directed in the Assay under *Belladonna Leaf*.

Assay preparation Weigh and finely powder not less than 20 Belladonna Leaf Extract Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of atropine and scopolamine, to a 60-mL separator, add 10.0 mL of *Diluent*, and shake vigorously to dissolve as much as possible of the sample. Proceed as directed for Assay preparation in the Assay under *Belladonna Leaf Extract*, beginning with "add 1.0 mL of *Internal standard solution*".

Procedure Proceed as directed for Procedure in the Assay under *Belladonna Leaf Extract*. Record from the Standard curves the quantities, in mg, of atropine and scopolamine in the weight of sample taken. Add the quantity, in mg, of atropine and scopolamine, to obtain the weight, in mg, of alkaloids in the portion of the Tablets taken.

Other requirements Comply with the requirements described under "Tablets" (Appendix 1.16).

BELLADONNA TINCTURE

Category Anticholinergic.

Belladonna Tincture contains, in each 100 mL, not less than 27 mg and not more than 33 mg of the alkaloids of belladonna leaf.

Belladonna Leaf, in *moderately coarse powder* 100 g
Ethanol (70 Per Cent) a sufficient quantity

Prepare about 900 mL of a tincture by *percolation* (Appendix 7.18). Determine the proportion of alkaloids in this tincture by the Assay and add, if necessary, sufficient Ethanol (70 Per Cent) to produce a Tincture containing, in each 100 mL, 30 mg of the alkaloids of belladonna leaf.

Strength available 0.3 mg per mL.

Dose Adults: 0.6 to 1 mL three or four times a day.
Children: 0.03 mL per kg of body weight in three or four divided doses.

Other relevant information

1. It may impair mental and/or physical abilities required for the performance of potentially hazardous tasks, patients should observe caution while driving or performing other tasks requiring alertness.

2. Transient stinging, dryness of mouth, blurred vision, photophobia with or without corneal staining, tachycardia, headaches, allergic reactions, and somnolence may appear during uses.

Ethanol content 65.0 to 70.0 per cent v/v (Method II, Appendix 6.5).

Assay Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

pH 9.5 phosphate buffer, Diluent, Internal standard preparation, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and **System suitability** Proceed as directed in the Assay under *Belladonna Leaf*.

Assay preparation Proceed as directed in the Assay under *Belladonna Leaf*, but pipette 2 mL of Belladonna Tincture (in place of "10 mL of this solution") into a 60-mL separator containing 10 mL of *Diluent*.

Procedure Proceed as directed in the Assay under *Belladonna Leaf*. Record from the Standard curve the quantities, in mg, of atropine and scopolamine in the sample. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids per 100 mL.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

CARDAMOM OIL

Thai name น้ำมันกระวานเทศ (NAM-MAN KRAWAN THET)

Category Carminative; pharmaceutic aid (flavouring agent).

Cardamom Oil is the volatile oil obtained by distillation from the crushed fruits of *Elettaria cardamomum* (L.) Maton (Family Zingiberaceae).

Origin of plant Cardamom Oil-yielding plant is native to India.

Constituents Cardamom Oil contains mainly α-terpinyl

acetate, 1,8-cineole, α-terpineol, and linalool.

Description Colourless or pale yellow liquid; odour, pungent and aromatic.

Solubility Miscible with *ethanol*.

Packaging and storage Cardamom Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Solubility test Soluble, at 25°, in 5 volumes of *ethanol (70 per cent)*.

Specific gravity 0.917 to 0.947 (Appendix 4.9).

Refractive index 1.461 to 1.467, at 20° (Appendix 4.7).

Optical rotation +22° to +44° (Appendix 4.8).

Ester value 90 to 156 (Appendix 5.5).

CARDAMOM OIL TINCTURE, COMPOUND

Category Carminative; pharmaceutic aid (flavouring agent).

Cardamom Oil	0.450 mL
Caraway Oil	0.400 mL
Cinnamon Oil	0.225 mL
Cochineal, in *moderately coarse powder*	7 g
Glycerol	50 mL
Ethanol (60 Per Cent) sufficient to produce	1000 mL

Moisten the Cochineal with a sufficient quantity of Ethanol (60 Per Cent) and prepare 900 mL of tincture by *percolation* (Appendix 7.18). Add the Cardamom Oil, the Caraway Oil, the Cinnamon Oil, the Glycerol, and sufficient Ethanol (60 Per Cent) to produce 1000 mL; mix. Filter, if necessary.

(**Note** A certified food red colour may be substituted for cochineal.)

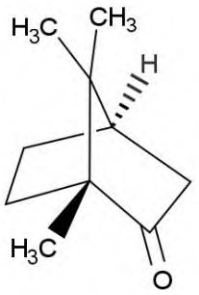
Weight per millilitre 0.925 to 0.937 g (Appendix 4.9).

Ethanol content 52 to 57 per cent v/v (Method II, Appendix 6.5).

Glycerol 4.5 to 5.5 per cent v/v when determined by the following method. Dilute 20.0 mL to 100.0 mL with *water*. To 20.0 mL of this solution add 100.0 mL of *water* and 1 g of *decolorizing charcoal* and boil under a reflux condenser for 15 minutes. Filter and wash the filter and charcoal with sufficient *water* to produce 150 ml. Add 0.25 mL of *bromocresol purple TS* and neutralize with 0.1 M *sodium hydroxide* or 0.05 M *sulfuric acid* to the blue colour of the indicator. Add 1.4 g of *sodium metaperiodate* and allow to stand for 15 minutes. Add 3 mL of *propylene glycol*, shake and allow to stand for 5 minutes. Add 0.25 mL of *bromocresol purple TS* and titrate with 0.1 M sodium hydroxide VS to the same blue colour. Each mL of 0.1 M *sodium hydroxide VS* is equivalent to 9.209 mg of glycerol. Calculate the percentage v/v of glycerol, taking its weight per mL as 1.260 g.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

CAMPHOR, NATURAL



$C_{10}H_{16}O$ 152.24 *464-49-3*

(1*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one.

Category Counter-irritant; pharmaceutic aid; antipruritic.

Natural Camphor is a ketone obtained from *Cinnamomum camphora* (L.) J. Presl (Family Lauraceae) and purified by sublimation.

Description Colourless, transparent crystals, crystalline masses, blocks of tough consistence or crumbly masses known as flowers of camphor; odour, strong and characteristic.

Solubility Slightly soluble in *water*, very soluble in *ethanol* and light petroleum, freely soluble in fixed oils, very slightly soluble in *glycerol*.

Other relevant information

1. Local application to the nostrils of infants may cause immediate collapse.

2. Liniment containing camphor should not be applied to broken or inflamed skin, mucous membranes, or near the eyes. It readily volatilizes at ordinary temperature.

Packaging and storage Natural Camphor shall be kept in tightly closed containers, and stored at a temperature not exceeding 25°.

Labelling The label on the container indicates whether it is obtained from natural sources or is prepared synthetically.

Identification

A. The infrared absorption spectrum is concordant with the spectrum obtained from Racemic Camphor RS or with the reference spectrum of Racemic Camphor.

B. It complies with the test for *Optical rotation*.

C. Dissolve 1 g in 30 mL of *methanol*, add 1 g of *hydroxylamine hydrochloride* and 1 g of *anhydrous sodium acetate*, boil under a reflux condenser for 2 hours, and allow to cool. Add 100 mL of *water*, filter, wash the precipitate with 10 mL of *water*, and recrystallize from 10 mL of a mixture of 4 volumes of *ethanol* and 6 volumes of water: the crystals, after drying over *phosphorus pentoxide desiccant* at a pressure of 1.5 to 2.2 kPa (about 11 to 19 Torr), melt between 118° and 121° (Appendix 4.3).

Melting range 174° to 181° (Appendix 4.3), but using a capillary tube with an internal diameter of not greater than 2 mm.

Optical rotation +40° to +43° at 20°, determined in a 10.0 per cent w/v solution in *ethanol* (Appendix 4.8).

Water Dissolve 1 g in 10 mL of *petroleum ether (boiling range, 40° to 60°)*: the solution is clear (Appendix 4.1).

Halogens Not more than 100 ppm. To 10.0 mL of a 10.0 per cent w/v solution in *2-propanol* in a distillation flask, add 1.5 mL of 2 M *sodium hydroxide* and 50 mg of *Raney nickel catalyst*, heat on a water-bath until the solvent has evaporated and allow to cool. Add 5 mL of *water*, mix, filter through a wet filter paper previously washed with *water* until free from chloride, and dilute the filtrate to 10.0 mL with *water*. To 5.0 mL of this solution add *nitric acid* dropwise until the precipitate that is produced dissolves and add sufficient *water* to produce 20 mL. The resulting solution shows no more chloride than that corresponds to 0.07 mL of 0.020 M *hydrochloric acid*.

Related substances Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution (a) Dilute 1.0 mL of *Test solution* to 100.0 mL with *heptane*.

Standard solution (b) Dilute 10.0 mL of *Standard solution* (a) to 20.0 mL with *heptane*.

Standard solution (c) Dissolve 500 mg of *borneol* in *heptane* and dilute to 25.0 mL with the same solvent. Dilute 5.0 mL of the solution to 50.0 mL with *heptane*.

Standard solution (d) Dissolve 50 mg of *linalool* and 50 mg of *bornyl acetate* in *heptane* and dilute to 100.0 mL with the same solvent.

Test solution Dissolve 2.5 g of the sample in *heptane* and dilute to 25.0 mL with the same solvent.

Chromatographic system.

DETECTOR Flame ionization

COLUMN A glass column (30 m × 0.25 mm) packed with *macrogol 20000* (0.25 µm).

TEMPERATURE

Column The temperature programme is as follows:

Time (Minutes)	Temperature (°)
0-10	50
10-35	50 → 100
35-45	100 → 200
45-55	200

Injection port 220°

Detector 250°

CARRIER GAS *Helium*

SPLIT RATIO 1:70

FLOW RATE 45 cm per second (1.3 mL per minute)

System suitability

SAMPLE *Standard solution (d)*

Suitability requirements

RESOLUTION Not less than 3.0 between bornyl acetate and linalool peaks.

Procedure Inject a portion (about 1 mL) of *Standard solution (a)*, *Standard solution (b)*, *Standard solution (c)*, *Standard solution (d)*, and *Test solution* into the chromatograph. Measure the area of the principal peak in each chromatogram.

Limits

— borneol: not more than the area of the principal peak in the chromatogram obtained from standard solution (c) (2.0 per cent).

— any impurity: not more than half of the area of the principal peak in the chromatogram obtained from standard solution (a) (0.5 per cent).

— total of other impurities: not more than 4 times the area of the principal peak in the chromatogram obtained from standard solution (a) (4.0 per cent).

— disregard limit: 0.1 times the area of the principal peak in the chromatogram obtained from standard solution (b) (0.05 per cent).

Non-volatile matter Not more than 0.1 per cent w/w when volatilized at 105° to constant weight; use about 5 g, accurately weighed.

Assay Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4).

Internal standard preparation Dissolve about 2 g of methyl salicylate, accurately weighed, in 50.0 mL of absolute ethanol.

Standard preparation Weigh accurately about 100 mg of Natural Camphor RS, add 5.0 mL of *Internal standard preparation*, dissolve in *absolute ethanol* and dilute to 100.0 mL with the same solvent.

Assay preparation Weigh accurately about 100 mg of Natural Camphor, add 5.0 mL of *Internal standard solution*, dissolve in *absolute ethanol* and dilute to 100.0 mL with the same solvent.

Chromatographic system

DETECTOR Flame ionization

COLUMN A glass column (3 m × 3 mm) packed

with 10 per cent of polyethylene glycol 20 M on silanized diatomaceous support 180- to 250-mesh.

TEMPERATURE
Column 160°
CARRIER GAS *Nitrogen*
FLOW RATE Adjust so that the retention time of natural camphor is about 6 minutes.

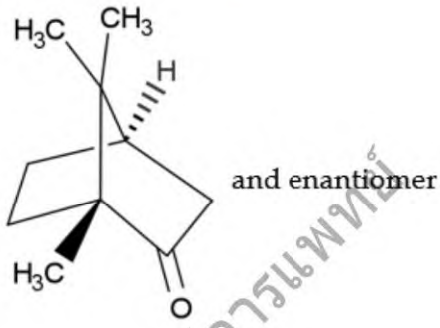
System suitability
SAMPLE *Standard preparation*

Suitability requirements
RESOLUTION Not less than 7 between natural camphor and methyl salicylate peaks.
RELATIVE STANDARD DEVIATIONS Chromatog-raph six injections of *Standard preparation*, and record peak areas. The analytical system is suitable for conducting this Assay if the relative standard deviation for the area ratios of natural camphor to methyl salicylate is not more than 1.0 per cent.

Procedure Separately inject equal volumes (about 2 µL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms and measure the areas of natural camphor and methyl salicylate peaks. Calculate the area ratios of natural camphor to methyl salicylate peaks.

Calculation Calculate the content of $C_{10}H_{16}O$ in the portion of Natural Camphor taken, using the declared content of $C_{10}H_{16}O$ in Natural Camphor RS.

CAMPHOR, RACEMIC



and enantiomer

$C_{10}H_{16}O$ 152.24 *76-22-2*

(1*RS*,4*RS*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one.

Category Counter-irritant; pharmaceutic aid; antipruritic.

Description White, crystalline powder or friable, crystalline masses; odour, strong and characteristic.

Solubility Slightly soluble in *water*, very soluble in *ether* and in *petroleum ether*; freely soluble in fixed oils; soluble in 1 part of *ethanol*; very slightly soluble in *glycerol*.

Other relevant information; Packaging and storage; Labelling See under *Camphor, Natural*.

Identification; Halogens Comply with the tests as described under *Camphor, Natural*.

Clarity and colour of solution A 10.0 per cent w/v solution in *ethanol* is clear (Appendix 4.1) and colourless.

Acidity or alkalinity To a 10.0 per cent w/v solution in *ethanol* add 0.1 mL of *phenolphthalein TS*. The solution is colourless and not more than 0.20 mL of 0.10 M *sodium hydroxide* is required to change the colour of the solution.

Water Dissolve 1 g in 10 mL of *petroleum ether (boiling range, 50° to 70°)*: the solution is clear (Appendix 4.1).

Optical rotation -0.15° to +0.15°, determined in a 10.0 per cent w/v solution in *ethanol* (Appendix 4.8).

Related substances Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution (a) Dissolve 50 mg of the sample and 50 mg of *bornyl acetate* in *hexane* and dilute to 50.0 mL with the same solvent.

Standard solution (b) Dilute 1.0 mL of the test solution to 200.0 mL with *hexane*.

Test solution Dissolve 50 mg of the sample in *hexane* and dilute to 50.0 mL with the same solvent.

Chromatographic system
DETECTOR Flame ionization
COLUMN A glass column (2 m × 2 mm) packed with *macrogol 20000* (0.25 µm)
TEMPERATURE
Column 130°
Injection port 200°
Detection 200°
CARRIER GAS *Nitrogen*
SPLIT RATIO 1:70
FLOW RATE 30 mL per minute

System suitability
SAMPLE *Standard solution (a)*

Suitability requirements
RESOLUTION Not less than 1.5 between the peaks due to camphor and bornyl acetate in the chromatogram obtained with *Standard solution (a)*.
SIGNAL-TO-NOISE RATIO Not less than 5 for the principal peak in the chromatogram obtained with *Standard solution (b)*.

Procedure Inject a portion (about 1 mL) of *Standard solution (a)*, *Standard solution (b)* and *Test solution* into the chromatograph. Measure the area of the principal peak in each chromatogram.

Limits
— any impurity: for each impurity, not more than 2 per cent of the area of the principal peak,
— total of other impurities: not more than 4 per cent of the area of the principal peak,
— disregard limit: any peak areas less than that of the principal peak in the chromatogram obtained from

standard solution (b).

Non-volatile matter Not more than 0.05 per cent w/w. Evaporate about 2 g, accurately weighed, to dryness on a water-bath and heat at 100° to 105° for 1 hour.

Assay Carry out the determination as described in the "Gas Chromatography" (Appendix 3.4).

Internal standard preparation and **Chromatographic system** Proceed as directed in the *Assay* under *Natural Camphor*.

Standard preparation Weigh accurately about 100 mg of Racemic Camphor RS, add 5.0 mL of *Internal standard preparation*, dissolve in *absolute ethanol* and dilute to 100.0 mL with the same solvent.

Assay preparation Weigh accurately about 100 mg of Racemic Camphor, add 5.0 mL of *Internal standard solution*, dissolve in *absolute ethanol* and dilute to 100.0 mL with the same solvent.

Suitability requirements

RESOLUTION Not less than 7 between racemic camphor and methyl salicylate peaks.

RELATIVE STANDARD DEVIATIONS Chromatograph six injections of *Standard preparation*, and record peak areas. The analytical system is suitable for conducting this Assay if the relative standard deviation for the area ratios of racemic camphor to methyl salicylate is not more than 1.0 per cent.

Procedure Separately inject equal volumes (about 2 µL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms and measure the areas of racemic camphor and methyl salicylate peaks. Calculate the area ratios of racemic camphor to methyl salicylate peaks.

Calculation Calculate the content of $C_{10}H_{16}O$ in the portion of Racemic Camphor taken, using the declared content of $C_{10}H_{16}O$ in Racemic Camphor RS.

CAMPHOR SPIRIT

Category Counter-irritant; pharmaceutic aid; antipruritic.

Camphor Spirit contains, in each 100 mL, not less than 9.0 g and not more than 11.0 g of camphor ($C_{10}H_{16}O$).

Camphor Spirit may be prepared as follows.

Camphor, Racemic or Natural	100 g
Ethanol, sufficient to produce	1000 mL

Dissolve Camphor in 800 mL of Ethanol, and add sufficient Ethanol to bring to final volume. Filter, if necessary.

Strength available 10 per cent w/v.

Dose Adults: *Topical*, to the affected area, not more than three or four times a day.

Ethanol content 80.0 to 87.0 per cent v/v (Method II, Appendix 6.5).

Assay

Assay preparation Pipette 2.0 mL of Camphor Spirit to a suitable pressure bottle containing 50 mL of freshly prepared *dinitrophenylhydrazine TS*.

Procedure Close the pressure bottle, immerse it in a water-bath, and maintain at about 75° for 16 hours. Cool to room temperature, and transfer the contents to a beaker with the aid of 100 mL of 3M *sulfuric acid*. Allow to stand at room temperature for not less than 12 hours, transfer the precipitate to a tared filter crucible, and wash with 100 mL of 3M *sulfuric acid* followed by 75 mL of cold water in divided portions. Continue the suction until the excess water is removed, dry the crucible and precipitate at 80° for 2 hours, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of camphor ($C_{10}H_{16}O$) in the portion taken.

Other requirements Complies with the requirements described under "Spirits" (Appendix 1.16).

CAMPHOR WATER, CONCENTRATED

Category Counter-irritant; rubefacient.

Racemic Camphor	40 g
Ethanol (90 Per Cent)	600 mL
Purified Water, sufficient to produce	1000 mL

Dissolve Racemic Camphor in Ethanol (90 Per Cent) and add, gradually, with vigorous shaking after each addition, sufficient Purified Water to produce 1000 mL.

Strength available 4 per cent w/v.

Dose Adults: *Topical*, to the affected area, not more than three or four times a day.

Ethanol content 51 to 55 per cent v/v (Method II, Appendix 6.5).

Other requirements Complies with the requirements described under "Aromatic Waters" (Appendix 1.16).

CATECHU, PALE

Gambier; Gambir

Thai name สีเสียดเทศ (SISIAT THET)

Category Intestinal astringent.

Pale Catechu is a dried aqueous extract prepared from the leaves and young shoots of *Uncaria gambier* (Hunter) Roxb. (Family Rubiaceae).

Constituents Pale Catechu contains polyphenolic

compounds mainly tannins and catechins. It also contains indole alkaloids such as gambirine, flavonoids and gambirtannin.

Description Odourless; taste, bitter and very astringent at first with sweetish aftertaste.

Macroscopical Usually occurs as cubes or brick-shaped pieces, about 2.5 cm long and wide, the brick-shaped ones up to 4 cm long, externally dull pale greyish brown to dark reddish brown, internally pale brown, friable and porous.

Microscopical Powdered drug of Pale Catechu shows yellowish brown masses of acicular catechin crystals, unicellular covering trichomes, epidermal cells of the leaves, corolla fragments, parenchyma, pollen grains, and fragments of cork. Acicular catechin crystals abundant. Unicellular covering trichomes lignified-walled, pitted at base, some with one or two thin transverse septa; fewer, smaller trichomes conical, with warty, unlignified walls. Epidermal cells of the leaves thin-walled with finely striated cuticle and paracytic stomata on the lower epidermis. Corolla fragment reddish brown with numerous covering trichomes and characteristic pitted and lignified cicatrices in the epidermis. Parenchyma containing calcium oxalate as cluster crystals and crystal sand. Pollen grain subspherical with three pores, three furrows and minutely pitted exine. Fragment of cork occasionally found.

Other relevant information The plant yielding pale catechu is native to Indonesia and Malaysia.

Packaging and storage Pale Catechu shall be kept in well-closed containers, protected from light and stored in a dry place.

Identification

A. To 200 mg of the powdered drug add 10 mL of *water*, warm in water-bath for 5 minutes with occasional shaking, and filter. To the filtrate, after cooling, add 2 to 3 drops of *gelatin TS*: a white turbidity or precipitate is produced.

B. Shake 100 mg of the powdered drug with 20 mL of *ethanol (50 per cent)* for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of *ethanol (50 per cent)*, and to the solution add 1 mL of *vanillin-hydrochloric acid TS*: a light red to red-brown colour develops.

C. Warm 300 mg of the powdered drug with 2 mL of *ethanol*, cool, and filter; to the filtrate add 2 mL of 5 M *sodium hydroxide*; shake, add 2 mL of *petroleum ether (boiling range, 40° to 60°)*, shake and allow to separate: a brilliant greenish fluorescence is produced in the upper layer.

Ethanol-insoluble matter Not more than 34.0 per cent w/w, calculated on the dried basis, determined by the following method. Macerate 5 g, accurately weighed, in *coarse powder*, with 100 mL of *ethanol*, shaking frequently during 6 hours and allowing to stand for 18 hours; filter, wash the residue with *ethanol*, and dry to constant weight at 100°.

Starch The residue obtained in the test for *Ethanol-insoluble matter* contains not more than an occasional starch granule.

Water-insoluble matter Not more than 33.0 per cent w/w, calculated on the dried basis, determined by the method for *Ethanol-insoluble matter*, using *water* in place of *ethanol*.

Loss on drying Not more than 15.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 8.0 per cent w/w (Appendix 7.7).

CHAMOMILE FLOWERS

Camomile Flowers; Chamomile; German Chamomile Flowers

Thai name ดอกหญ้าหอม (DOK YAA HOM)

Category Anti-inflammatory.

Chamomile Flowers consist of the dried flower heads of *Matricaria chamomilla* L. (*Matricaria recutita* L., *Chamomilla recutita* (L.) Rauschert) (Family Asteraceae alt. Compositae). They contain not less than 0.4 per cent v/w of blue volatile oil.

Origin of plant The plant of chamomile flowers is native to northern Europe and grows wildly in central European countries, especially abundant in eastern Europe. It is also found in western Asia, the Mediterranean region of northern Africa, and the United States of America.

Constitutents Chamomile flowers contain the blue volatile oils which yield terpenoids, *a*-bisabolol (levomenol) and its oxides, and azulenes including chamazulene and guaiazulene. They also contain matricin, en-yne-dicycloether, coumarins, and flavonoid glucosides such as apigenin-7-glucoside.

Description Odour, strong, pleasant, characteristic; taste, bitter and aromatic.

Macroscopical Flower head hemispherical, about 6 mm in diameter, consisting of a few ray florets and numerous disk florets on a receptacle with surrounding involucre. Involucre 2 to 3 rows of imbricated bracts; bract lanceolate, with blunt apex and scarious whitish

edges, green, glabrous. Ray floret comprising 10 to 20 pistils, caducous; corolla about 6 mm long, about 2 mm wide, 3-toothed, traversed by 4 major veins, white becoming darken, ligulate. Disk floret perfect, about 2 mm long, yellow; corolla tubular, 5-toothed; stamens 5, epipetalous, syngenesious. Receptacle hemispherical when young, becoming conical in old flower head, hollowed, 3 to 10 mm in diameter; paleae absent. Fruit, if found, achene, ovoid, 3- to 5-longitudinally ribbed.

Microscopical

Involucral bract Outer, abaxial epidermis, a single layer of radially elongated cells; central part composed of chlorophyll tissue covered by elongated epidermal cells with sinuous lateral walls, stomata and secretory trichomes. Vascular bundle surrounded by numerous elongated, pitted sclereids with fairly large lumens.

Ligulate and tubular corollas Surface view shows isodiametric or elongated cells with wavy walls and a few glandular trichomes.

Ligulate florets Outer epidermis comprising papillary cells with striations radiating from the apex. Mesophyll containing very small clusters of calcium oxalate crystals; four major veins longitudinally running through the entire mesophyll, occasionally accompanied by 1 or 2 other shorter veins running in parallel to the major veins; each of the two median veins splitting into two near the apex and, with the lateral veins, anastomosing two by two to form 3 arcs at the three major terminal teeth of the ligule. Ovary oval to globose, bearing a sclerous ring consisting of a layer of cells at the base; epidermis of the ovary composed of elongated cells with sinuous walls bearing secretory trichomes between the walls; numerous, very small clusters of calcium oxalate are found. Stigmata bearing papillose epidermal cells at the ends. Pollen grains rounded and triangular, about 30 µm in diameter, with 3 germinal pores and spiny exile.

Packaging and storage Chamomile Flowers shall be kept in well-closed containers, protected from light and stored at a temperature not exceeding 25°.

Identification

A. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution A solution containing 1.0 mg per mL of *borneol*, 2.0 mg per mL of *bornyl acetate*, and 400 µg per mL of *guaiazulene* in *toluene*.

Test solution Reduce 1.0 g of the sample to a *course powder*, using a porcelain pestle and mortar. Transfer to a 1.5-cm × 15-cm chromatographic column, and tamp lightly with a short length of rubber hose. Rinse the pestle and mortar twice, each time with 10 mL of *dichloromethane*. Pour the rinsings into the column. Collect the percolate in a flask with a long, narrow neck, and remove the solvent by evaporation on a water-bath. Dissolve the residue in 0.5 mL of *toluene*.

Adsorbent *Silica gel GF254*

Mobile phase *Chloroform*

Application Apply 10 µL of each solution, as 20-mm bands.

Development and drying Develop the plate and dry in air.

Detection Examine the plate under ultraviolet light (254 nm). Spray the plate with *anisaldehyde TS*, heat at 105° for 5 to 10 minutes and examine under visible light. Observe the result.

Results When examined under ultraviolet light (254 nm), the test solution shows a largest quenching band, due to en-yne-dicycloether, corresponding in R_f to the band due to bornyl acetate in the standard solution; a quenching band due to matricin near the line of application. Several quenching bands are also observed.

When examined under visible light, the standard solution shows a brownish yellow band, that becomes violet-grey after a few hours, due to borneol in the lower third; a yellowish brown to grey band due to bornyl acetate in the middle; and a deep red band with a blue edge due to guaiazulene in the upper third of the chromatogram. The test solution shows a blue band due to matricin near the line of application; one violet-red band due to bisabolol, at R_f value between those of borneol and bornyl acetate bands; red bands of terpenes at R_f values similar to that of the guaizulene band obtained from the standard solution. Other violet-red bands are also present.

B. Dissolve 250 mg of *4-dimethylaminobenzal-dehyde* in a mixture of 5 mL of *phosphoric acid*, 45 mL of *acetic acid*, and 45 mL of *water*. Transfer 2.5 mL of this solution and 0.1 mL of *Test solution*, to a test tube. Heat on a water-bath for 2 minutes, and allow to cool. Add 5 mL of *solvent hexane*, and shake: the aqueous layer has a distinct greenish blue or blue colour.

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Total ash Not more than 13.0 per cent w/w (Appendix 7.7).

Apigenin-7-glucoside content Not less than 0.3 per cent w/w. Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Mobile phase:

Dilute phosphoric acid Mix 5.0 mL of *phosphoric acid* in 50 mL of *water*. Dilute with *water* to 100 mL.

Mobile phase A Prepare 0.005 M *potassium dihydrogenphosphate*. Adjust with *Dilute phosphoric acid* to

a pH of 2.55±0.05.

Mobile phase B *Acetonitrile* and *methanol* (13:7)

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0	74	26
3	74	26
22	15	85
27	74	26
30	74	26

Standard solution A solution of 25.0 µg per mL of Apigenin-7-glucoside RS and 10.0 µg per mL of *7-methoxycoumarin* in *methanol* and *water* (1:1).

Test solution Transfer about 1 g, accurately weighed, to a suitable flask fitted with a reflux condenser and a stirrer. Add 80.0 mL of *methanol*, and reflux the mixture with stirring for 1 hour. Cool the flask to room temperature, pass the extract through a folded filter paper, and collect the filtrate in a 100-mL volumetric flask. Rinse the flask with 3 mL of *methanol*, pour the methanolic rinsings through the filter paper, and add the filtrate to the volumetric flask. Dilute with *methanol* to volume, and mix. Transfer 25.0 mL of the filtered solution to a round-bottomed flask fitted with a reflux condenser and a stirrer. Add 5.0 mL of *sodium hydroxide TS* and reflux the mixture for 25 minutes. Cool the flask and adjust the solution with *hydrochloric acid* to a pH of 5.0 to 6.2. Quantitatively transfer the solution to a 50-mL volumetric flask, dilute with *methanol* to volume and filter, discarding the first 10 mL of the filtrate.

Chromatographic system

DETECTOR Ultraviolet light (335 nm)

COLUMN A stainless steel column (12.5 cm × 4.6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm).

COLUMN TEMPERATURE 43°±1°

FLOW RATE 1.0 mL per minute. (**Note** Make adjustments, if necessary, to obtain relative retention times of 0.63 for apigenin-7-glucoside and 1.0 for 7-methoxycoumarin.)

System suitability

SAMPLE *Standard solution* (Note The relative retention times for apigenin-7-glucoside, 7-methoxycoumarin, apigenin, *trans*-spiroether, and *cis*-spiroether are about 0.63, 1.0, 1.2, 1.6, and 1.8, respectively.)

Suitability requirements

RESOLUTION Not less than 3.5 between apigenin-7-glucoside and 7-methoxycoumarin.

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for apigenin-7-glucoside.

Procedure Separately inject equal volumes (about 15 µL) of *Standard solution* and *Test solution* into the chromatograph and measure the responses for the major peaks. (**Note** Allow the test solution to elute for not less than 6 times the retention time of apigenin-7-glucoside.

Calculation Calculate the content of $C_{21}H_{20}O_{10}$ in the portion of the Chamomile Flowers taken, using the declared content of $C_{21}H_{20}O_{10}$ in Apigenin-7-glucoside RS.

Bisabolane derivatives content Not less than 0.15 per cent w/w. Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution A solution containing 1 mg per mL of Levomenol RS in *cyclohexane*.

Test solution Transfer the volatile oils obtained in the Assay to a 25-mL volumetric flask, rinse the graduated tube of the apparatus with a small portion of *cyclohexane*, transfer the rinsing to the 25-mL volumetric flask, add *cyclohexane* to volume, and mix.

Chromatographic system

DETECTOR Flame ionization

COLUMN A fused-silica capillary column (30 m × 0.32 mm), coated with a 0.25-µm film of polyethylene glycol compound (average molecular weight about 15,000)

TEMPERATURE

Column Initial Temperature 70°
Temperature Ramp 4° per minute
Final Temperature 230°
Hold Time at Final Temperature 10 minutes

Detector 250°

Injection port 220°

CARRIER GAS *Helium*

FLOW RATE 1.0 mL per minute

System suitability

SAMPLE *Standard solution*

Suitability requirements

SYMMETRY FACTOR Not more than 1.8 for levomenol

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent determined from the chamomile peak

Procedure Separately inject equal volumes (about 1 µL) of *Standard solution* and *Test solution* into the chromatograph and measure the responses for the major peaks. Identify the peaks due to levomenol, bisabolol oxide B, bisabolol oxide, and bisabolol oxide A in the test solution, using the retention time of levomenol in the standard solution and the approximate relative retention times of 0.89, 0.97, and 1.1 for bisabolol oxide B, bisabolol oxide, and bisabolol oxide A, respectively, with reference to the levomenol (bisabolol) peak.

Calculation Calculate the content of bisabolane derivatives (bisabolol oxide B, bisabolol oxide, and bisabolol oxide A) in the portion of the Chamomile

Flowers taken, calculated as levomenol.

Assay Carry out the "Determination of Volatile Oil" (Appendix 7.3). Use 60 g, in *coarse powder*, freshly prepared and accurately weighed. Use 300 mL of *water* as the distillation liquid and a 2-L round-bottomed flask. Distil at a rate of 3 to 4 mL per minute for 5 hours. Use 0.5 mL of *xylene* in the graduated tube. Calculate the content of volatile oil, in mL, in the portion of the Chamomile Flowers taken. (**Note** Retain the volatile oils for use in the test for *Bisabolane derivatives content*.)

CINCHONA BARK

Cinchona; Peruvian Bark; Red Cinchona Bark

Thai name เปลือกซินโคนา (PLUEAK CINCHONA)

Category Antimalarial and bitter.

Cinchona Bark consists of the dried bark of *Cinchona pubescens* Vahl (*C. succirubra* Pav. ex Klotzsch), or of its varieties or its hybrids (Family Rubiaceae). It contains not less than 6.5 per cent of total alkaloids, of which 30 to 60 per cent are alkaloids of the quinine-type, calculated on the dried basis.

Origin of plant Cinchona is native to Central America and South America, but hybrids are cultivated in India, Africa and the Far East.

Constituents Cinchona Bark contains the quinine-type alkaloids, of which quinine and quinidine are its major components. It also contains the cinchonine-type alkaloids, quinic acid and quinovose.

Description Odour, mild, characteristic; taste, intensely bitter and somewhat astringent.

Macroscopical Quilled or curved pieces of dried stems or barks, 2 to 6 mm thick; externally usually rough with transverse fissures and longitudinal furrows or wrinkles, dull brownish grey or grey and frequently with lichens; some varieties experience exfoliation of the outer surface; internally deep reddish brown and striated; the external fracture is short and the inner fracture is fibrous.

Microscopical Transverse section of the cork shows thin-walled cells with red brown contents, cortex, phloem, medullary rays, and sclereids. Cortex, narrow, consisting of tangentially elongated pitted cells containing starch granules or amorphous red-brown matter, with scattered idioblasts containing microprisms of calcium oxalate and large secretory cells. Phloem: narrow sieve tubes, transverse sieve plates and phloem parenchyma, and large characteristic spindle-shaped phloem fibres occurring in isolation or in irregular radial rows. Medullary rays, thin-walled, elongated cells radially arranged. Sclereids, very rare.

Powdered drug of Cinchona Bark possesses the microscopical characteristic of the unground drug. Starch granules, mostly simple but occasionally with 2 or 3 components, free or included in parenchyma cells.

Other relevant information

It is contra-indicated in pregnant women.

It is incompatible with iodides and salicylates.

Packaging and storage Cinchona Bark shall be kept in well-closed containers, protected from light, and stored in a dry place.

Identification

A. Shake 100 mg for 1 minute with 5 mL of 1 M *sulfuric acid* and filter. To 1 mL of the filtrate add 0.2 mL of *mercuric-potassium iodide TS*. A precipitate is formed. Dilute the remainder of the filtrate to 10 mL with *water*. The resulting solution, when examined under ultraviolet light (366 nm) shows a blue fluorescence, which disappears on the addition of *hydrochloric acid*.

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 17.5 mg of *quinine*, 2.5 mg of *quinidine*, 10 mg of *cinchonine* and 10 mg of *cinchonidine* in 5 mL of *anhydrous ethanol*.

Test solution To 100 mg, in *fine powder*, add 0.1 mL of *strong ammonia solution* and 5 mL of *dichloromethane*, shake for 30 minutes and filter. Evaporate the filtrate to dryness on water-bath and dissolve the residue in 1 mL of *anhydrous ethanol*.

Adsorbent *Silica gel G*, use a typical plate (20×20 cm).

Mobile phase *Diethylamine*, *ethyl acetate*, and *toluene* (10:20:70).

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Twice over the distance of 15 cm. Dry the developed plate at 105°, and then allow to cool.

Detection A Spray the plate with *anhydrous formic acid*, allow to dry in air and examine under ultraviolet light (366 nm).

Results A When examined under ultraviolet light (366 nm), the test solution shows a blue fluorescent band due to quinine in the lower third and a blue fluorescent band due to quinidine around the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution.

Detection B Spray the plate with *iodoplatinate TS*.

Results B The test solution shows a violet band that becomes violet grey due to quinine in the lower third, an intense dark blue band, and two violet bands that becomes violet grey due to cinchonidine, quinidine and cinchonine, respectively around the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° for 2 hours (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Assay

Standard quinine preparation Dissolve about 30 mg of *quinine*, accurately weighed, in 0.1 M *hydrochloric acid* and dilute to 1000.0 mL with 0.1 M *hydrochloric acid*.

Standard cinchonine preparation Dissolve about 30 mg of *cinchonine*, accurately weighed, in 0.1 M *hydrochloric acid* and dilute to 1000.0 mL with 0.1 M *hydrochloric acid*.

Assay preparation Mix about 1 g, in *fine powder* and accurately weighed, with 10 mL of *water* and 7 mL of *dilute hydrochloric acid* in a 250 mL conical flask. Heat in a water-bath for 30 minutes, allow to cool. Add 25 mL of *dichloromethane*, 50 mL of *ether* and 5 mL of a 20 per cent w/v solution of *sodium hydroxide*. Shake for 30 minutes and add 3 g of *tragacanth* and shake until the mixture becomes clear. Filter through a plug of *absorbent cotton* and rinse the flask with five 20-mL portions of a mixture of 1 volume of *dichloromethane* and 2 volumes of *ether*. Combine the filtrate and washings, evaporate the filtrate to dryness and dissolve the residue in 10.0 mL of *anhydrous ethanol*. Evaporate 5.0 mL of this solution to dryness, dissolve the residue in 0.1 M *hydrochloric acid* and dilute to 1000.0 mL with 0.1 M *hydrochloric acid*.

Procedure Measure the absorbances of *Standard quinine preparation*, *Standard cinchonine preparation* and *Assay preparation* at the maximum at about 316 nm and 348 nm, using 0.1 M *hydrochloric acid* as the blank (Appendix 2.2).

Calculation Calculate the percentage content of alkaloids, in the portion of Cinchona Bark taken by the expression:

$$x = \frac{(A_{316} \times A_{348c}) - (A_{316c} \times A_{348})}{(A_{316q} \times A_{348c}) - (A_{316c} \times A_{348q})} \times \frac{100}{w} \times \frac{2}{1000}$$

$$y = \frac{(A_{316} \times A_{348q}) - (A_{316q} \times A_{348})}{(A_{316c} \times A_{348q}) - (A_{316q} \times A_{348c})} \times \frac{100}{w} \times \frac{2}{1000}$$

where x = percentage content of quinine-type alkaloids,

y = percentage content of cinchonine-type alkaloids,

w = the weight, in g, of Cinchona Bark in *Assay preparation*,

A_{316} and A_{348} = the absorbances of *Assay preparation* at 316 and 348 nm, respectively,

A_{316q} and A_{348q} = the absorbances of *Standard quinine preparation* at 316 and 348 nm, respectively, corrected to a concentration of 1 µg per mL,

A_{316c} and A_{348c} = the absorbances of *Standard cinchonine preparation* at 316 and 348 nm, respectively, corrected to a concentration of 1 µg per mL.

Calculate the content, of total alkaloids ($x+y$), and calculate the relative content of quinine-type alkaloids, using the expression:

$$\frac{100x}{x+y}.$$

COLOPHONY

Rosin

Thai name ชันสน (CHAN SON)

Category Pharmaceutic necessity for certain plaster masses and collodions.

Colophony is the residue left after distilling the volatile oil from the oleo-resin obtained from *Pinus palustris* Mill., *P. merkusii* Jungh. & de Vriese and other species of *Pinus* (Family Pinaceae).

Origin of plant Colophony-yielding plants are native to Asia, Europe and North America.

Constituents Colophony contains about 90 per cent of resin acids or isomeric diterpene acids.

Description Translucent, pale yellow to brownish yellow, with conchoidal markings on the surface, angular, irregularly-shaped, glassy pieces of different sizes, brittle. Odour, mild, pine-like.

Other relevant information Caution should be exercised when handling colophony as it may cause contact dermatitis.

Packaging and storage Colophony shall be kept in well-closed containers, preferably in the unground state.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 mg of *thymol*, and 10 mg of *linalool* in 10 mL of *methanol*.

Test solution Dissolve 1 g in 10 mL of *methanol* by gently warming.

Adsorbent *Silica gel G*, use a typical plate (20×20 cm).

Mobile phase *Dichloromethane*.

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate in air.

Detection Spray the plate with *anisaldehyde TS*, heat at 105° for 10 minutes and examine under visible light. Observe the result.

Results When examined under visible light, the test solution shows a purple band of linalool in the lower third of the chromatogram, corresponding in colour and R_f to the band shown by the standard solution. Several purple bands around the middle and in the upper third of the chromatogram also appear.

Total ash Not more than 0.2 per cent w/w (Appendix 7.7).

Acid value 145 to 180; use 1 g (Appendix 5.4).

CORIANDER

Coriander-seed; Coliander; Coriander Fruit

Thai name ลูกผักชี (LUK PHAK CHI)

Category Carminative; pharmaceutic acid.

Coriander is the dried cremocarp of *Coriandrum sativum* L. (Family Umbelliferae). It yields not less than 0.3 mL of volatile oil from each 100 g of drug, calculated on the dried basis.

Origin of plant Coriander is native to the Middle East and the Mediterranean region, typically Italy.

Constituents Coriander contains a volatile oil, consisting mainly of linalool. It also contains flavonoids, coumarins, etc.

Description Odour of the crushed cremocarp, aromatic; taste, spicy, pungent and characteristic.

Macroscopical Entire cremocarp usually with tightly connected mericarps, globose, subglobose or oval, 2 to 6 mm long, 1.5 to 5 mm in diameter, brown or light brown, glabrous with 10 wavy, slightly raised primary ridges and 8 straight, more prominent secondary ridges; mericarp concave on the internal surface; stylopod crowning the apex and a small fragment of pedicel may be present.

Microscopical Powdered drug of Coriander shows oil droplets, fragments of endosperm, fragments of sclerenchymatous layer of mesocarp, fragments of parenchyma of mesocarp, fragments of epicarp in surface view, fragments of secretory cells with brown cells, and fragments of vascular bundles. Oil droplets, numerous. Fragment of endosperm: small, thick-walled cells containing microrosettes and microcrystals of calcium oxalate, and oil droplets. Fragment of endocarp: very narrow cells parquetry-like arranged and usually associated with a layer of thin-walled or thick-walled rectangular sclereids of mesocarp. Fragments of sclerenchymatous layer of mesocarp: short, strongly thickened, pitted, fusiform cells occurring in layers with the cells of adjacent layers approximately at right angles to one another. Fragments of parenchyma of mesocarp: small, slightly thick-walled cells with the remnants of secretory canals and sclereids. Fragments of epicarp in surface view: thin-walled polyhedral cells, some containing small prismatic crystals of calcium oxalate. Fragments of secretory canals with brown cells, rarely present. Fragments of vascular bundles, occasionally found.

Packaging and Storage Coriander shall be kept in tightly closed containers and stored in a dry place at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 µL of *linalool*, and 2 µL of *geranyl acetate* in 1.0 mL of *toluene*.

Test solution To 500 mg, in *No. 355 powder*, add 5.0 mL of *methanol*. Sonicate for 10 minutes, filter or centrifuge and use the filtrate or supernatant.

Adsorbent *Silica gel F254* (TLC or HPTLC plate).

Mobile phase *Ethyl acetate* and *toluene* (5:95).

Application Apply 10 µL (or 2 µL) each of *Standard solution* and *Test solution* as 15-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 10 cm (or 6 cm) above the line of application. Dry the developed plate in air for 5 minutes.

Detection Spray the plate with *anisaldehyde TS*, heat at 105° for 5 minutes and examine under visible light. Observe the result.

Results When examined under visible light, the test solution shows a violet band of linalool in the lower third, corresponding in colour and R_f to the band shown by the standard solution. A violet-blue band near the starting line and a bluish violet band in the upper third of the chromatogram are present.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° for 2 hours; use 1 g, in *No. 355 powder* (Appendix 4.15).

Foreign matter None of the cremocarps show perforations due to insects (Appendix 7.2).

Total ash Not more than 8.0 per cent w/w (Appendix 7.7).

Assay Carry out the method for the "Determination of Volatile Oil" (Appendix 7.3), using 30 g, in *coarse powder*, freshly prepared and accurately weighed. Use 200 mL of *water* as the distillation liquid and a 500-mL round-bottomed flask. Distil at a rate of 2 to 3 mL per minute for 2 hours. Use 0.5 mL of *xylene* in the graduated tube.

CORIANDER, POWDERED

Complies with the requirements for Foreign matter and Total ash stated under Coriander and with the following requirements.

It yields not less than 0.2 mL of volatile oil from each 100 g of drug.

Description Fawn to brown. Diagnostic structures: epidermal cells of the pericarp, if present, crossing layers of fusiform sclerenchymatous cells and thin-walled lignified cells of the mesocarp; inner epidermis; fragments of brown epithelium of vittae; endosperm containing fixed oil and aleurone grains containing calcium oxalate. Trichomes and starch absent.

Packaging and storage Powdered Coriander shall be kept in tightly closed containers and stored at a temperature not exceeding 25°.

Assay Carry out the Assay described under *Coriander*.

CORIANDER OIL

Thai name น้ำมันลูกผักชี (NAM MAN LUK PHAK CHI)

Category Carminative; pharmaceutic aid.

Coriander Oil is the volatile oil distilled with steam from the dried ripe fruit of *Coriandrum sativum* L. (Family Umbelliferae).

Constituents Coriander Oil contains predominantly monoterpene alcohols as linalool. It also contains terpenes (e.g., α-pinene, limonene, γ-terpinene), and camphor.

Description Clear colourless or pale yellow liquid; odourless.

Solubility test Soluble, at 20°, in 3 volumes of *ethanol (70 per cent)*.

Packaging and storage Coriander Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification

Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 µL of *linalool*, and 2 µL of *geranyl acetate* in 1.0 mL of *toluene*.

Test solution Dissolve 10 µL in *toluene* and dilute to 1.0 mL with the same solvent.

Adsorbent *Silica gel F254* (TLC or HPLC plate).

Mobile phase *Ethyl acetate* and *toluene* (5:95).

Application Apply 10 µL (or 2 µL) each of *Standard solution* and *Test solution* as 15-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 10 cm (or 6 cm) above the line of application. Dry the developed plate in air for 5 minutes.

Detection Spray the plate with *anisaldehyde TS*, heat at 105° for 5 minutes and examine under visible light. Observe the result.

Results When examined under visible light, the test solution shows a violet band due to linalool in the lower third, corresponding in colour and R_f to the band shown by the standard solution. A violet-blue band near the starting line and a bluish violet band in the upper third of the chromatogram is present.

The retention time of the major peaks in the chromatogram of the Coriander Oil correspond to those in the chromatogram of the Standard solution (a), as obtained in the *Chromatographic profile*.

Specific gravity 0.860 to 0.880 (Appendix 4.9).

Optical rotation + 7° to + 13° (Appendix 4.8).

Refractive index 1.462 to 1.470, at 20° (Appendix 4.7).

Acid value Not more than 3.0; use 5 g (Appendix 5.4)

Chromatographic profile Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution (a) Dissolve 10 µL of *α-pinene*, 10 µL of *limonene*, 10 µL of *γ-terpinene*, 10 µL of *p-cymene*, 10 mg of *camphor*, 20 µL of *linalool*, 10 µL of *α-terpineol*, 10 µL of *geranyl acetate* and 10 µL of *geraniol* in 1 mL of *heptane*.

Standard solution (b) Dissolve 5 µL of *geraniol* in *heptane* and dilute to 10.0 mL with the same solvent.

Test solution Use Coriander oil.

Chromatographic system

DETECTOR Flame ionization

COLUMN A fused-silica capillary column (60 m × 0.25 mm) packed with *macrogol 20000* (0.25 µm).

TEMPERATURE

Column The temperature programme is as follows:

Time (Minutes)	Temperature (°)
0-10	60
10-75	60 → 190
75-120	190

Injection port 220°

Detector 240°

CARRIER GAS *Helium*

SPLIT RATIO 1:65

FLOW RATE 1 mL per minute

System suitability

SAMPLE *Standard solution (a)*

Suitability requirements

RESOLUTION Not less than 1.5 between linalool and camphor peaks.

Procedure Inject a portion (about 0.2 mL) of *Standard*

solution (a), *Standard solution (b)* and *Test solution* into the chromatograph. Identify the peaks due to the components in Coriander Oil, using the retention times determined from the chromatogram obtained from the standard solution (a). Measure the areas of the principal peaks in the chromatogram obtained from the Coriander Oil.

Calculation Calculate the percentage content of the components of Coriander Oil taken by normalization procedure.

Limits

– *α*-pinene: 3.0 per cent to 7.0 per cent.
– limonene: 1.5 per cent to 5.0 per cent.
– *γ*-terpinene: 1.5 per cent to 8.0 per cent.
– *p*-cymene: 0.5 per cent to 4.0 per cent.
– camphor: 3.0 per cent to 6.0 per cent.
– linalool: 65.0 per cent to 78.0 per cent.
– *α*-terpineol: 0.1 per cent to 1.5 per cent.
– geranyl acetate: 0.5 per cent to 4.0 per cent.
– geraniol: 0.5 per cent to 3.0 per cent.
– disregard limit: area of the peak in the chromatogram obtained from the standard solution (b) (0.05 per cent).

Chiral purity Not more than 14 per cent of (*R*)-linalool. Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution Dissolve 10 µL of *linalool* and 5 mg of *borneol* in *pentane* and dilute to 10.0 mL with the same solvent.

Test solution Dissolve 20 mg of Coriander Oil in *pentane* and dilute to 10.0 mL with the same solvent.

Chromatographic system

DETECTOR Flame ionization

COLUMN A fused-silica capillary column (25 m × 0.25 mm) packed with *modified b-cyclodextrin for chiral chromatography* (0.25 µm).

TEMPERATURE

Column The temperature programme is as follows:

Time (Minutes)	Temperature (°)
0-65	50 → 180

Injection port 230°
Detector 230°

CARRIER GAS *Helium*

SPLIT RATIO 1:30

FLOW RATE 1.3 mL per minute

System suitability

SAMPLE *Standard solution*

Suitability requirements

RESOLUTION Not less than 5.5 between(*R*)-linalool (first peak) and (*S*)-linalool (second peak) and not less than 2.9 between the peaks due to (*S*)-linalool and borneol (third peak).

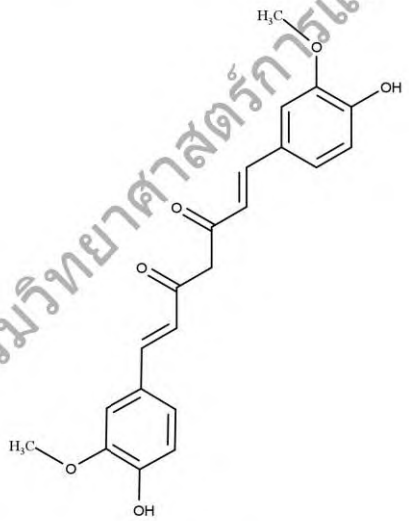
Procedure Separately inject equal volumes (about 1 µL) of *Standard solution* and *Test solution* into the chromatograph. Measure the responses for the major peaks.

Calculation Calculate the percentage content of (*R*)-linalool using the expression:

$$\frac{A_R}{A_S + A_R} \times 100$$

where A_S = area of the peak due to (*S*)-linalool;
A_R = area of the peak due to (*R*)-linalool.

CURCUMIN



$C_{21}H_{20}O_6$ 368.38 *458-37-7*

(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)- hepta-1,6-diene-3,5-dione.

Category Anti-inflammatory, antioxidant.

Curcumin is the curcuminoid extracted from the dried rhizomes of *Curcuma longa* L. (*C. domestica* Valeton) (Family Acanthaceae). It contains not less than 95.0 per cent w/w of $C_{21}H_{20}O_6$.

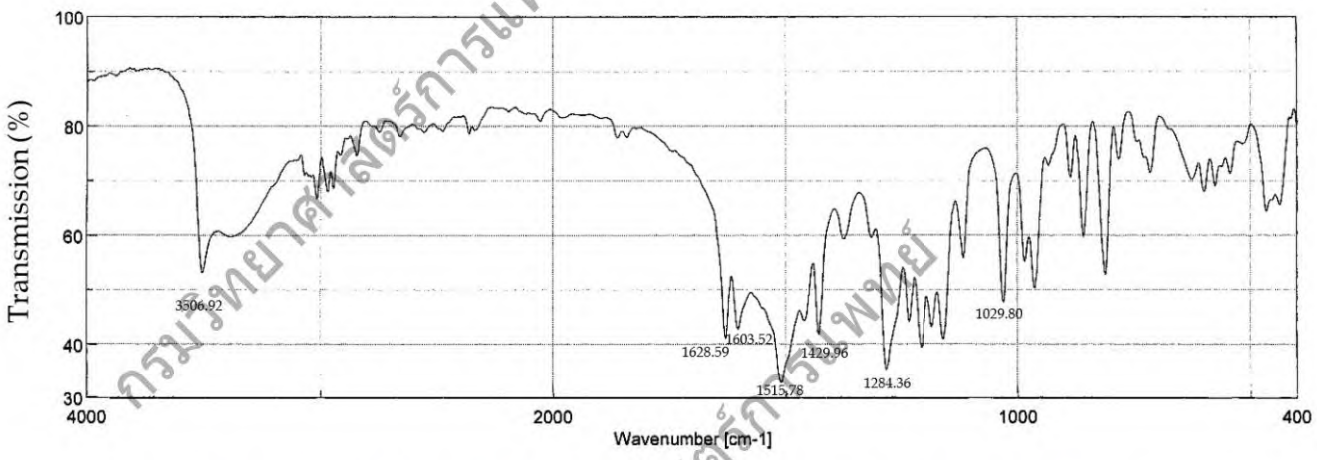
Description Orange-yellow needles.

Solubility Practically insoluble in *water* and soluble in alkalis.

Packaging and storage Curcumin shall be kept in well-closed containers, protected from light and stored at a temperature of -20°.

Identification The infrared absorption spectrum corresponds to the spectrum obtained from Curcumin RS (Fig. 1).

Melting temperature 179° to 183° (Appendix 4.3).



Frequency (cm^{-1})	Band and Vibration Mode	Functional Group
3506.92	OH stretching	Hydroxyl group
1628.59	C=O stretching	Carbonyl group
1603.52	C=C stretching	Alkenes
1515.78	C=C	Alkenes
1429.96	C-H	Alkanes
1284.36	C=O stretching	Ethers
1029.80	C-O-C stretching	Ethers

Fig. 1 Infrared Spectrum of Curcumin and Its Characteristic Peaks
(Fourier Transform Using Diffuse Reflectance Technique with Potassium Bromide as Window Material)

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5). (**Note** Filter *Standard preparations (a), (b)*, *Assay preparation* and *Mobile phases* through a membrane having a 0.22-µm porosity or finer before subjecting into the chromatograph.)

Standard stock preparation (a) A solution containing 400 µg of Curcumin RS per mL in *acetonitrile.*

Standard stock preparation (b) A solution containing 400 µg of Curcuminoids RS per mL in *acetonitrile.*

Standard preparation (a) Prepare a solution containing 40 µg of curcumin per mL by quantitatively diluting *Standard stock preparation (a)* with *Mobile phase.*

Standard preparation (b) Prepare a solution containing 40 µg of Curcuminoids per mL by quantitatively diluting *Standard stock preparation (b)* with *Mobile phase.*

Assay preparation Weigh accurately a suitable quantity of Curcumin, dissolve in and dilute with *acetonitrile* to obtain a solution containing 400 µg per mL of curcumin. Dilute this solution quantitatively with *Mobile phase* to have a solution of a known concentration of about 40 µg per mL of curcumin.

Mobile Phase A 0.1 per cent v/v solution of *formic acid* and *acetonitrile for chromatography* (60:40).

Chromatographic system

DETECTOR Ultraviolet light (420 nm)

COLUMN A stainless steel column (10 cm × 2.1 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (1.7 µm). (Waters-Acquity® or equivalent is suitable)

COLUMN TEMPERATURE 40°

FLOW RATE 0.5 mL per minute

System suitability

SAMPLE *Standard preparation (a)* and *Standard preparation (b).*

SUITABILITY REQUIREMENTS The chromatogram of the standard preparation (b) is concordant with the reference chromatogram (Fig. 2). The approximate relative retention times of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin are 1.00, 0.87 and 0.76 respectively, with reference to the curcumin peak.

COLUMN EFFICIENCY Not less than 5000 theoretical plates for the curcumin peak in the chromatogram of the standard preparation (a).

SYMMETRY FACTOR Not more than 1.5 for the curcumin peak in the chromatogram of the standard preparation (a).

RESOLUTION Not less than 2 between the curcumin and desmethoxycurcumin peaks in the chromatogram of the standard preparation (b).

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for curcumin in the chromatogram of the

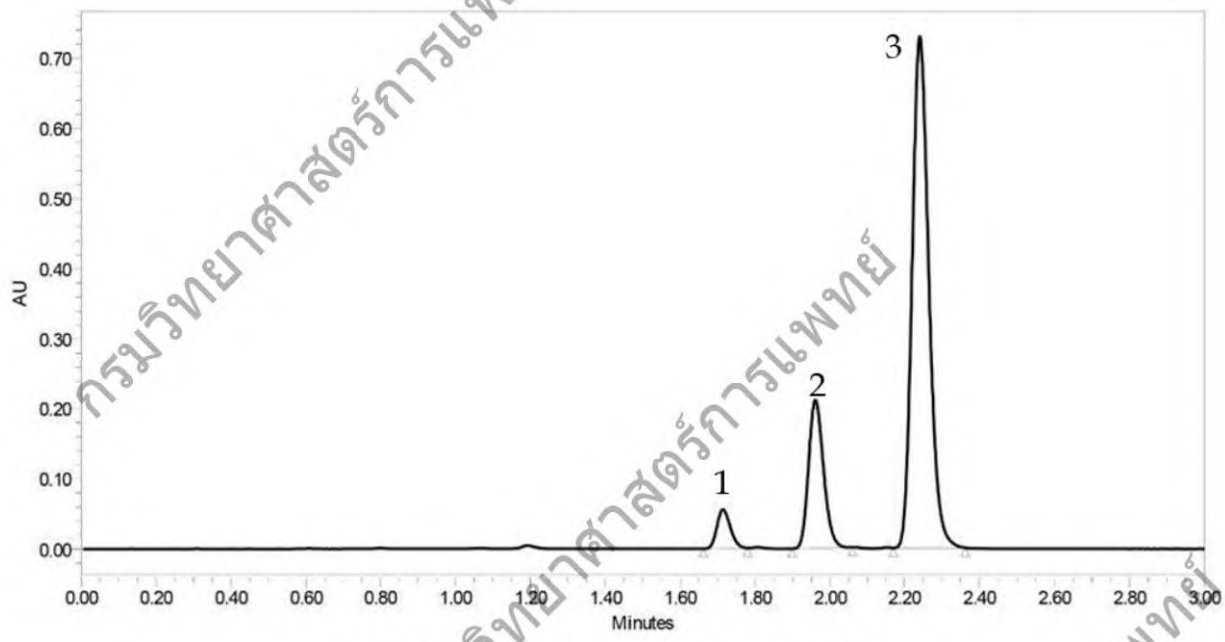


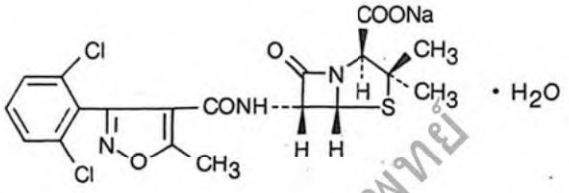
Fig. 2 Chromatogram of Curcuminoids RS
1. Bisdesmethoxycurcumin 2. Desmethoxycurcumin 3. Curcumin

standard preparation (a).

Procedure Separately inject equal volumes (about 2 µL) of *Standard preparation (a)* and *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the major peaks.

Calculation Calculate the content of $C_{21}H_{20}O_6$ in the portion of the Curcumin taken, using the declared content of $C_{21}H_{20}O_6$ in Curcumin RS.

DICLOXACILLIN SODIUM



$C_{19}H_{16}Cl_2N_3O_5S{\cdot}Na{\cdot}H_2O$ 510.32 *13412-64-1*

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolyl] carbonyl]amino]-3,3-dimethyl-7-oxo-, [2*S*-(2α,5α,6β)]-, monosodium salt, monohydrate.

Anhydrous 492.30 *343-55-5*

Category Antibacterial (of the penicillin class).

Dicloxacillin Sodium contains not less than 850 µg of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$, per mg.

Description White to off-white, crystalline powder.

Solubility Freely soluble in *water*.

Contra-indication It is contra-indicated in patients who have shown hypersensitivity to any member of the penicillins or of the cephalosporins or other related allergens.

Warning

1. Hypersensitivity reactions are the most common adverse effects noted with the penicillins. Serious and occasionally fatal hypersensitivity (anaphylactoid) reactions may occur, regardless of the route of administration or the dose.

2. Serious superinfections with resistant organisms, especially gram-negative bacteria (e.g., *Pseudomonas* and *Proteus*) and *Candida*, may occur following long-term therapy with penicillins.

3. Massive doses of penicillins may cause hematologic abnormalities (including anemia, thrombocytopenia, leukopenia, neutropenia, and eosinophilia).

4. *Clostridium difficile* colitis may develop in some patients.

5. Concurrent use with any bacteriostatic drug (e.g., tetracyclines, chloramphenicol, etc.) is not recommended for the treatment of meningitis or other situations where a rapid bactericidal effect is necessary.

Precaution Periodic assessment of renal, hepatic, and hematopoietic systems is recommended during therapy.

Additional information

1. Dicloxacillin Sodium should not be used in the treatment of meningitis since it penetrates poorly into cerebrospinal fluid.

2. Group A beta-hemolytic streptococcal infections should be treated for at least 10 days to help prevent the

development of acute rheumatic fever or acute glomerulonephritis.

Packaging and storage Dicloxacillin Sodium shall be kept in tightly closed containers and stored at a temperature not exceeding 25°.

Labelling The label on the container states (1) the expiration date; (2) the storage condition.

Identification

The infrared absorption spectrum is concordant with the spectrum obtained from Dicloxacillin Sodium RS (Appendix 2.1) or with the reference spectrum of Dicloxacillin Sodium.

The retention time of the major peak of the Assay preparation corresponds to that of the Standard preparation, as obtained in the *Assay*.

It yields the *reactions* characteristic of sodium salts (Appendix 5.1).

pH 4.5 to 7.5, in a 1.0 per cent w/v solution (Appendix 4.11).

Water Not less than 3.0 per cent w/w and not more than 5.0 per cent w/w (Karl Fischer Method, Appendix 4.12).

N,N-Dimethylaniline Not more than 20 ppm. (Appendix 5.16)

Organic impurities Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5). (**Note** Protect solutions containing dicloxacillin from light.

Diluent, System suitability stock solution, Mobile phase A, Mobile phase B, and **Chromatographic System** Proceed as directed in the *Assay*.

System suitability solution A solution containing 10 µg per mL of Dicloxacillin Related Compound D RS from *System suitability stock solution* and 1 mg per mL of Dicloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Standard solution A solution containing 10 µg per mL of Dicloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

Test solution Weigh accurately a suitable quantity of Dicloxacillin Sodium, dissolve in and dilute with *Diluent* to obtain a solution containing 1 mg per mL of dicloxacillin sodium. Sonitcate as needed to dissolve. Store this solution at 4°.

Mobile phase

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0	70	30
10	70	30
30	50	50
35	35	65
45	25	75

Return to the original conditions and re-equilibrate the system.

(**Note** The relative retention times for dicloxacillin and dicloxacillin related compound D are about 1.0 and 1.1, respectively.)

System Suitability

SAMPLE *System suitability solution* and *Standard solution*.

Suitability requirements

SYMMETRY FACTOR Between 0.8 and 1.5 for the dicloxacillin peak in the chromatogram of the standard solution.

RESOLUTION Not less than 1.5 between dicloxacillin related compound D and dicloxacillin peaks in the chromatogram of the system suitability solution.

RELATIVE STANDARD DEVIATION Not more than 2.5 per cent for the dicloxacillin peak in the chromatogram of the standard solution.

Procedure Separately inject equal volumes (about 10 mL) of *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculation Calculate the percentage of each impurity in the portion of the Dicloxacillin Sodium taken, with reference to the relative retention times and relative response factors as described in the following table using the expression:

$$(r_u/r_s) \times (C_s/C_u) \times P \times (F_1/F_2) \times 100$$

where r_u = peak response of each impurity from the Test solution

r_s = peak response from the Standard solution

C_s = concentration, in mg/mL, of Dicloxacillin Sodium RS in the Standard solution

C_u = concentration, in mg/mL, of Dicloxacillin Sodium in the Test solution

P = potency, in µg/mg, of dicloxacillin in Dicloxacillin Sodium RS

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see Table 1)

(**Note** The reporting threshold is 0.05 per cent).

Limits

— individual or any other impurities: not more than 1.0 per cent,

—total impurities: not more than 5.0 per cent.

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5). (**Note** Protect solutions containing dicloxacillin from light.

Diluent *Acetonitrile* and *water* (50:50).

Standard preparation A solution containing 100 µg per mL of Dicloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

System suitability stock solution A solution containing 100 µg per mL of Dicloxacillin Related Compound D in *Diluent*. Sonicate as needed to dissolve.

System suitability solution A solution containing 1 µg per mL of Dicloxacillin Related Compound D RS from *System suitability stock solution* and 100 µg per mL of Dicloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

Assay preparation Weigh accurately a suitable quantity of Dicloxacillin Sodium, dissolve in and dilute with *Diluent* to obtain a solution containing 100 µg per mL of dicloxacillin sodium. Sonicate as needed to dissolve. Store this solution at 4°.

Mobile phase

MOBILE PHASE A Prepare a solution containing 1.18 mg per mL of *sodium 1-hexanesulfonate monohydrate* and 0.8 µL per mL of *ammonium hydroxide*; adjusted with *phosphoric acid* to a pH of 2.9 to 3.1.

MOBILE PHASE B *Acetonitrile.*

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0	45	55
2	45	55
2.5	35	65
5	35	65

Return to the original conditions and re-equilibrate the system.

Chromatographic System

DETECTOR Ultraviolet light (225 nm).

COLUMN A stainless steel column (25 cm × 4.6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm).

TEMPERATURES

Autosampler 4°.

Column 40°.

FLOW RATE 1.5 mL per minute.

(**Note** The relative retention times for dicloxacillin and dicloxacillin related compound D are about 1.0 and 1.1, respectively.)

System Suitability

SAMPLE *System suitability solution* and *Standard preparation.*

Suitability requirements

SYMMETRY FACTOR Between 0.8 and 1.5 for the dicloxacillin peak in the chromatogram of the standard preparation.

RESOLUTION Not less than 1.5 between dicloxacillin and dicloxacillin related compound D peaks in the chromatogram of the system suitability solution.

Table 1 Relative retention times and relative response factors

Name	Relative Retention Time	Relative Response Factor
Dicloxacillin penicilloic acid[a,b]	0.23 0.25	1.0
Dicloxacillin N-acetyl penicilloic acid[c]	0.43	1.0
Dicloxacillin penilloic acid[a,d]	0.61 0.69	0.75
Dicloxacillin related compound D[e]	0.90	1.4
Dicloxacillin	1.0	-

[a]The system resolves two isomers.

[b](4*S*)-2-{Carboxy[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[c](2*R*,4*S*)-3-Acetyl-2-{(*R*)-carboxy[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[d](4*S*)-2-{[3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxamido]methyl}5,5-dimethylthiazolidine-4-carboxylic acid.

[e]3-(2,6-Dichlorophenyl)-5-methylisoxazole-4-carboxylic acid.

RELATIVE STANDARD DEVIATION Not more than 0.73 per cent for the dicloxacillin peak in the chromatogram of the standard preparation.

Procedure Separately inject equal volumes (about 10 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculation Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the portion of the Dicloxacillin Sodium taken, using the declare content of $C_{19}H_{17}Cl_2N_3O_5S$ in Dicloxacillin Sodium RS.

DICLOXACILLIN SODIUM FOR ORAL SUSPENSION

Category Antibacterial.

Dicloxacillin Sodium for Oral Suspension is a dry mixture of Dicloxacillin Sodium and one or more suitable buffers, colours, flavours, and preservatives. It contains the equivalent of not less than 90.0 per cent and not more than 120.0 per cent of the labelled amount of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$) when constituted as directed.

Strength available 62.5 mg (base) and 125 mg (base) per 5 mL.

Dose Dosage of Dicloxacillin Sodium for Oral Suspension is expressed in terms of dicloxacillin base.

Adults and children 40 kg of body weight and over: 125 to 500 mg every 6 hours.

Children up to 40 kg of body weight: 3.125 to 6.25 mg per kg of body weight every 6 hours.

Contra-indication; Warning; Precaution; Additional information See under *Dicloxacillin Sodium*.

Packaging and storage Dicloxacillin Sodium for Oral Suspension shall be kept in tightly closed containers and stored at a temperature not exceeding 30°.

Labelling The label on the container states (1) the quantity equivalent to the amount of dicloxacillin; (2) the expiration date.

Identification The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation, as obtained in the *Assay*.

pH 4.5 to 7.5, in the suspension constituted as directed in the labelling (Appendix 4.11).

Water Not more than 2.0 per cent w/w (Karl Fischer Method, Appendix 4.12).

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Diluent A solution containing 2 mg per mL of *dibasic potassium phosphate* and 8 mg per mL of *monobasic potassium phosphate*.

Buffer Prepare a solution containing 2.72 mg per mL of *monobasic potassium phosphate*, adjusted with 8 M *potassium hydroxide* to a pH of 4.9 to 5.1.

Standard preparation Transfer about 52 mg of Dicloxacillin Sodium RS, accurately weighed, to a 250-mL volumetric flask, add 40.0 mL of *dimethylformamide*, 10.0 mL of *ethanol*, and 100 mL of *Diluent*, and shake for 5 minutes or until completely dissolved. Dilute with *Diluent* to volume, and mix. Filter through a 0.45-µm membrane filter and use the filtrate. (**Note** Use the standard preparation promptly, or refrigerate and use on the day prepared.)

Assay preparation Constitute Dicloxacillin Sodium for Oral Suspension as directed in the labelling. Transfer an accurately weighed portion of the constituted suspension, equivalent to about 50 mg of dicloxacillin, to a 250-mL volumetric flask. Add 40.0 mL of *dimethylformamide* and 10.0 mL of *ethanol*, and shake for 15 minutes. Add 100 mL of *Diluent*, and shake for an additional 30 minutes. Dilute with *Diluent* to volume and mix. Filter through a 0.45-µm membrane filter and use the filtrate. (**Note** Use the assay preparation promptly, or refrigerate and use on the day prepared.

Mobile phase *Acetonitrile* and *Buffer* (26:74)

Chromatographic System

DETECTOR Ultraviolet light (225 nm).

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm).

FLOW RATE 1.5 mL per minute

System Suitability

SAMPLE *Standard preparation*

Suitability requirements

CAPACITY FACTOR Between 4 and 11 for the dicloxacillin peak.

COLUMN EFFICIENCY Not less than 700 theoretical plates for the dicloxacillin peak.

SYMMETRY FACTOR Not more than 2.0 for the dicloxacillin peak.

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for the dicloxacillin peak.

Procedure Separately inject equal volumes (about 10 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculation Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the portion of the constituted Oral Suspension taken, using the declared content of $C_{19}H_{17}Cl_2N_3O_5S$ in the Dicloxacillin Sodium RS.

Other requirements Complies with the requirements described under "Oral Liquids" (Appendix 1.16).

DICLOXACILLIN SODIUM CAPSULES

Category Antibacterial.

Dicloxacillin Sodium Capsules contain not less than 90.0 per cent and not more than 120.0 per cent of the labelled amount of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$).

Strength available 250 mg and 500 mg (base).

Dose Dosage of Dicloxacillin Sodium Capsules is expressed in terms of dicloxacillin base.

Adults and children 40 kg of body weight and over: 125 to 500 mg every 6 hours.

Children up to 40 kg of body weight: 3.125 to 6.25 mg per kg of body weight every 6 hours.

Contra-indication; Warning; Precaution; Additional information See under *Dicloxacillin Sodium*.

Packaging and storage Dicloxacillin Sodium Capsules shall be kept in tightly closed containers and stored at a temperature not exceeding 30°.

Labelling The label on the container states (1) the quantity equivalent to the amount of dicloxacillin; (2) the expiration date.

Identification The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that of the Standard preparation, as obtained in the *Assay*.

Water Not more than 5.0 per cent w/w (Karl Fischer Method, Appendix 4.12).

Dissolution Carry out the test as described in the "Dissolution Test" (Appendix 4.24).

Dissolution medium: *water*; 900 mL.

Apparatus 1: 100 rpm.

Time: 30 minutes.

Determine the amount of dicloxacillin dissolved using the following method.

Mobile phase and **Chromatographic system** Prepare as directed in the *Assay*.

Standard solution Weigh accurately a suitable quantity of Dicloxacillin Sodium RS, dissolve in and dilute with *Dissolution medium* to obtain a solution having a known concentration of about 280 µg per mL of dicloxacillin sodium. Filter through a 0.45-µm membrane filter and use the filtrate. (**Note** Use the standard solution promptly, or refrigerate and use on the day prepared.)

Test solution If necessary, dilute a filtered portion of the test solution with Dissolution medium to obtain a solution having a concentration of about 280 µg of dicloxacillin sodium per mL, and mix.

Procedure Inject about 6 µL each of *Standard solution* and *Test solution* into the chromatograph, record the chromatogram, and measure the response for the major peak.

Calculation Calculate the quantity of $C_{19}H_{17}Cl_2N_3O_5S$ dissolved by comparing this peak response with the major peak response similarly obtained on chromatographing *Standard solution*.

Tolerances Not less than 75 per cent (Q) of the labelled amount of $C_{19}H_{17}Cl_2N_3O_5S$ is dissolved in 30 minutes.

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Buffer Prepare a solution containing 2.72 mg per mL of *monobasic potassium phosphate*, adjusted with 8 M *potassium hydroxide* to a pH of 4.9 to 5.1.

Standard preparation Weigh accurately a suitable quantity of Dicloxacillin Sodium RS, dissolve in and dilute with *Buffer* to obtain a solution containing about 1.1 mg per mL of Dicloxacillin Sodium. Filter through a 0.45-µm membrane filter and use the filtrate. (**Note** Use the standard preparation promptly, or refrigerate and use on the day prepared.)

Assay preparation Remove, as completely as possible, the contents of not less than 10 Dicloxacillin Sodium Capsules and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity, containing about 50 mg of dicloxacillin, to a 50-mL volumetric flask. Add 30 mL of *Buffer*, sonicate for 5 minutes, and dilute with *Buffer* to volume. Filter through a 0.45-µm membrane filter and use the filtrate. (**Note** Use the assay preparation promptly, or refrigerate and use on the day prepared.)

Mobile phase *Acetonitrile* and *Buffer* (1:3)

Chromatographic System

DETECTOR Ultraviolet light (225 nm).

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm).

FLOW RATE 2.0 mL per minute.

System Suitability

SAMPLE *Standard preparation*

Suitability requirements

CAPACITY FACTOR Between 4 and 11 for the dicloxacillin peak.

COLUMN EFFICIENCY Not less than 700 theoretical plates for the dicloxacillin peak.

SYMMETRY FACTOR Not more than 2.0 for the dicloxacillin peak.

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for the dicloxacillin peak.

Procedure Separately inject equal volumes (about 2 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks.

Calculation Calculate the content of $C_{19}H_{17}Cl_2N_3O_5S$ in the portion of the Capsules taken, using the declared

content of $C_{19}H_{17}Cl_2N_3O_5S$ in Dicloxacillin Sodium RS.

Other requirements Comply with the requirements described under "Capsules" (Appendix 1.16).

DIGITALIS LEAF

Digitalis

Category Antiarrhythmic; cardiotonic

Digitalis Leaf is the dried leaf of *Digitalis purpurea* L. (Family Scrophulariaceae). The leaves are rapidly dried, as soon as possible after collection, at a temperature not exceeding 60°. It contains not less than 0.3 per cent of cardenolic glycosides, calculated as digitoxin $C_{41}H_{64}O_{13}$, on the dried basis.

Origin of plant Digitalis is native to Europe, the Mediterranean region, and the Canary Islands.

Constituents Digitalis contains cardenolic glycosides, including digitoxin, gitoxin, purpurea glycoside A and purpurea glycoside B. It also contains steroid saponin (e.g., desgalactotigonin, digitonine), anthraquinones and other four glycosides, namely acteoside, purpureaside A, calceolarioside B, and plaintainside D.

Description Odour, mild, characteristic; taste, bitter.

Macroscopical Broken, brittle leaves, apex subacute, margin irregularly crenate, dentate or serrate, base decurrent; upper surface rugose and pubescent; lower surface densely pubescent with prominent lateral veins and raised veinlets.

Microscopical Powdered drug of Digitalis shows fragments of upper epidermis, palisade parenchyma and fragments of lower epidermis. Fragments of upper epidermis: thick-walled, slightly sinuous-walled or pitted-walled cells covered with cuticle, and some with scars of covering trichomes. Palisade parenchyma underlined the upper epidermis. Fragments of lower epidermis: in surface view, distinctly prominent cells and anomocytic stomata; multicellular uniseriate covering trichomes, usually 3- to 5-celled and often with one or more collapsed cells; unicellular and/or multicellular uniseriate glandular trichomes with stalks and unicellular or multicellular heads.

Other relevant information When given in large and quickly repeated doses, it may be toxic and even life-threatening.

Packaging and storage Digitalis Leaf shall be kept in well-closed containers, protected from light and moisture.

Identification

A. To 1 g, in *fine powder*, add 20 mL of *ethanol (50 per cent)* and 10 mL of *lead acetate TS*, boil for 2 minutes, allow to cool, centrifuge, and extract the clear supernatant liquid with two 15-mL portions of *chloroform*, separating the layers, if necessary, by centrifuging. Dry the combined chloroform extracts over *anhydrous sodium sulfate* and filter (solution A). Reserve 15 mL of solution A for use in tests B and C, and evaporate 5 mL of the remainder to dryness on a water-bath. To the residue add 2 mL of a 2 per cent w/v solution of *3,5-dinitrobenzoic acid* in *ethanol* and 1 mL of 1 M *sodium hydroxide*: a reddish violet colour is produced within 15 minutes.

B. Evaporate 5 mL of solution A to dryness on a water-bath; to the residue add 3 mL of *xanthydrol TS* and heat for 3 minutes on a water-bath: a red colour is produced.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 5 mg of Purpureaglycoside A RS, 2 mg of Purpureaglycoside B RS, 5 mg of Digitoxin RS and 2 mg of Gitoxin RS in a mixture of equal volumes of *chloroform* and *methanol* and dilute to 10 mL with the same solvent.

Test solution Evaporate 10 mL of solution A to dryness on a water-bath and dissolve the residue in 1 mL of a mixture of equal volumes of *chloroform* and *methanol*.

Adsorbent *Silica gel G*.

Mobile phase *Water*, *methanol*, and *ethyl acetate* (7.5:10:75).

Application Apply 20 µL each of *Standard solution* and *Test solution* as (2-cm × 0.3-cm) bands.

Development and drying Allow the solvent front to ascend 10 cm above the line of application.

Dry the developed plate at room temperature until the solvents have evaporated.

Detection Spray with a mixture of 2 volumes of a 1 per cent w/v solution of *chloramine T* and 8 volumes of a 25 per cent w/v solution of *trichloroacetic acid* in *ethanol*, then heat at 105° for 10 minutes and examine under ultraviolet light (366 nm).

Results When examined under ultraviolet light (366 nm), the test solution shows a light blue fluorescent band due to purpureaglycoside B in the lower part, a brownish-yellow fluorescent band due to purpureaglycoside A just above it, a light blue fluorescent band due to gitoxin around the middle of the chromatogram and a brownish yellow fluorescent band due to digitoxin just above it, corresponding in colour and R_f to the bands shown by the standard solution.

***Digitalis lanata* Ehrh.** Leaves with few or no trichomes and with parallel venation or cells of the abaxial epidermis with beaded anticlinal walls and of cells of the adaxial epidermis with numerous stomata are found.

Loss on drying Not more than 6.0 per cent w/w after

drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 5.0 per cent w/w (Appendix 7.6).

Total ash Not more than 12.0 per cent w/w (Appendix 7.7).

Assay

Standard preparation Dissolve about 50 mg of Digitoxin RS, accurately weighed, in sufficient *ethanol* to produce 50.0 mL. Dilute 5.0 mL of this solution to 50.0 mL with *ethanol*. To 5.0 mL of the resulting solution add 25 mL of *water* and 3 mL of 4 M *hydrochloric acid*. Heat on a water-bath under a reflux condenser for 1 hour and transfer to a separator, rinsing the flask with two 5-mL portions of *water*. Extract with three 25-mL portions of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulfate* and dilute to 100.0 mL with *chloroform*. Evaporate 40.0 mL of the chloroform solution and dissolve the residue in 7 mL of *ethanol (50 per cent)*. Add 2 mL of *dinitrobenzoic acid TS* and 1 mL of 1 M *sodium hydroxide*.

Assay preparation Shake about 250 mg of Digitalis Leaf, in *fine powder* and accurately weighed, with 50 mL of *water* for 1 hour. Add 5 mL of a 15 per cent w/v solution of *lead acetate*, and shake. After a few minutes, add 7.5 mL of a 4 per cent w/v solution of *disodium hydrogen phosphate dodecahydrate* and filter. To 50.0 mL of the filtrate add 5 mL of *hydrochloric acid*, heat on a water-bath under a reflux condenser for 1 hour and transfer to a separator, rinsing the flask with two 5-mL portions of *water*. Extract with three 25-mL portions of *chloroform*. Dry the combined chloroform extracts over *anhydrous sodium sulfate* and dilute to 100.0 mL with *chloroform*. Evaporate 40.0 mL of the chloroform solution and dissolve the residue in 7 mL of *ethanol (50 per cent)*. Add 2 mL of *dinitrobenzoic acid TS* and 1 mL of 1 M *sodium hydroxide*.

Procedure Measure the absorbances of *Standard preparation* and *Assay preparation* at 540 nm several times during the first 12 minutes until the maximum is reached (Appendix 2.2), using a mixture of 7 mL of *ethanol (50 per cent)*, 2 mL of *dinitrobenzoic acid TS* and 1 mL of 1 M *sodium hydroxide* as the blank.

Calculation Calculate the content of $C_{41}H_{64}O_{13}$ in the portion of Digitalis Leaf taken, using the declared content of $C_{41}H_{64}O_{13}$ in Digitoxin RS.

EUCALYPTUS OIL

Thai name น้ำมันโกฐจุฬารส (NAMMAN KOT CHULA ROT)

Category Counter-irritant; expectorant; pharmaceutic aid.

Eucalyptus Oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus globulus* Labill., *E. polybractea* R.T. Baker, and *E. smithii* R.T. Baker (Family Myrtaceae). It contains not less than 70.0 per cent w/w and not more than 95.0 per cent w/w of cineole, $C_{10}H_{18}O$.

Origin of plant Eucalyptus Oil-yielding plant is native to Australia.

Constituents Eucalyptus Oil contains cineole (eucalyptol), camphene, *p*-cymene, α-pinene, α-terpineol, γ-terpinene, camphene, etc.

Description Odour aromatic, reminiscent of eucalyptus leaves; taste, pungent, camphoraceous, followed by a cold sensation.

Other relevant information Eucalyptus oil should not be administered internally to children or patients with gastro-intestinal tract inflammation, gall bladder disease, or impaired liver function.

Packaging and storage Eucalyptus Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 20 µL of *α-terpineol* and 50 µL of *cineole* in *toluene* and dilute to 5 mL with *toluene*.

Test solution Dissolve 100 mg of the sample in *toluene* and dilute to 10 mL with the same solvent.

Adsorbent *Silica gel G* (TLC or HPTLC plate).

Mobile phase *Ethyl acetate* and *toluene* (10:90).

Application Apply 10 µL (or 2 µL) each of *Standard solution* and *Test solution* as 10-mm (or 6-mm) bands.

Development and drying Allow the solvent front to ascend 15 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection Spray the plate with *anisaldehyde TS*, heat at 100° for 5 minutes, and examine in daylight.

Results When examined in daylight, the test solution shows an intense violet brown band due to 1,8-cineole in the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution.

Solubility test Soluble, at 20°, in 5 volumes of *ethanol (70 per cent)*.

Relative density 0.906 to 0.927 (Appendix 4.9).

Refractive index 1.458 to 1.470, at 20° (Appendix 4.7).

Optical rotation 0° to +10° (Appendix 4.8).

Assay Carry out the determination as described in the

"Gas Chromatography" (Appendix 3.4).

Internal standard preparation Dissolve 1 volume of *anisole* in 250 volumes of *hexane*.

Standard preparation Weigh accurately about 100 mg of Cineole RS and dissolve in 25.0 mL of *hexane*. Pipette 5.0 mL of this solution, add 5.0 mL of *Internal standard preparation* and add *hexane* to 100.0 mL.

Assay preparation Weigh accurately about 100 mg of Eucalyptus Oil and dissolve in 25.0 mL of *hexane*. Pipette 5.0 mL of this solution, add 5.0 mL of *Internal standard preparation* and add *hexane* to 100.0 mL.

System suitability preparation Dissolve sufficient quantities of *cineole* and *limonene* in *hexane* to obtain a solution containing about 200 µg per mL each.

Chromatographic system

DETECTOR Flame ionization.

COLUMN A glass column (5 m × 3 mm), packed with a 10 per cent w/w of *alkylene glycol phthalate ester* on *silanized diatomaceous support* (150 to 180 µm).

TEMPERATURE

Column 120°

Injection port 120°

Detector 120°

CARRIER GAS *Nitrogen*.

FLOW RATE Adjust so that the retention time of cineole is about 11 minutes.

System suitability

SAMPLE *System suitability preparation* and *Standard preparation*.

Suitability requirements

RESOLUTION Not less than 1.5 between cineole and limonene peaks.

RELATIVE STANDARD DEVIATION The relative standard deviation is not more than 2.0 per cent for the cineole peak.

Procedure Separately inject equal volumes (about 2 µL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of cineole and anisole peaks. Calculate the area ratios of cineole to anisole peaks.

Calculation Calculate the content of $C_{10}H_{18}O$ in the portion of Eucalyptus Oil taken, using the declared content of $C_{10}H_{18}O$ in Cineole RS.

GENTIAN

Gentian Root

Category Bitter

Gentian consists of the dried underground organs of *Gentiana lutea* L. (Family Gentianaceae).

Origin of plant Gentian is native to Central and Southern Europe.

Constituents Gentian contains bitter secoiridoid monoterpenes including gentiopicroside swirtiamarin, sweroside, and its acyl glucoside derivatives, amarogentin. It also contains xanthones (e.g., gensitin, isogensitin, gentianose, gentioside), alkaloid gentianine, volatile oil, etc.

Description Odour, characteristic; taste, sweet, followed by persistently bitter aftertaste.

Macroscopical Single or branched subcylindrical of underground parts, varied in length, usually 5 to 40 mm in diameter; externally yellowish brown or greyish brown; internally yellowish or reddish yellow. Root brittle, longitudinally wrinkled and occasionally bearing rootlet scars. Rhizome brittle; rhizome branch usually bearing terminal bud and encircled by closely arranged root scars.

Microscopical Powdered drug of Gentian shows cork, fragments of dermal tissue, parenchyma, vessels, and xylem. Cork: yellowish brown, thin-walled, polygonal cells. Fragments of dermal tissue: yellowish brown thin-wall cork cells and thick-walled collenchyma. Parenchyma: moderately thick-walled cells containing oil droplets and small prismatic and minute needle-shaped crystals of calcium oxalate. Vessels: lignified reticulate or spiral vessels. Xylem: vessels and moderately thick-walled parenchyma containing oil droplets.

Other relevant information

1. It is contra-indicated in pregnant, nursing women, young children, and in patients with gastric or duodenal ulcer, hypertension and hyperacidity.
2. Overdose may result in nausea and vomiting.

Packaging and storage Gentian shall be kept in well-closed containers, protected from light.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 5 mg of *hyperoside*, and 5 mg of *phenazone* in 10 mL of *methanol*.

Test solution To 1 g, in *No. 355 powder*, add 25 mL of *methanol*, shake for 15 minutes and filter. Evaporate the filtrate to dryness under reduced pressure at a temperature not exceeding 50°. Dissolve the residue with a small portions of *methanol* to obtain 5 mL of a solution, which may contain a sediment.

Adsorbent *Silica gel F254*.

Mobile phase *Water, anhydrous formic acid, ethyl formate* (4:8:88).

Application Apply 20 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Allow the solvent front to ascend 8 cm above the line of application in an unsaturated tank. Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (254 nm).

Results A When examined under ultraviolet light (254 nm), the test solution shows a prominent quenching band due to gentiopicroside in the lower third of the chromatogram, corresponding in R_f to the quenching band of hyperoside shown by the standard solution. There is also a weak quenching band due to amarogentin in the middle and a prominent quenching band in the upper third of the chromatogram.

A quenching band of phenazone obtained from the standard solution appears also in the upper third with R_f value between that of amarogentin and of the prominent quenching band from the test solution.

Detection B Spray the plate with a 10 per cent w/v of *potassium hydroxide* in *methanol* and then spray with a freshly prepared 0.2 per cent w/v solution of *fast blue B salt* in a mixture of equal volumes of *anhydrous ethanol* and *water*. Examine the plate under visible light.

Results B When examined under visible light, the test solution shows a weak light brown band due to gentiopicroside in the lower third of the chromatogram, corresponding in R_f to the brownish red band due to hyperoside shown by the standard solution. A violet-red band due to amarogentin and a dark violet band of higher R_f value in the upper third of the chromatogram are present.

Acid-insoluble ash Not more than 3.0 per cent w/w (Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Water-soluble extractive Not less than 33.0 per cent w/w (Appendix 7.12). Use 5.0 g, in *No. 710 powder*, add 200 mL of boiling *water*, allow to stand for 10 minutes, shake occasionally. Allow to cool, dilute to 200.0 mL with *water* and filter. Evaporate 20.0 mL of the filtrate to dryness on a water-bath. Dry the residue at 105°.

GENTIAN, POWDERED

Complies with the requirements for Packaging and storage, Identification, Acid-insoluble ash, Total ash, and Water-soluble extractive stated under Gentian, and with the following requirement.

Description Yellowish brown to brown. Diagnostic structures: a small number of lignified, reticulately thickened vessels; small acicular crystals of calcium oxalate; droplets of oil, parenchymatous cells, numerous; starch granules rare.

GENTIAN TINCTURE

Gentian	1 part
Ethanol (70 Per Cent)	5 parts

Prepare by a suitable procedure (Appendix 7.18).

Description Yellowish brown or reddish brown liquid; taste, bitter.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 5 mg of *hyperoside*, and 5 mg of *phenazone* in 10 mL of *methanol*.

Test solution Use Gentian Tincture.

Adsorbent *Silica gel F254*.

Mobile phase *Water, anhydrous formic acid* and *ethyl formate* (4:8:88).

Application Apply 20 µL each of *Standard solution* and the tincture as 10-mm bands.

Development and drying Allow the solvent front to ascend 8 cm above the line of application in an unsaturated tank. Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (254 nm).

Results A When examined under ultraviolet light (254 nm), the test solution shows a prominent quenching band due to gentiopicroside in the lower third of the chromatogram, corresponding in R_f to the quenching band of hyperoside shown by the standard solution. There is also a weak quenching band due to amarogentin in the middle and a prominent quenching band in the upper third of the chromatogram.

A quenching band of phenazone obtained from the standard solution appears also in the upper third with R_f value between that of amarogentin and of the prominent quenching band from the test solution

Detection B Spray the plate with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and then spray with a freshly prepared 0.2 per cent w/v solution of *fast blue B salt* in a mixture of equal volumes of *anhydrous ethanol* and *water*. Examine the plate under visible light.

Results B When examined under visible light, the test solution shows a weak light brown band due to gentiopicroside in the lower third of the chromatogram, corresponding in R_f to the brownish red band due to hyperoside shown by the standard solution. A violet-red band due to amarogentin and a dark violet band of higher R_f value in the upper third of the chromatogram are present.

Ethanol content 62 to 67 per cent v/v (Method II, Appendix 6.5).

Dry residue Not more than 5 per cent w/w. Introduce rapidly 3.0 g of the Gentian Tincture in a flat-bottomed dish, about 50 mm in diameter and about 360 mm deep.

Evaporate to dryness on a water-bath and dry at 105° for 3 hours. Allow to cool in a desiccator over *self-indicating silica gel* and weigh.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

IPECACUANHA

Ipecac; Ipecacuanha Root

Category Emetic; expectorant.

Ipecacuanha is the fragmented and dried underground organs of *Carapichea ipecacuanha* (Brot.) L. Anderson [*Cephaelis ipecacuanha* (Brot.) A. Rich., *C. acuminata* H. Karst]. (Family Rubiaceae), from Mato Grosso or Costa Rica. It contains not less than 2.0 per cent of total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$), and on the dried basis.

Origin of plant Ipecacuanha is native to Central America, such as Costa Rica and Nicaragua, and South America, particularly Brazil.

Constituents Ipecacuanha contains major isoquinoline alkaloids as emetine and cephaeline.

Description Odour, mild, characteristic and sternutatory; taste, bitter and unpleasant.

Macroscopical

Mato Grosso Ipecacuanha A mixture of whole or fragments of rhizomes and roots. Root somewhat tortuous, usually up to 15 cm long, 3 to 6.5(-9) mm in diameter and 6 mm thick; externally closely annulated, with rounded ridges completely encircling the root. Fragment of root, short in the bark and splintery in the wood; internally with a wide greyish bark and a small uniformly dense wood. Rhizome usually attached to root, short, cylindrical, up to 2 mm in diameter, with fine longitudinal wrinkles and pith occupying approximately one-sixth of the entire diameter.

Costa Rica Ipecacuanha Root generally resembles that of Mato Grosso Ipecacuanha, but often up to 9 mm thick; externally greyish or reddish brown with transverse ridges at intervals of usually 1 to 3 mm extending about half-way round the circumference and fading at the extremities into the general surface level.

Microscopical Powdered drug of Ipecacuanha shows parenchyma, raphide crystals of calcium oxalate, fragments of tracheids and vessels, larger vessels and sclereids from rhizome, cork, fragments of dermal tissue, fibres. Parenchyma, ovate cells, some containing starch granules. Starch granules, simple or 2- to 10- compound; the simple granules being up to 15 µm in diameter in Costa Rica ipecacuanha and up to 22 µm in diameter in Mato Grosso ipecacuanha. Raphide crystals of calcium oxalate, scattered or in bundles. Fragments of tracheids and vessels: bordered-pitted vessels accompanied by ligneous elongated parenchyma. Vessels and sclereids from rhizomes usually larger than those of the roots. Fragments of dermal tissue: reddish brown cork and phelloderm, some containing raphide crystals of calcium oxalate.

Other relevant information

1. It is contra-indicated in pregnant women.
2. It shall not be used to induce emesis following ingestion of corrosive substances (e.g., strong acid or alkali), central nervous system stimulants, strychnine, and petroleum distillate; and when airway-protective reflexes are compromised.

Packaging and storage Ipecacuanha shall be kept in well-closed container, protected from light, and stored in a dry place.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 2.5 mg of Emetine hydrochloride RS and 3 mg of Cephaeline hydrochloride RS in *methanol* and dilute to 20 mL with the same solvent.

Test solution To 100 mg, in *No. 180 powder*, in a test-tube add 50 µL of *strong ammonia solution* and 5 mL of *ether* and stir the mixture vigorously with a glass rod. Allow to stand for 30 minutes. Filter and use the filtrate.

Adsorbent *Silica gel F254* (TLC or HPTLC plate).

Mobile phase *Strong ammonia solution, methanol, ethyl acetate,* and *toluene* (2:15:18:65).

Application Apply 10 µL (or 5 µL) each of *Standard solution* and *Test solution* as 10-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 10 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (366 nm).

Results A When examined under ultraviolet light (366 nm), the test solution of Costa Rica Ipecacuanha shows a blue fluorescent band due to cephaeline and a blue fluorescent band due to emetine in the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. The test solution of Mato Grosso Ipecacuanha shows a faint blue fluorescent band due to cephaeline and a blue fluorescent band due to emetine in the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution.

Detection B Spray the plate with a 0.5 per cent w/v solution of *iodine* in *ethanol*, heat at 60° for 10 minutes and examine under ultraviolet light (366 nm). Observe the result.

Results B When examined under ultraviolet light

(366 nm), the test solution of Costa Rica Ipecacuanha or of Mato Grosso Ipecacuanha shows a blue fluorescent band due to cephaeline and a yellow fluorescent band due to emetine, clearly separated around the middle of the chromatogram. Both bands correspond in colour and R_f to the bands shown by the standard solution.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight; use 1 g (Appendix 4.15).

Foreign matter Not more than 2.0 per cent w/w (Appendix 7.2).

Acid-insoluble ash Not more than 3.0 per cent w/w (Appendix 7.6).

Total ash Not more than 5.0 per cent w/w (Appendix 7.7).

Assay To about 7.5 g, in *fine powder* and accurately weighed, in a dry flask add 100 mL of *ether* and shake for 5 minutes. Add 5 mL of 6 M *ammonia*, shake frequently for 1 hour, add 5 mL of *water*, shake vigorously, and decant the ethereal layer into a dry flask through a plug of absorbent cotton. Wash the residue in the flask with two 25-mL portions of *ether*, decanting each portion through the same plug of absorbent cotton. Combine the ethereal solutions and remove most of the ether by distillation and the remainder by gently warming in a current of air. Dissolve the residue in 2 mL of *ethanol (90 per cent)*, evaporate the ethanol and heat at 100° for 5 minutes. Dissolve the residue in 5 mL of previously neutralized *ethanol (90 per cent)* by warming on a water-bath, add 15 mL of 0.1 M *hydrochloric acid VS* and titrate the excess of acid with 0.1 M *sodium hydroxide VS* using 0.5 mL of *methyl red-methylene blue TS* as indicator. Each mL of 0.1 M *hydrochloric acid VS* is equivalent to 24.03 mg of total alkaloids, calculated as emetine.

IPECACUANHA, POWDERED

Powdered Ipecac; Powdered Ipecacuanha Root

Complies with the requirements for total alkaloids, Packaging and storage, Identification, Loss on drying, Foreign matter, Acid-insoluble ash, and Total ash stated under Ipecacuanha and with the following requirement.

Description Light grey to yellowish brown. Diagnostic structures: parenchyma, raphide crystals of calcium oxalate, fragments of tracheids and vessels, larger vessels and sclereids from rhizome, cork, fragments of dermal tissue, fibres. Parenchyma, ovate cells, some containing starch granules. Starch granules, simple or 2- to 10-compound; the simple granules being up to 15 µm in diameter in Costa Rica ipecacuanha and up to 22 µm in diameter in Mato Grosso ipecacuanha. Raphide crystals of calcium oxalate, scattered or in bundles. Fragments of tracheids and vessels: bordered-pitted vessels accompanied by ligneous elongated parenchyma. Vessels and sclereids from rhizomes usually larger than those of the roots. Fragments of dermal tissue: reddish brown cork and phelloderm, some containing raphide crystals of calcium oxalate.

IPECACUANHA LIQUID EXTRACT

Ipecacuanha Liquid Extract contains not less than 1.90 per cent and not more than 2.10 per cent of total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$).

Ipecacuanha, in *fine powder* 1000 g
Ethanol (80 Per Cent) a sufficient quantity

Exhaust the Ipecacuanha by percolation (Appendix 7.18) with Ethanol (80 Per Cent), reserving the first 750 mL of the percolate. Remove the ethanol from the remainder of the percolate by evaporation under reduced pressure at a temperature not exceeding 60° and dissolve the residual extract in the reserved portion. Determine the proportion of alkaloids in the liquid thus obtained by the Assay described below. To the remainder of the liquid add sufficient Ethanol (80 Per Cent) to produce an Ipecacuanha Liquid Extract containing 2 per cent w/v of total alkaloids calculated as emetine. Allow to stand for not less than 24 hours and filter.

Description Dark brown liquid.

Ethanol content 63 to 69 per cent v/v (Method II, Appendix 6.5).

Relative density 0.910 to 0.960 (Appendix 4.9).

Assay To 5.0 mL of Ipecacuanha Liquid Extract in a separator add 20 mL of *water*, 5 mL of 1 M *sulfuric acid* and 10 mL of *chloroform* and shake well. Transfer the chloroform extract to a second separator containing a mixture of 4 mL of *ethanol* and 20 mL of 0.05 M *sulfuric acid*, shake, allow to separate and discard the chloroform layer. Continue the extraction of the liquid in the first separator with two 10-mL portions of *chloroform*, transferring the chloroform solution each time to the second separator and washing as before. Transfer the acidic liquid from the second separator to the first separator, make distinctly alkaline with *ammonia TS* and shake with successive portions of *chloroform* until complete extraction of the alkaloids is effected, washing each chloroform solution with the same 10 mL of *water* contained in a third separator. Remove the chloroform, add to the residue 2 mL of *ethanol*, evaporate to dryness and dry for 5 minutes at 80° in a current of air. Dissolve the residue in 2 mL of *ethanol*, previously neutralized to *methyl red TS*, add 10.0 mL of 0.05 M *sulfuric acid VS* and

titrate with 0.1 M *sodium hydroxide VS* using *methyl red-methylene blue TS* as indicator. Each mL of 0.05 M *sulfuric acid VS* is equivalent to 24.03 mg of total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$).

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16).

IPECACUANHA SYRUP

Ipecacuanha Syrup contains, in each 100 mL, not less than 0.12 g and not more than 0.15 g of the total alkaloids, calculated as emetine ($C_{29}H_{40}N_2O_4$) and cephaeline ($C_{28}H_{38}N_2O_4$).

Ipecacuanha, in *coarse powder* a suitable quantity
Glycerol 100 mL
Simple Syrup, sufficient to produce 1000 mL

Prepare the liquid extract using a mixture of 3 volumes of Ethanol and 1 volume of Purified Water to extract Ipecacuanha. If necessary, evaporate the mixture under reduced pressure or add a suitable amount of Ethanol or Purified Water to obtain a solution containing, in each 100 mL, 1.7 to 2.1 g of the total alkaloids, emetine and cephaeline. To 70 mL of this solution, add Glycerol 100 mL and Simple Syrup to make 1000 mL.

Description Yellowish brown, viscous liquid; taste, sweet with a bitter after taste. It has a sweet taste and a bitter aftertaste.

Identification Take 2 mL of the syrup into an evaporating dish, mix with 1 mL of *hydrochloric acid*, and add small pieces of chlorinated lime: circumference of it turns orange.

Microbial limit Complies with the requirements for Category 4A in the "Limit for Microbial Contamination" (Appendix 10.5).

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation Dissolve about 10 mg of Emetine Hydrochloride RS, previously dried over *phosphorus pentoxide desiccant* at 50° *in vacuum* for 5 hours and accurately weighed, in 0.01 M *hydrochloric acid* and dilute to 100.0 mL with the same solvent.

Assay preparation Dilute 5.0 mL of Ipecac Syrup to 50.0 mL with 0.01 M *hydrochloric acid*.

Resolution solution Dissolve 1 mg each of Emetine Hydrochloride RS and *cephaeline hydrobromide* in 10 mL of 0.01 M *hydrochloric acid*.

Mobile phase A 0.4 per cent w/v solution of *sodium 1-heptane sulfonate* and *methanol* (1:1), prepared by dissolving 2.0 g of *sodium 1-heptane sulfonate* in 500 mL of *water*, adjusting the pH to 4.0 with *glacial acetic acid*, and adding 500 mL of *methanol*.

Chromatographic System

DETECTOR Ultraviolet light (283 nm).

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilyl bonded to porous silica or ceramic microparticles (5 µm).

COLUMN TEMPERATURE 50°

FLOW RATE Adjust so that the retention time of emetine is about 14 minutes.

System Suitability

SAMPLE *Resolution solution*

Suitability requirements

RESOLUTION Not less than 5.0 between cephaeline and emetine peaks.

RELATIVE STANDARD DEVIATION Not more than 1.5 per cent for emetine.

Procedure Separately inject equal volumes (about 10 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Identify the peaks due to cephaeline and emetine in the assay preparation using the retention times of emetine and cephaeline in the standard preparation and the resolution solution.

Calculation Calculate the content of total alkaloids, calculated as cephaeline and emetine, in the portion of the Ipecacuanha Syrup taken by the expression,

$$W[A_{U1} + (A_{U2} \times 0.971)]/A_S \times 1/2 \times 0.868$$

in which A_{U1} and A_{U2} are the areas of the peaks due to emetine and cephaeline, respectively, obtained from *Assay preparation*; A_S is the area of the peak due to emetine obtained from *Standard preparation*; W is the weight, in mg, of Emetine Hydrochloride RS in *Standard preparation*. (**Note** 1 mg of emetine hydrochloride contains 0.868 mg of emetine and correction factor for cephaeline is 0.971.)

Other requirements Complies with the requirements described under "Oral Liquids" (Appendix 1.16).

LAVENDER OIL

Thai name น้ำมันลาเวนเดอร์ (NAMMAN LAVENDER)

Category Pharmaceutic aid.

Lavender Oil is the volatile oil distilled with steam from the flowering tops of *Lavandula angustifolia* Mill. (*L. officinalis* Chaix) (Family Lamiaceae).

Origin of plant Lavender Oil-yielding plant is native to Mediterranean region, particularly France, Italy, and Spain.

Constituents Lavender Oil contains predominantly esters such as linalyl acetate and a-terpinyl acetate, and monoterpenes, e.g., camphene, 1,8-cineole, limonene,

and linalool.

Description Colourless, pale yellow, or yellowish green liquid; odour aromatic, reminiscent of the flowers; taste bitter, somewhat camphor-like.

Packaging and storage Lavender Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dilute 10 µL of *cineole*, 5 µL of *linalool*, and 5 µL of *linalyl acetate* to 1 mL with *toluene*.

Test solution Dilute 20 µL of the sample to 1 mL with *toluene*.

Adsorbent *Silica gel F254* (TLC or HPTLC plate).

Mobile phase *Ethyl acetate* and *toluene* (5:95).

Application Apply 6 µL (or 2 µL) each of *Standard solution* and *Test solution* as 10-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 10 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection Spray the plate with *anisaldehyde TS*, heat at 100° for 5 minutes, and examine immediately in daylight.

Results When examined in daylight, the test solution shows a dark violet band due to linalool and possibly a pale violet-brown band due to 1,8-cineole in the lower third of the chromatogram, and a dark violet band due to linalyl acetate in the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. A greyish or brownish band in the lower third of the chromatogram, a pink band in the middle of the chromatogram and a violet band in the upper third of the chromatogram are present.

Relative density 0.878 to 0.892 (Appendix 4.9).

Refractive index 1.455 to 1.466, at 20° (Appendix 4.7).

Optical rotation –12.5° to –6° (Appendix 4.8).

Acid value Not more than 1.0; use 5 g (Appendix 5.4).

LEMON OIL

Thai name น้ำมันมะนาวฝรั่ง (NAMMAN MA-NAO FA-RANG)

Category Pharmaceutic aid (flavouring agent).

Lemon Oil is the volatile oil obtained by expression from the outer part of the fresh pericarp of the ripe or nearly ripe fruit of *Citrus* × *limon* (L.) Osbeck (Family Rutaceae). It contains not less than 3.5 per cent w/w of aldehydes, calculated as citral, $C_{10}H_{16}O$.

Origin of plant Lemon Oil-yielding plants are native to South Asia, particularly northwestern and northeastern India, and other parts of Asia.

Constituents Lemon Oil contains monoterpenes, predominantly limonene, citral, *β-pinene*, *γ*-terpinene, coumarins (e.g., citropten and 5-geranyloxy-7-methoxycoumarin), and furanocoumarin such as bergamottin, byakangelicin, and psoralen.

Description Pale yellow or greenish yellow liquid, visibly free from water; odour, that of lemon.

Packaging and storage Lemon Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 mg of *citropten* and 50 µL of *citral* in *toluene* and dilute to 10 mL with the same solvent.

Test solution Mix 1 mL of the sample with 1 mL of *toluene*.

Adsorbent *Silica gel F254*.

Mobile phase *Ethyl acetate* and *toluene* (15:85).

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (254 nm).

Results A When examined under ultraviolet light (254 nm), the test solution shows a light blue fluorescent band due to citropten in the middle of the chromatogram, a quenching band due to citral in the upper third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. There are also quenching bands of byakangelicin and psoralen derivative lower than the citropten band, in the lower third of the chromatogram; a dark blue band of 5-geranyloxy-7-methoxycoumarin in the middle of the chromatogram above the citropten band and a quenching band of bergamottin above the citral band, in the upper third of the chromatogram.

Detection B Examine the plate under ultraviolet light (366 nm).

Results B When examined under ultraviolet light (366 nm), the test solution shows a bright violet-blue fluorescent band due to citropten in the middle of the chromatogram, a quenching band due to citral in the upper third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. There are also an orange band of byakangelicin and a yellow fluorescent band of psoralen derivative lower than citropten band, in the lower third of the chromatogram; a bright blue fluorescent band of 5-geranyloxy-7-methoxycoumarin above the citropten band, in the middle of the chromatogram; a yellow fluorescent band of bergamottin above the citral band, in

the upper third of the chromatogram.

Weight per millilitre 0.850 to 0.856 g (Appendix 4.9).

Refractive index 1.473 to 1.476, at 20° (Appendix 4.7).

Optical rotation +57° to +70°, at 20° (Appendix 4.8).

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 10 g, omitting the *toluene* and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 76.73 mg of $C_{10}H_{16}O$.

LEMON OIL, TERPENELESS

Category Pharmaceutic aid.

Terpeneless Lemon Oil may be prepared by concentrating Lemon Oil under reduced pressure until most of the terpenes have been removed or by solvent partition. It contains not less than 40 per cent w/w of aldehydes, calculated as citral, $C_{10}H_{16}O$.

Description Clear, colourless or pale yellow, liquid, visibly free from water; odour and taste, that of lemon.

Packaging and storage Terpeneless Lemon Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Solubility test Soluble, at 20°, in 1 volume of *ethanol (80 per cent)*.

Weight per millilitre 0.880 to 0.895 g (Appendix 4.9).

Refractive index 1.475 to 1.485, at 20° (Appendix 4.7).

Optical rotation -5° to +2°, at 20° (Appendix 4.8).

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 1 g, omitting the *toluene* and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 76.73 mg of $C_{10}H_{16}O$.

LEMON PEEL, DRIED

Thai name เปลือกมะนาวฝรั่งแห้ง (PLUEAK MA-NAO FA-RANG HAENG)

Category Pharmaceutic aid.

Dried Lemon Peel is the dried outer part of the pericarp of the ripe, or nearly ripe, fruit of *Citrus* × *limon* (L.) Osbeck (Family Rutaceae). It yields not less than 2.0 mL of lemon oil from each 100 g of drug.

Constituents Dried Lemon Peel contains flavonoids and their glycosides such as naringin, neohesperidin, hesperidin, and rutin. Others are coumarins, monosaccharides, phenolic acids namely caffeic acid, etc.

Description Odour aromatic, characteristic; taste aromatic and bitter.

Macroscopical Strips or pieces with a marked thickening of the epicarp around the calyx; externally yellow and somewhat rough from the presence of numerous minute pits, each corresponding to an oil gland; internally with a small remnant of white spongy pericarp. Fracture short.

Macroscopical Transverse section of the dried peel shows epidermis, parenchyma, vascular bundle, and prismatic crystal of calcium oxalate. Epidermis: small thin-walled cells with underlying parenchyma containing numerous large oil glands and scattered small strands of vascular tissue. Prismatic crystals of calcium oxalate occurring throughout the parenchyma, particular abundant in the layers adjacent to the epidermis.

Packaging and storage Dried Lemon Peel shall be kept in tightly closed containers, at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Prepare a solution containing 0.01 per cent w/v each of *caffeic acid* and *naringin*, and 0.025 per cent w/v of *rutin*, in *methanol*.

Test solution Add 1 g of freshly cut peel to 10 mL of *methanol* and heat in a water-bath at 65° for 5 minutes, shaking frequently. Allow to cool and filter.

Adsorbent *Silica gel G*.

Mobile phase *Water, anhydrous formic acid, 2-butanone, ethyl acetate* (10:10:30:50).

Application Apply 20 µL of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate at 105°.

Detection Spray the warm plate with a 1 per cent w/v solution of *diphenylboric acid aminoethyl ester* in *methanol*, then with a 5 per cent w/v solution of *polyethylene glycol 400* in *methanol*, allow the plate to dry in air for about 30 minutes and examine under ultraviolet light (366 nm). Observe the result.

Results When examined under ultraviolet light (366 nm), the test solution shows a yellowish brown fluorescent band due to rutin and above it, an intense red fluorescent band and further above, a very weak greenish fluorescent band due to naringin in the

chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. Other coloured bands are also shown in the chromatogram.

Assay Carry out the method for the "Determination of Volatile Oil" (Appendix 7.3), using 20 g, accurately weighed and crushed under *water*. Use 300 mL of *water* as the distillation liquid and a 500-mL round-bottomed flask. Distil at a rate of 2 to 3 mL per minute for 3 hours. Use 0.2 ml of *xylene* in the graduated tube.

LEMON SPIRIT

Category Pharmaceutic aid (flavouring agent).

Lemon Spirit contains not less than 3.45 per cent w/v and not more than 4.60 per cent w/v of aldehydes, calculated as citral, $C_{10}H_{16}O$.

Lemon Spirit may be prepared as follow.

Terpeneless Lemon Oil	100	mL
Ethanol, sufficient to produce	1000	mL

Mix.

Packaging and storage Lemon Spirit shall be kept in tightly closed containers of glass or other suitable material and stored at a temperature not exceeding 25°.

Weight per millilitre 0.814 to 0.823 g (Appendix 4.9).

Ethanol content 84 to 88 per cent v/v (Method II, Appendix 6.5).

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 10.0 mL of Lemon Spirit, omitting the *toluene* and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 76.73 mg of $C_{10}H_{16}O$.

Other requirements Complies with the requirements described under "Spirits" (Appendix 1.16).

LEMON SYRUP

Category Pharmaceutic aid (flavoring agent).

Lemon Syrup contains not less than 2.2 per cent w/v and not more than 2.6 per cent w/v of citric acid monohydrate, $C_6H_8O_7{\cdot}H_2O$.

Lemon Syrup may be prepared as follows.

Lemon Spirit	5	mL
Citric Acid Monohydrate	25	g
Invert Syrup	100	mL
Syrup, sufficient to produce	1000	mL

Dissolve the Citric Acid Monohydrate in some of the Syrup, add the Invert Syrup, the Lemon Spirit, and sufficient Syrup to produce 1000 mL and mix.

Packaging and storage Lemon Syrup shall be kept in well-closed containers and stored at a temperature not exceeding 25°.

Weight per millilitre About 1.33 g (Appendix 4.9).

Assay Mix about 8 g of Lemon Syrup, accurately weighed, with 100 mL of *water* and titrate with 0.1 M *sodium hydroxide VS* using a 1.0 per cent w/v solution of *phenolphthalein* in *ethanol* as indicator. Each mL of 0.1 M *sodium hydroxide VS* is equivalent to 7.005 mg of $C_6H_8O_7{\cdot}H_2O$. Determine the weight per mL as described in the "Determination of Weight per Millilitre, Specific Gravity and Relative Density" (Appendix 4.9), and calculate the content of $C_6H_8O_7{\cdot}H_2O$, weight in volume.

Other requirements Complies with the requirements described under "Oral Liquids" (Appendix 1.16).

MYRRH

Thai name มดยอบ (MOT YOP)

Category Astringent.

Myrrh is an oleo-gum-resin obtained from the stem and branch of *Commiphora myrrha* (Nees) Engl. and possibly other species of *Commiphora* (Family Burseraceae). It yields not less than 7.0 mL of volatile oil from each 100 g of drug.

Constituents Myrrh contains a water-soluble gum, resins and volatile oil. The major constituents of the oil are furanosesquiterpenes such as curzerene and furanodione. It also contains monoterpenes, a-pinene and limonene.

Description Odour, aromatic and characteristic; taste, bitter and acrid.

Macroscopical Irregular or roundish grains or pieces, vary in size and shape, pale to dark orange-brown covered with grey or yellowish brown dust.

Microscopical Powdered drug of the oleo-gum-resin shows a few tissue fragments and substances from the plant including fragments of cork, stone cells, parenchyma, fibres, and calcium oxalate crystals. Cork fragments reddish brown. Stone cells polyhedral cells with partly or strongly thickened, pitted, lignified-walls, and with brownish content, occurring as solitary cells or arranged in group. Parenchyma thin-walled. Fibre sclerenchymatous. Calcium oxalate crystals irregularly prismatic or polyhedral.

Other relevant information The plant yielding myrrh is native to southern Arabia, northeastern Africa (mainly Somalia and Ethiopia) and southern Africa (e.g., Kenya).

Packaging and storage Myrrh shall be stored in a cool and dry place.

Identification

Triturate 400 mg of the crushed drug with 1 g of washed sand, shake for a few minutes with 10 mL of *ether* and filter. Evaporate the filtrate to dryness in a porcelain dish, and add a few drops of *nitric acid* to the residue: a purple-violet colour is produced immediately.

Transfer 100 mg of the powdered drug to a test-tube, and add 1 ml of *nitric acid*: a red colour is produced. Upon addition of a crystal of *vanillin*, the red colour deepens and does not diminish when water is added.

Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 mg of *thymol* and 40 µL of *anethole* in 10 mL of *ethanol*.

Test solution To 200 mg of the powdered drug add 4 mL of *ethanol*, sonicate for 10 minutes and filter.

Adsorbent *Silica gel F254* (HPTLC plate)

Mobile phase *Glacial acetic acid*, *di-isopropyl ether*, and *cyclohexane* (10:40:60).

Application Apply 5 µL each of *Standard solution* and *Test solution* as 8-mm bands.

Development and drying Allow the solvent front to ascend 6 cm above the line of application. Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (366 nm).

Results A When examined under ultraviolet light (366 nm), the test solution shows no blue or violet fluorescent bands in the lower third of the chromatogram.

Detection B Spray the plate with *anisaldehyde TS*, heat at 100° for 2 minutes and examine under visible light. Observe the result.

Results B When examined under visible light, the standard solution shows a violet band due to anethole in the upper third and an orange band due to thymol around the middle of the chromatogram. The test solution shows a reddish violet band above the anethole band and a red band between the anethole and the thymol bands obtained from the standard solution; one violet and one brown bands appear in the lower third of the chromatogram.

Ethanol-insoluble matter Not more than 75.0 per cent w/w, determined by the following method. Transfer about 1 g, accurately weighed, to a suitable flask. Add 30 mL of *ethanol* and shake vigorously for 10 minutes. Filter the supernatant through a tared sintered-glass filter, avoiding the transfer of sediment from the flask. Repeat the extraction with two 20-ml portions of *ethanol*. Quantitatively transfer the sediment to the filter by rinsing the flask with *ethanol*. Dry the residue at 105° to constant weight.

Loss on drying Not more than 15.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Total ash Not more than 7.0 per cent w/w (Appendix 7.7).

Assay Carry out the method for the "Determination of Volatile Oil" (Appendix 7.3), using 7 g, accurately weighed, in *No. 710 powder*. Use 150 mL of *water* as the distillation liquid and a 500-mL round-bottomed flask. Distil at the rate of 2 to 3 mL per minute for 4 hours. Use 0.5 mL of *xylene* in the graduated tube.

MYRRH TINCTURE

Category Astringent.

Myrrh Tincture may be prepared as follows.

Myrrh	1 part
Ethanol (90 Per Cent)	5 parts

Mix. Filter if necessary.

Dose *Topical*, 1 mL, paint or dab on the affected area(s) 2 to 3 times a day. Do not dilute the tincture.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution, **Adsorbent**, **Mobile phase**, **Application**, **Development and drying**, **Detection**, and **Result** Proceed as directed in the Identification under Myrrh.

Test solution Dilute 5 mL of the tincture to 10 mL with *ethanol*, sonicate for 10 minutes and filter.

Ethanol content 82 to 88 per cent v/v (Appendix 6.5).

Total solids Not less than 4.0 per cent w/w (Appendix 7.21).

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

NUX VOMICA

Thai name โกฐกะกลิ้ง (KOT KA-KLING)

Category Bitter.

Nux Vomica consists of the dried ripe seeds of *Strychnos nux-vomica* L. (Family Loganiaceae). It contains not less than 1.50 per cent for the sum of brucine ($C_{23}H_{26}N_2O_4$) and strychnine ($C_{21}H_{22}N_2O_2$), of which 43 per cent to 67 per cent is strychnine, calculated on the dried basis.

Constituents Nux Vomica contains alkaloids, predominantly indole alkaloids i.e., strychnine and brucine, are its major components. It also contains

catechol, secoxyloganin and squalene.

Origin of plant Nux vomica is native to India, Bangladesh, Sri Lanka, and Southeast Asia.

Description Odourless; taste, very bitter and persisting.

Macroscopical Discoid, 10 to 30 mm in diameter, 4 to 6 mm thick, with a slightly raised margin, one surface slightly convex, the other one slightly concave, or sometimes irregularly curved, light grey to greenish grey, satiny; one surface bearing hilum at the centre, from which extends a radial ridge ending in a slight protuberance on the edge (the micropyle). Endosperm abundant, translucent, horny with a disc-shaped cavity in the centre. Embryo bearing two small thin cordate cotyledons in the central cavity next to micropyle and with a cylindrical radicle.

Microscopical Powdered drug of the seed shows fragments of outer testa in surface view, fragments of the lignified ridges of the hairs and fragments of outer layer of endosperm. Outer testa: cells with enlarged, sclerified, strongly thickened and pitted base, which transformed into curved or straight hairs; hair usually broken, with 7 to 10 lignified ridges and round lignified apex. Fragments of the lignified ridges of hairs appearing as rods or strips, highly variable in length. Fragments of Endosperm, numerous, consisting of thick-walled polyhedral cells, some of which containing oil and aleurone grains. Fragments of outer layer of endosperm, a few; in surface view, polyhedral cells, sometimes associated with the brown pigmented layer of the testa of cells with indistinct wall; in transverse section, elongated cells, sometimes with the pigmented layer and the outer testa.

Other relevant information

1. Nux vomica shall be properly processed in order to reduce its toxicity before it is compounded in the pharmaceutical preparation.

2. When reducing the drug to a powder, take special precautions in handling.

Packaging and storage Nux Vomica shall be kept in well-closed containers.

Identification

A. To 3 g of the powdered drug add 3 mL of *ammonia TS* and 20 mL of *chloroform*, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water-bath, add 5 mL of diluted *sulfuric acid* (1 in 10), and warm on a water-bath while shaking well until the odour of chloroform is no longer perceptible. After cooling, filter through a pledget of *absorbent cotton*, and add 2 mL of *nitric acid* to 1 mL of the filtrate: a red colour develops.

B. To the remaining filtrate obtained in *test A* add 1 mL of *potassium dichromate TS*, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of *water*. Transfer a part of the precipitate to a small test-tube, add 1 mL of *water*, dissolve by warming, cool, and add 5 drops of *sulfuric acid* dropwise carefully along the wall of the test-tube: the layer of sulfuric acid shows a purple colour which turns immediately red to red-brown.

C. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 mg of *brucine* and 10 mg of *strychnine* in 10 mL of *ethanol*.

Test solution To 2.0 g of the powdered drug add 20 mL of *ethanol (70 per cent)*, allow to macerate for 15 minutes at room temperature, with stirring, and centrifuge. Use the supernatant.

Adsorbent *Silica gel G* (TLC or HPTLC plate).

Mobile phase *Strong ammonia solution*, *methanol*, and *dichloromethane* (1:5:95); use the lower layer.

Application Apply 10 µL (or 5 µL) each of *Standard solution* and *Test solution* as bands.

Development and drying Allow the solvent front to ascend 15 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection Spray the plate with *iodoplatinate TS*, heat at 105° for 15 minutes and examine immediately under visible light. Observe the result.

Results When examined under visible light, the test solution shows a blue band due to brucine in the lower third and violet band due to strychnine around the middle of the chromatogram, corresponding in R_f to the bands shown by the standard solution.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Foreign matter Not more than 1.0 per cent w/w (Appendix 7.2).

Total ash Not more than 3.0 per cent w/w (Appendix 7.7).

***Strychnos ignatii* P.J. Bergius** Irregularly shaped seeds, neither discoid nor flattened; when examined the powdered drug under a microscope, sclerified hairs, usually sheared off, not thickened at the base and with walls composed of small, oblique, sclerified strips, tightly fused longitudinally are found.

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation Dissolve about 10 mg of Brucine RS and 10 mg of Strychnine RS, accurately weighed, in *acetonitrile* and dilute to 10.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with *Mobile phase A*.

Assay preparation Weigh accurately about 1 g of the powdered Nux Vomica, add 10.0 mL of *ethanol (60 per*

cent). Boil gently, under a reflux condenser, with stirring. After 30 minutes, cool and filter into a 20-mL volumetric flask. Wash the filter with *ethanol (60 per cent)* and dilute to 20.0 mL with the same solvent. Dilute 1.0 mL of the solution to 20.0 mL with *Mobile phase A*.

Mobile phase

MOBILE PHASE A *Triethylamine, acetonitrile, methanol, tris(hydroxymethyl)-aminomethane buffer solution pH 9.0* (0.1:7.5:7.5:85)

MOBILE PHASE B *Triethylamine, tris(hydroxymethyl) aminomethane buffer solution pH 9.0, acetonitrile, methanol* (0.1:15:42.5:42.5)

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0 – 5	100	0
5 - 25	100 → 70	0 → 30
25 - 30	70 → 65	30 → 35
30 - 31	65 → 0	35 → 100
31 - 32	0	100

Chromatographic system

DETECTOR Ultraviolet light (260 nm)

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilyl bonded to porous silica or ceramic microparticles (3.5 µm), end-capped with ethylene-bridged hybrid particles containing both inorganic (silica) and organic (organosiloxanes) components.

COLUMN TEMPERATURE 35°

FLOW RATE 1.0 mL per minute

System suitability

SAMPLE *Standard preparation*

Suitability requirements

RESOLUTION Not less than 3.0 between brucine and strychnine peaks.

RELATIVE STANDARD DEVIATION Not more than 2.0 per cent for brucine and for strychnine.

Procedure Separately inject equal volumes (about 10 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Identify the peaks due to brucine and strychnine in the assay preparation using the retention time of brucine and strychnine in the standard preparation.

Calculation Calculate the contents of brucine and strychnine, in the portion of the Nux Vomica taken by the expression:

$$A_u W(2P) / A_s w,$$

in which A_u is the area of the peak due to brucine or to strychnine obtained from *Assay preparation*; A_s is the area of the peak due to brucine or to strychnine obtained from *Standard preparation*; w is the weight, in g, of Nux Vomica in *Assay preparation*, W is the weight, in g, of Brucine RS or of Strychnine RS in *Standard preparation*; P is the declared content of $C_{23}H_{26}N_2O_4$ in Brucine RS, or $C_{21}H_{22}N_2O_2$ in Strychnine RS.

NUX VOMICA TINCTURE

Category Bitter.

Nux Vomica Tincture contains not less than 0.15 per cent w/w and not more than 0.30 per cent w/w for the sum of brucine ($C_{23}H_{26}N_2O_4$) and strychnine ($C_{21}H_{22}N_2O_2$), of which 43 per cent to 67 per cent is strychnine.

It may be prepared as follows.

Nux Vomica, in *No. 710 powder*	1 part
Ethanol (70 Per Cent)	10 parts

Mix the powdered Nux Vomica with *ethanol (70 per cent)* as prescribed. Macerate for at least 10 days with sufficient shaking. Separate the residue from the ethanol and strain under pressure if necessary. Allow the combined liquid to stand for 48 hours and filter.

Dose 0.6 to 2.4 mL.

Other relevant information Taking more medication than recommended doses of nux vomica tincture may produce convulsions of all voluntary muscles due to strychnine toxicity.

See also under Nux Vomica.

Packaging and storage Nux Vomica Tincture shall be kept in tightly closed containers, protected from light.

Identification Complies with the test for Identification C described under *Nux Vomica*.

Ethanol content 60 to 70 per cent v/v (Method II, Appendix 6.5).

Assay Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation, Mobile phase, Chromatographic System, System Suitability, and **Procedure** Proceed as directed in the *Assay* under Nux Vomica.

Assay preparation Transfer about 2 g of Nux Vomica Tincture, accurately weighed, to a 20-mL volumetric flask, add sufficient amount of *ethanol (60 per cent)*, mix, and dilute with the same solvent to volume.

Calculation Calculate the contents of brucine and strychnine in the portion of the Tincture taken by the expression:

$$A_u W P / A_s w 10,$$

in which A_u is the area of the peak due to brucine or

to strychnine obtained from *Assay preparation*; A_s is the area of the peak due to brucine or to strychnine obtained from *Standard preparation*; *w* is the weight, in g, of Nux Vomica Tincture in *Assay preparation*; *W* is the weight, in g, of Brucine RS or of Strychnine RS in *Standard preparation*; *P* is the declared content of $C_{23}H_{26}N_2O_4$ in Brucine RS, or $C_{21}H_{22}N_2O_2$ in Strychnine RS.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

ORANGE OIL

Thai name น้ำมันผิวส้ม (NAMMAN PHIU SOM)

Category Pharmaceutic aid.

Orange Oil is obtained by mechanical means from the fresh peel of the fruit of *Citrus sinensis* (L.) Osbeck (Family Rutaceae). It contains not less than 1.0 per cent w/w of aldehydes, calculated as decanal, $C_{10}H_{20}O$.

Origin of plant Orange Oil-yielding plant is possibly not a naturally occurring species. According to the genetic analysis evidence, most experts agree that the plant is a hybrid originated by an intentional cross between *Citrus reticulata* Blanco and *Citrus maxima* (Burm.) Merr. It is, however, believed to be originally found near the border between China and Vietnam, and is cultivated across the subtropical and tropical regions, as well as Florida, California, and Brazil.

Constituents Orange Oil contains predominantly limonene. It also contains other monoterpenoids, e.g., α-pinene, β-pinene, β-myrcene, sabinene, and linalool.

Description Yellow to yellowish brown liquid, visibly free from water; odour, that of orange.

Other relevant information It shall not be used if it has terebinthine odour.

Packaging and storage Orange Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Solubility test Soluble, at 20°, in 7 volumes of *ethanol (90 per cent)*.

Weight per millilitre 0.842 to 0.848 g (Appendix 4.9).

Refractive index 1.472 to 1.476, at 20° (Appendix 4.7).

Optical rotation +94° to +99° (Appendix 4.8).

Residue on evaporation 1.0 to 5.0 per cent w/w when determined by the method for "Residue on Evaporation of Volatile Oils" (Appendix 7.13). Use 2 g and heat for 4 hours.

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 10 g, omitting the *toluene* and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 78.76 mg of $C_{10}H_{20}O$.

ORANGE OIL, TERPENELESS

Category Pharmaceutic aid.

Terpeneless Orange Oil may be prepared by concentrating Orange Oil under reduced pressure until most of the terpenes have been removed or by solvent partition. It contains not less than 18 per cent w/w of aldehydes, calculated as decanal, $C_{10}H_{20}O$.

Description Clear, yellow or orange yellow, liquid, visibly free from water.

Packaging and storage Terpeneless Orange Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Solubility test Soluble, at 20°, in 1 volume of *ethanol (90 per cent)*.

Weight per millilitre 0.855 to 0.880 g (Appendix 4.9).

Refractive index 1.461 to 1.473, at 20° (Appendix 4.7).

Optical rotation Not more than +60° (Appendix 4.8).

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 1.5 g, omitting the *toluene*, and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 78.76 mg of $C_{10}H_{20}O$.

PEPPERMINT LEAF

Category Carminative.

Peppermint Leaf consists of the dried leaf of *Mentha* × *piperita* L. (Family Lamiaceae). It yields not less than 1.2 mL of volatile oil from each 100 g of drug, calculated on the anhydrous basis.

Origin of plant Peppermint is native to Europe and Middle East and is commercially cultivated in eastern and northern Europe and the United States of America. It is also found in Africa.

Constituents The major constituent of Peppermint Leaf is a volatile oil containing predominantly menthol and menthone. It also contains flavonoids (e.g., luteolin and

rutin), phenolic acids, including rosmarinic acid, and a small amount of triterpenes.

Description Odour, aromatic, penetrating, menthol-like; taste, aromatic, characteristic.

Macroscopical Entire, cut, or broken leaves, thin, fragile, and usually crumpled, green to brownish green, and in some varieties, with brownish purple veins. Entire leaf ovate or lanceolate, 3 to 9 cm long, 1 to 3 cm wide, apex acuminate, base uneven, margin shapely dentate, venation prominently pinnate on the lower surface, with lateral veins leaving the midrib at about 45°. Petiole green or brownish violet, grooved, typically 0.5 to 1 cm long, up to 1 mm in diameter.

Microscopical Powdered drug of Peppermint Leaf shows adaxial epidermis, abaxial epidermis, covering trichomes, glandular trichomes, fragments from near the leaf margin, fragments of dorsiventral mesophyll, and crystals of menthol. Adaxial epidermis: sinuous-wavy walled cells with cuticle striated over the veins associated with palisade parenchyma. Abaxial epidermis with diacytic stomata. Covering trichome: 3 to 8 elongated cells, uniseriate and with striated cuticle. Glandular trichomes of 2 types: 1) unicellular stalk with small rounded unicellular head and 2) unicellular stalk with enlarged oval head consisting of 8 radiating cells. Fragments from near the leaf margin: isodiametric cells with beaded and more or less straight anticlinal walls; covering trichomes, unicellular or bicellular, short, conical. Fragments of dorsiventral mesophyll: a layer of palisade cells and 4 to 6 layers of spongy parenchyma. Crystals of menthol yellowish, present under the cuticle of secretory cells.

Other relevant information Peppermint leaf should not be used in patients with gallstones unless under medical supervision.

Packaging and storage Peppermint Leaf shall be kept in well-closed containers, protected from light.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 2 mg of *luteolin-7-glucoside*, 2 mg of *rutoside trihydrate*, and 5 mg of *rosmarinic acid* in 10 mL of *methanol*.

Test solution To 0.5 g, in *No. 355 powder*, add 2.5 mL of *methanol* and 2.5 mL of *water*, sonicate for 5 minutes, and filter.

Adsorbent *Silica gel F254*.

Mobile phase *Acetic acid, anhydrous formic acid, water,* and *ethyl acetate* (7:7:18:68).

Application Apply 4 µL each of *Standard solution* and *Test solution* as 8-mm bands.

Development and drying Allow the solvent front to ascend 6 cm above the line of application. Dry the developed plate in air.

Detection Heat at 105° for 5 minutes, spray the warm plate with a 1 per cent w/v of *diphenylboric acid aminoethyl ester* in *methanol*, and then with a 5 per cent w/v of *macrogol 400* in *methanol*. Alternatively, dip the warm plate in a 0.5 per cent w/v of *diphenylboric acid aminoethyl ester* in *ethyl acetate,* then in a 5 per cent w/v of *macrogol 400* in *dichloromethane*. Allow to dry in air for about 1 minute and examine the plate under ultraviolet light (366 nm).

Results When examined under ultraviolet light (366 nm), the test solution shows a light blue fluorescent band due to rosmarinic acid in the upper third of the chromatogram, corresponding in colour and R_f to the band shown by the standard solution. Two orange-yellow fluorescent bands, a red fluorescent band in the middle of the chromatogram and an orange-yellow fluorescent band in the lower third of the chromatogram are present.

Two orange fluorescent bands of luteolin-7-glucoside and rutoside obtained from the standard solution appear around the middle of the chromatogram.

Foreign matter Not more than 5 per cent of stem; diameter of the stems, not greater than 1.5 mm; not more than 2 per cent of foreign elements; not more than 8 per cent of leaf showing brown stains due to *Puccinia menthae* (Appendix 7.2); use 10 g.

Total ash Not more than 15.0 per cent w/w (Appendix 7.7).

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Water Not more than 11.0 per cent v/w (Azeotropic Distillation Method, Appendix 4.12); use 20 g.

Assay Carry out the method for the "Determination of Volatile Oil" (Appendix 7.3), using 20 g, accurately weighed. Use 200 mL of *water* as the distillation liquid and a 500-mL round-bottomed flask. Distill at a rate of 2 to 4 mL per minute for 2 hours. Using 0.5 mL of *xylene* in the graduated tube.

PEPPERMINT OIL

Category Carminative; pharmaceutic aid.

Peppermint Oil is obtained by steam distillation from the aerial parts of the flowering plant of *Mentha* × *piperita* L. (Family Lamiaceae).

Constituents Peppermint Oil contains menthol and menthone as the major compounds. Other monoterpenes include cineole, menthyl acetate and limonene.

Description Colourless, pale yellow or pale greenish yellow liquid; odour, characteristic; taste, characteristic followed by a sensation of cold.

Solubility Miscible with *ethanol* and with *dichloromethane*.

Other relevant information It darkens in colour and becomes viscous on keeping. On cooling to a low temperature, separation of menthol occurs, especially when a few crystals of this substance are added to start crystallization.

Packaging and storage Peppermint Oil shall be kept in well-filled, tightly closed containers, protected from light, and at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 50 mg of *menthol*, 20 µL of *cineole*, 10 mg of *thymol*, and 10 µL of *menthyl acetate* in *toluene* and dilute to 10 mL with the same solvent.

Test solution Mix 100 mg of the sample with *toluene* and dilute to 10 mL with the same solvent.

Adsorbent *Silica gel F254* (TLC or HPTLC plate).

Mobile phase *Ethyl acetate* and *toluene* (5:95).

Application Apply 10 µL (or 1 µL) of *Standard solution* and 20 µL (or 2 µL) of *Test solution* as 10-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 15 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (254 nm).

Results A When examined under ultraviolet light (254 nm), the test solution may show a quenching band of carvone or pulegone in the middle of the chromatogram. A quenching band of thymol obtained from the standard solution appears in the middle of the chromatogram.

Detection B Spray the plate with *anisaldehyde TS* and heat at 105° for 5 to 10 minutes. Examine immediately in daylight.

Results B When examined under visible light, the test solution shows a violet-blue band due to menthyl acetate, a violet-blue or brown band due to cineole in the middle of the chromatogram, and an intense blue or violet band due to menthol in the lower third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. An intense violet-red band of hydrocarbons, a brownish yellow band of menthofuran in the upper third of the chromatogram, a greenish blue band of menthone in the middle of the chromatogram are present; a light pink or greyish blue or greyish green band of carvone, pulegone, isomenthone in the middle of the chromatogram may be present.

The standard solution shows a pink band of thymol in the middle of the chromatogram.

The retention time of the peaks due to limonene, cineole, menthone, menthofuran, isomenthone, menthyl acetate, and menthol in the chromatogram of the Peppermint Oil correspond to those in the chromatogram of the Standard solution, as obtained in the *Chromatographic profile*. Isopulegol, pulegone and carvone may be present in the chromatogram.

Relative density 0.900 to 0.916 (Appendix 4.9).

Optical rotation −30° to −10° (Appendix 4.8).

Refractive index 1.457 to 1.467 (Appendix 4.7).

Acid value Not more than 1.4; use 5.0 g (Appendix 5.4).

Chromatographic profile Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution (a) Dissolve 10 µL of *limonene*, 20 µL of *cineole*, 40 µL of *menthone*, 10 µL of *menthofuran*, 10 µL of (+)-*isomenthone*, 40 µL of *menthyl acetate*, 20 µL of *isopulegol*, 60 mg of *menthol*, 20 µL of *pulegone*, 10 µL of *piperitone*, and 10 µL of *carvone* in *heptane* and dilute to 10.0 mL with the same solvent.

Standard solution (b) Dissolve 5 µL of *isopulegol* in *heptane* and dilute to 10.0 mL with the same solvent. Dilute 0.1 mL of the solution to 5.0 mL with *heptane*.

Test solution Mix 0.20 mL of Peppermint Oil with *heptane* and dilute to 10.0 mL with the same solvent.

Chromatographic system

DETECTOR Flame ionization

COLUMN A fused-silica capillary column (60 m × 0.25 mm) packed with *macrogol 20000* (0.25 µm).

TEMPERATURE

Column The temperature programmed is as follows:

Time (Minutes)	Temperature (°)
0-10	60
10-70	60 → 180
70-75	180

Injection port 200°

Detector 220°

CARRIER GAS *Helium*

SPLIT RATIO 1:50

FLOW RATE 1.5 mL per minute

System suitability

SAMPLE *Standard solution (a)*

Suitability requirements

RESOLUTION Not less than 1.5 between limonene and cineole peaks and not less than 1.5 between piperitone and carvone peaks.

Procedure Inject a portion (about 1 mL) of *Standard solution (a)*, *Standard solution (b)* and *Test solution* into the chromatograph. Identify the peaks due to the components in Peppermint Oil, using the retention time determined from the chromatogram obtained from the standard solution (a). Measure the areas of the principal peaks in the chromatogram obtained from the test solution.

Calculation Calculate the percentage content of the components of Peppermint Oil taken by normalization procedure.

Limits

— limonene: 1.0 per cent to 3.5 per cent,
— 1,8-cineole: 3.5 per cent to 8.0 per cent,
— menthone: 14.0 per cent to 32.0 per cent,
— menthofuran: 1.0 per cent to 8.0 per cent,
— isomenthone: 1.5 per cent to 10.0 per cent,
— menthyl acetate: 2.8 per cent to 10.0 per cent,
— isopulegol: not more than 0.2 per cent,
— menthol: 30.0 per cent to 55.0 per cent,
— pulegone: not more than 3.0 per cent,
— carvone: not more than 1.0 per cent,

disregard limit: the area of principal peak in the chromatogram obtained from the standard solution (b) (0.05 per cent).

The ratio of cineole content to limonene content is a minimum of 2.

RHUBARB

Rheum

Thai name โกฐน้ำเต้า (KOT NAM-TAO)

Category Laxative.

Rhubarb consists of the dried underground parts of *Rheum palmatum* L.; *R. tanguticum* Maxim. Ex Balf., *R. officinale* Baill. or their interspecific hybrids, or mixture of these (Family Polygonaceae), separated from the stem, rootlets and most of the bark, frequently divided. It contains not less than 0.25 per cent of sennoside A, $C_{42}H_{38}O_{20}$ calculated on the dried basis.

Origin of plant Rhubarb is native to Asia, specifically China. It is also widely cultivated in China, North Korea and South Korea.

Constituents Rhubarb contains hydroxyanthracene derivatives including emodin, physcione, aloe-emodin, and chrysophanol glycosides. The dimeric sennosides A-F are also its major active principles.

Description Odour, aromatic and characteristic; taste, bitter and slightly astringent.

Macroscopical Ovoid, oblong-ovoid to cylindrical rhizome, often cut transversely or longitudinally, up to 15 cm long, up to 10 cm wide; surface shows a pinkish tinge with a reticulum of darker lines; transversely cut surface shows narrow outer zone; fracture granular.

Microscopical Transverse section of the rhizome shows parenchyma, medullary ray, phloem, xylem vessels. Parenchyma: thin-walled round or polygonal cells with abundant simple or compound starch granules, star-shaped hilum or cluster of calcium oxalate crystals. Medullary ray: 1- to 4-celled cells containing yellowish red or brownish orange amorphous mass. Phloem: sieve tubes, frequently obliterated. Xylem vessel, in group of 2 to 5, reticulate, thickened. Sclereids and fibres absent.

Other relevant information According to its geographical origin, the appearance of rhubarb varies from subcylindrical, barrel-shaped, or irregularly formed pieces to cubes or rectangular pieces known as "rhubarb fingers". It is gritty between the teeth and colours the saliva yellow upon chewing.

Packaging and storage Rhubarb shall be kept in well-closed containers, protected from light and moisture.

Identification

A. To 50 mg of the powdered drug, add 25 mL of 2 M *hydrochloric acid*, heat on a water-bath for 15 minutes, allow to cool, shake with 20 mL of *ether*, separate the ether layer and shake it with 10 mL of 6 M *ammonia*: the aqueous layer turns red.

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 5 mg of *emodin* in 5 mL of *ether*.

Test solution Heat 50 mg of the powdered drug in a water-bath for 15 minutes with a mixture of 1 mL of *hydrochloric acid* and 30 mL of *water*. Allow to cool, add 25 mL of *ether* and shake well. Dry the ether layer over *anhydrous sodium sulfate* and filter. Evaporate the ether layer to dryness and dissolve the residue in 0.5 mL of *ether*.

Adsorbent *Silica gel G*.

Mobile phase *Anhydrous formic acid, ethyl acetate,* and *light petroleum* (1:15:75).

Application Apply 20 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Allow the solvent front to ascend 10 cm above the line of application. Dry the developed plate in air.

Detection Examine the plate under ultraviolet light (366 nm), spray the plate with a 10 per cent w/v solution of *potassium hydroxide* in *methanol* and examine under visible light. Observe the result.

Results When examined under ultraviolet light (366 nm), the test solution shows an orange fluorescent band due to emodin in the middle of the chromatogram

corresponding in R_f to the band shown by the standard solution; above the emodin band, two bands of similar fluorescence (physcione and chrysophanol, in order of increasing R_f value); below the emodin band, also two bands of of similar fluorescence (rhein and aloe-emodin, in order of decreasing R_f value). All the bands become red to violet after spraying with a 10 per cent w/v solution of *potassium hydroxide* in *methanol*.

Rheum rhaponticum Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 10 mg of *rhaponticin* in 10 mL of *methanol*.

Test solution Heat 200 mg of the powdered drug, with 2 mL of *methanol* and boil under a reflux condenser for 5 minutes. Allow to cool and filter. Use the filtrate.

Adsorbent *Silica gel G*.

Mobile phase *Methanol* and *dichloromethane* (20:80).

Application Apply 20 µL each of *Standard solution* and *Test solution* as 3-mm bands.

Development and drying Allow the solvent front to ascend 12 cm above the line of application. Dry the developed plate in air.

Detection Spray the plate with *phosphomolybdic acid TS* and examine under visible light. Observe the result.

Results The chromatogram obtained with the test solution does not show a blue band near the line of application corresponding to that shown in the chromatogram obtained with the standard solution.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° to constant weight (Appendix 4.15).

Acid-insoluble ash Not more than 2.0 per cent w/w (Appendix 7.6).

Total ash Not more than 13.0 per cent w/w (Appendix 7.7).

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Mobile phase Diluted *acetic acid* (1 in 80) and *acetonitrile* (4:1).

Standard preparation A solution containing 50 µg per mL of Sennoside A RS in a 0.1 per cent w/v solution of *sodium hydrogen carbonate*.

System suitability preparation Dissolve 1 mg each of Sennoside A RS and *naringin* in a 0.1 per cent w/v solution of *sodium hydrogen carbonate* to make 10 mL.

Assay preparation Weigh accurately about 500 mg of the powdered Rhubarb, add 50 mL of a 0.1 per cent w/v solution of *sodium hydrogen carbonate*, shake for 30 minutes and filter.

Chromatographic system

DETECTOR Ultraviolet light (340 nm).

COLUMN A stainless steel column (15 cm × 4 to 6 mm), packed with octadecylsilane chemically bonded to porous silica or ceramic microparticles (5 µm).

COLUMN TEMPERATURE 40°

FLOW RATE Adjust so that the retention time of sennosides A is about 15 minutes.

System suitability

SAMPLE *System suitability preparation*

Suitability requirements

RESOLUTION Not less than 3 between sennoside A and naringin peaks.

RELATIVE STANDARD DEVIATION Not more than 1.5 per cent determined from the sennoside A peak.

Procedure Separately inject equal volumes (about 10 µL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the major peaks.

Calculation Calculate the content of $C_{42}H_{38}O_{20}$ in the portion of the Rhubarb taken, using the declared content of $C_{42}H_{38}O_{20}$ in Sennoside A RS.

RHUBARB, POWDERED

Thai name โกฐน้ำเต้าผง (KOT NAM-TAO PHONG)

Complies with the requirements for content of sennoside A, Packaging and storage, Identification, *Rheum rhaponticum*, Loss on drying, Acid-insoluble ash, and Total ash stated under Rhubarb and with the following requirement.

Description Orange or yellow-brown. Diagnostic structures: calcium oxalate cluster crystals, up to 100 µm or more in diameter, or their fragments; unlignified, reticulately thickened vessels, 20 to 175 µm in width, mostly 60 to 100 µm; starch granules abundant; sclereids and fibres absent.

ROSE OIL

Thai name น้ำมันกุหลาบ (NAMMAN KULAP)

Category Pharmaceutic aid.

Rose Oil is the volatile oil distilled with steam from the fresh flowers of *Rosa gallica* L., *Rosa* × *damascena* Herrm., *R. alba* L., *R. centifolia* L., and varieties of these species (Family Rosaceae).

Origin of plant Rose Oil-yielding plants (*Rosa gallica* L.) are native to Mediterranean to East Central, East Europe, and Caucasus. While *Rosa gallica* L. is a naturally occurring plant, other plants species-yielding Rose Oil are artificially crossed. In other words, *Rosa* × *damascena* Herrm. is an artificial hybrid between *R. gallica* L. and *R. moschata* Herrm. in Europe; *Rosa alba* L. is the hybrid between *Rosa gallica* L. and *Rosa corymbifera* Borkh., both hybrid species found in Europe. Another

specie yielding Rose Oil is *Rosa centifolia* L., with the hybrid formula of *Rosa canina* × *R. gallica* × *R. moschata*.

Constituents Rose Oil contains predominantly geraniol and citronellol. It also contains other monoterpenes such as α-pinene, sabinene, β-pinene and linalool, flavonoid glycosides, mucilages, etc.

Description Colourless or yellow liquid; odour, fragrant; taste, characteristic of rose. At 25°, it is a viscous liquid. Upon gradual cooling, it changes to a translucent, crystalline mass, easily liquefied by warming.

Packaging and storage Rose Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Solubility test One mL is miscible with 1 mL of *chloroform* without turbidity. Add 20 mL of *ethanol (90 per cent)* to this mixture: the resulting liquid is neutral or acid to moistened *litmus paper* and, upon standing at 20°, deposits crystals within 5 minutes.

Specific gravity 0.848 to 0.863, at 30° compared with *water* at 15° (Appendix 4.9).

Refractive index 1.457 to 1.463, at 30° (Appendix 4.7).

Optical rotation –1° to –4° (Appendix 4.8).

ROSEMARY OIL

Thai name น้ำมันโรสแมรี (NAMMAN ROSEMARY)

Category Pharmaceutic aid.

Rosemary Oil is the volatile oil distilled with steam from the flowering aerial parts of *Rosmarinus officinalis* L. (Family Lamiaceae).

Origin of plant Rosemary Oil-yielding plant is native to Mediterranean region, Turkey, and some eastern European countries such as Albania.

Constituents Rosemary Oil contains monoterpenes and their esters such as borneol, bornyl acetate, camphene, 1,8-cineole, limonene, and pinene. It also contains bitter substances and flavonoids.

Description Colourless to pale yellow liquid; odour aromatic, charcteristic; taste bitter and camphor-like.

Other relevant information Oral use is not recommended for pregnant and nursing women.

Packaging and storage Rosemary Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 50 mg of *borneol* and 50 mg of *bornyl acetate* and 100 µL of *cineole* in *toluene* and dilute to 10 mL with the same solvent.

Test solution Dissolve 0.5 mL of the sample in *toluene* and dilute to 10 mL with the same solvent.

Adsorbent *Silica gel G.*

Mobile phase *Ethyl acetate* and *toluene* (5:95).

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate in air.

Detection Spray the plate with *vanillin-sulfuric acid TS1*, heat at 105° for 10 minutes, and examine immediately in daylight.

Results When examined in daylight, the test solution shows a violet-blue band due to borneol and a dark blue band due to cineole in the lower third of the chromatogram and a pale bluish grey band due to bornyl acetate in the middle of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. A violet-pink band in the middle of the chromatogram, a violet-grey band and a dark violet band in the upper third of the chromatogram are present.

Solubility test Soluble, at 20°, in 1 part of *ethanol (90 per cent)*.

Relative density 0.893 to 0.910 (Appendix 4.9).

Refractive index 1.464 to 1.473, at 20° (Appendix 4.7).

Optical rotation 5° to +8° (Appendix 4.8).

Acid value Not more than 1.0 (Appendix 5.4).

SENEGA ROOT

Senega

Category Expectorant.

Senega Root consists of the dried, whole or fragmented root and root crown of *Polygala senega* L. or root of *Polygala tenuifolia* Willd., with rootlets removed (Family Polygalaceae).

Origin of plant Senega is native to North America, including eastern Canada and north-eastern United States of America.

Constituents Senega Root contains triterpene saponins as its major components. It also contains methyl salicylate.

Description Odour, mild, sweet, slightly rancid, or reminiscent of methyl salicylate; taste, sweet at first, with acrid aftertaste which irritating to the throat.

Macroscopical

Polygala senega L. Dried whole or fragmented of root

and root crown, with rootlets removed. A whole root crown greyish brown and distinctively wider than the root, forming an irregular head of up to 3 cm in diameter consisting of numerous tightly packed remnants or scars of stems and violet-brown buds. The tap roots are flexuous and tortuous, brown or yellow, single or 2- to 3-branched, 1 to 8 mm in diameter at the crown, tapering to the apex; externally transversely and longitudinally striated with more or less decurrent spiral ridge; internally paler yellow.

Polygala tenuifolia Willd. Dried whole root or fragment of cylindrical slice of a root with rootlets removed. Whole root straight or curved, 3 to 15 cm long, 3 to 8 mm in diameter, pale yellowish brown or brown with numerous transverse wrinkles and fissures and lesser longitudinal striations; round rootlet scars are also often visible; xylem light yellow and readily detached, with some fragments consisting of rolls of bark and their folded inwards edges. The fracture shows a yellowish cortex and a pale yellow, usually circular, central woody area. Fragmented root: externally yellowish grey to brownish grey; internally solid or hollow in the central part where the xylem has detached.

Microscopical

Polygala senega L. Transverse section of the root is more or less triangular, showing cortex and central woody area. Cortex, yellowish, with different degree of thickness surrounding the woody area. Woody area, pale yellow, irregular in shape, especially at the ridge.

Powdered drug of Senega Root from *Polygala senega* possesses the microscopical characteristic of the unground drug. Fragments of lignified tissues made up of a number of tracheids and slightly larger reticulate and bordered-pitted vessels, yellowish parenchyma containing oil droplets, and fragments of cork which occasionally accompanied by phelloderm, and parenchyma some containing oil droplets are observed. Epidermis from bud scales with stomata and unicellular trichomes may also be found.

Polygala tenuifolia Willd. Transverse section of the root shows a yellowish brown outer part and a round, pale yellow central woody area.

Powdered drug of Senega Root from *Polygala tenuifolia* possesses the microscopical characteristic of the unground drug and is mostly similar to that of the powdered drug of Senega Root from *P. senega*. However, calcium oxalate cluster crystal tissues, isolated or included in parenchyma cells, and long, fine thick-walled lignified fibres, most often fragmented, in cluster or associated with vessels, are also found.

Other relevant information

Caution should be exercised when using senega root in pregnant women.

Since solid evidence on the use of senega root in nursing mothers and pediatrics has yet been established. Senega root should not be administered during lactation or to children without medical supervision.

It is recommended to seek medical advice if coughing persists after 7 days of use.

Senega root may exacerbate existing gastro-intestinal inflammation such as gastritis or gastric ulcers, and excessive consumption may cause vomiting. Therefore, caution should be exercised when using the drug in patients with the said conditions.

Packaging and storage Senega Root shall be kept in well-closed containers, protected from light and moisture.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution (a) Dissolve 1 mg of *rutoside trihydrate* and 30 mg of *puerarin* in *methanol* and dilute to 25.0 mL with the same solvent.

Standard solution (b) Dilute 2.5 mL of *Standard solution (a)* to 10.0 mL with *methanol*.

Standard solution (c) Dissolve 2 mg of *chlorogenic acid* and 30 mg of *puerarin* in *methanol* and dilute to 25.0 mL with the same solvent.

Test solution To 500 mg, in *No. 355 powder*, add 5.0 mL of *ethanol (70 per cent)*. Sonicate at 80° for 15 minutes, filter or centrifuge and use the filtrate or supernatant.

Adsorbent *Silica gel F254*.

Mobile phase *Anhydrous formic acid*, *glacial acetic acid*, *water*, and *ethyl acetate* (11:11:26:100).

Application Apply 3 µL of *Standard solutions* and *Test solution* as 8-mm bands.

Development and drying Allow the solvent front to ascend 7 cm above from the lower edge of the plate. Dry the developed plate at 105° for 3 minutes.

Detection Spray the warm plate with a 1 per cent w/v solution of *diphenylboric acid aminoethyl ester* in *methanol*, then with a 5 per cent w/v solution of *macrogol 400* in *methanol* or, alternatively, dip the warm plate in a 0.5 per cent w/v solution of *diphenylboric acid aminoethyl ester* in *ethyl acetate*, then in a 5 per cent w/v of *macrogol 400* in *dichloromethane*, allow the plate to dry in air for about 1 minute and examine under ultraviolet light (366 nm).

Results for *Polygala senega* L. When examined under ultraviolet light (366 nm), the test solution shows a faint, orange fluorescent band of rutoside in the middle and a blue fluorescent band of puerarin in the upper third of the chromatogram, corresponding in R_f and colour to the bands shown by the standard solution. Other blue fluorescent bands may be present

in the middle and the lower third of the chromatogram.

Results for *Polygala tenuifolia* Willd. When examined under ultraviolet light (366 nm), the test solution shows a blue fluorescent band due to puerarin in the upper third of the chromatogram, corresponding in R_f and colour to the band shown by the standard solution. A greenish blue fluorescent band at the border between the middle and lower third and other blue fluorescent bands in the middle and the lower third of the chromatogram are present.

Loss on drying Not more than 12.0 per cent w/w after drying at 105° for 2 hours; use 1 g, in *No. 355 powder* (Appendix 4.15).

Acid-insoluble ash Not more than 3.0 per cent w/w (Method II, Appendix 7.6).

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

Extractable matter Not less than 20 per cent w/w. To about 1 g, in *No. 355 powder* and accurately weighed, add a mixture of 4.5 g of *ethanol* and 15.5 g of *water* and allow to macerate for 3 hours, shaking frequently. Filter. Evaporate 5.0 g of the filtrate to dryness on a water-bath and dry at 105° for 2 hours. The residue weighs a minimum of 50 mg.

Foam index Not more than 3.5; use 1 g, in *No. 355 powder*. (Appendix ==)

SENEGA ROOT, POWDERED

Senega, Powdered

Complies with the requirements for Packaging and storage, Identification, Loss on drying, Acid-insoluble ash, Total ash, Extractable matter, and Foam index stated under Senega Root and with the following requirement.

Description Light brown; odour, irritant and sternutatory. Powdered drug of Senega Root possesses the microscopical characteristic of the unground drug. Fragments of lignified tissues made up of a number of tracheids and slightly larger reticulate and bordered-pitted vessels, yellowish parenchyma containing oil droplets, and fragments of cork which occasionally accompanied by phelloderm, and parenchyma some containing oil droplets are observed. Epidermis from bud scales with stomata and unicellular trichomes may also be found.

SENEGA LIQUID EXTRACT

Category Expectorant.

Senega Liquid Extract may be prepared as follows.

Senega Root, in *moderately coarse powder* 1000 g
Dilute Ammonia Solution a sufficient quantity
Ethanol (60 Per Cent) a sufficient quantity

Exhaust the Senega in *moderately coarse powder* with Ethanol (60 Per Cent) by *percolation* (Appendix 7.18). Reserve the first 800 mL of percolate; evaporate the subsequent percolate to the consistency of a soft extract, and dissolve it in the reserved portion. Add sufficient *dilute ammonia solution* to make the product slightly alkaline, and add sufficient Ethanol (60 Per Cent) to produce 1000 mL. Allow to stand for not less than 48 hours and filter.

Dose 0.3 to 1 mL.

Weight per milliliter 1.03 to 1.05 g (Appendix 4.9).

Ethanol content 38.0 to 42.0 per cent v/v (Method II, Appendix 6.5).

Total solids 26.0 to 34.0 per cent w/v (Appendix 7.21); use 500 mg.

Other requirements Complies with the requirements described under "Extracts" (Appendix 1.16).

SENEGA TINCTURE

Category Expectorant.

Senega Tincture may be prepared as follows.

Senega Liquid Extract 200 mL
Ethanol (60 Per Cent) to 1000 mL

Mix, allow to stand for not less than 12 hours, and filter.

Dose 2.5 to 5 mL.

Weight per millilitre 0.930 to 0.940 g (Appendix 4.9).

Ethanol content 54.0 to 58.0 per cent v/v (Appendix 6.5).

Total solids 4.9 to 7.0 per cent w/v (Appendix 7.21); use 5 mL.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

SPEARMINT

Thai name สะระแหน่ญวน (SARANAE YUAN)

Category Pharmaceutic aid (flavouring agent).

Spearmint consists of the dried leaf and flowering top of *Mentha spicata* L. (*Mentha crispa* L.) or of *Mentha* × *cardiaca* (Gray) Bak. (Family Lamiaceae).

Origin of plant Spearmint is native to Europe and Asia.

Constituents The major constituent of Spearmint is a volatile oil containing predominantly carvone.

Description Odour, aromatic, and characteristic; taste slightly pungent and characteristic, not followed by a cooling sensation in the mouth.

Macroscopical More or less crumpled. Leaves sessile or subsessile, opposite, ovate-lanceolate blade, 3 to 7 cm long, with an acute or acuminate apex; base of the leaf, narrowed or rounded; margin, sharply and unequally serrate; colour, bright green; stems, nearly glabrous, sometimes green, but generally dusky red, or purplish colour, oppositely branched, quadrangular, ridged, and from 1 to 3 mm in diameter. Verticillasters, occurring on more or less crowded or interrupted, lanceolate, terminal spikes, 4 to 10 mm in diameter; bracts, linear-lanceolate, subulate, ciliate, 7 to 10 mm long; calyx, campanulate (*M. spicata*) to tubular-campanulate (*M* × *cardiaca*), subequally five-toothed, glandular-punctate, pubescent on teeth, green (*M. spicata*) to purplish red (*M* × *cardiaca*); corolla, glabrous, nearly white or from moderate yellowish brown to weak yellowish orange to pinkish to lilac and purple, tubular-campanulate; stamens in fours, equal; style, exserted, two-cleft at the summit; nutlets, ellipsoidal, minutely grained ((*M spicata*)or warty (*M* × *cardiaca*)), dark brown, about 500 µm in diameter.

Microscopical Green to olive-green. It closely resembles powdered peppermint except for the absence of crystals from the globular heads of the glandular hairs and for the nonglandular hairs which are usually up to six-celled.

Packaging and storage Spearmint shall be kept in well-closed containers and protected from light.

Foreign matter Not more than 2.0 per cent w/w of stems over 3 mm in diameter or other foreign matter (Appendix 7.2).

SPEARMINT OIL

Thai name น้ำมันสะระแหน่ญวน (NAMMAN SARANAE YUAN)

Category Pharmaceutic aid (flavouring agent).

Spearmint Oil is obtained by steam distillation from the fresh, overground parts of the flowering plants of *Mentha spicata* L. and *Mentha* × *cardiaca* (Gray) Bak. (Family Lamiaceae).

Constituents Spearmint Oil contains predominantly carvone. It also contains limonene, pulegole, cineole, etc.

Description Clear colourless, pale yellow, or greenish yellow liquid when recently distilled, but becoming darker and viscous on keeping; odour, that of spearmint.

Labelling The label on the container states whether the oil is American-type oil or Chinese-type oil.

Identification The retention time of the principal peaks in the chromatogram of the Spearmint Oil correspond to those in the chromatogram of the Standard solution, as obtained in the *Chromatographic profile*.

Packaging and storage Spearmint Oil shall be kept in well-filled, tightly closed containers, protected from light, and at a temperature below 25°.

Weight per millilitre American-type oil 0.917 to 0.934; Chinese-type oil 0.935 to 0.952 (Appendix 4.9).

Optical rotation American-type oil −45° to −60 °; Chinese-type oil −50° to −62° (Appendix 4.8).

Refractive index 1.484 to 1.491, at 20° (Appendix 4.7).

Solubility test Soluble, at 20°, in 1 volume of *ethanol (80 per cent)*; the solution may become cloudy when diluted.

Chromatographic profile Carry out the test as described in the "Gas Chromatography" (Appendix 3.4).

Standard solution Mix carefully 0.1 g of *limonene*, 0.2 g of *1,8-cineole*, 0.4 g of *menthone*, 0.1 g of *(+)-isomenthone*, 0.4 g of *menthyl acetate*, 0.2 g of *pulegone*, 0.6 g of *menthol*, and 0.1 g of *carvone* with 1 g of *hexane*.

Test solution Use Spearmint Oil.

Chromatographic system

DETECTOR Flame ionization

COLUMN A glass capillary column (25 m to 60 m × 0.25 mm) packed with *macrogol 20000* (0.25 µm) (Carbowax 20M® or equivalent is suitable).

TEMPERATURE

Column The temperature programme is as follows:

Time (Minutes)	Temperature (°)
0-6	55
6-38	55 → 180
38-40	180

Injection port 220°

Detector 230°

CARRIER GAS *Helium*

FLOW RATE 1.5 mL per minute.

System suitability

SAMPLE *Standard solution.*

Suitability requirements

COLUMN EFFICIENCY Not less than 30,000 theoretical plates for the limonene peak in the chromatogram of the standard solution.

RESOLUTION Not less than 1.5 between limonene and cineole peaks.

Procedure Inject a portion (about 0.1 mL) of *Standard solution* and Spearmint Oil into the chromatograph. Identify the peaks due to the components in Spearmint

Oil, using the retention times determined from the chromatogram obtained from the standard solution. Measure the areas of the principal peaks in the chromatogram obtained from the Spearmint Oil.

Calculation Calculate the percentage content of the components of Spearmint Oil taken by normalization procedure.

Limits

- limonene: 2.0 per cent to 25 per cent,
- cineole: not more than 2.5 per cent,
- menthone: not more than 2.5 per cent,
- isomenthone: not more than 1.0 per cent,
- menthyl acetate: not more than 1.0 per cent,
- pulegone: not more than 0.5 per cent,
- menthol: not more than 2 per cent,
- carvone: not less than 55 per cent.

SQUILL

Scilla

Category Expectorant.

Squill consists of the bulb of *Drimia maritima* (L.) Stearn [*Urginea maritima* (L.) Baker] (Family Asparagaceae), collected soon after the plant has flowered, divested of its dry outer, membranous coats, cut into transverse slices and dried. It is known in commerce as white squill.

Origin of plant Squill is native to Mediterranean region and the Canary Islands.

Constituents Squill contains steroid glycosides, namely bufadienolides, most importantly scillaren A and proscillaridin A. It also contains flavone glycosides such as vitexin and orientin, sterols, and mucilage.

Description Odourless or almost odourless.

Macroscopical Transverse slices, occurring as straight or curved triangular pieces, 5 to 50 mm long, 3 to 8 mm wide at mid-point, tapering towards each end, 5 to 8 mm thick, yellowish white, texture horny, somewhat translucent, breaking with an almost glassy fracture when quite dry, but becoming tough and flexible when absorbing moisture. Transversely cut surface showing a row of prominent vascular bundles near the concave edge and numerous smaller bundles scattered throughout the mesophyll.

Microscopical Powdered drug of Squill shows epidermis, mesophyll and vascular bundles. Epidermis: axially elongated polygonal cells; cuticle stratified; stomata very rare, anomocytic, circular in outline. Mesophyll: colourless thin-walled parenchyma cells containing, many of which containing bundles of acicular crystals of calcium oxalate embedded in mucilage; the rests containing sinistrin, and very occasionally starch granules. Vascular bundles: collateral, scattered throughout the mesophyll; xylem vessels with spiral and annular wall thickening; trichome absent.

Other relevant information

It is contra-indicated in patients who are on digitalis glycosides, and those with potassium deficiency, hypercalcemia, or hyperkalemia.

It is contra-indicated in pregnant women as it may lead to spontaneous abortion.

The plant, when overdosing, is an irritant poison that may cause nausea and vomiting, bloody urine, convulsions, and even death from paralysis of the heart in systole.

Packaging and storage Squill shall be stored in a dry place at a temperature not exceeding 25°.

Identification The mucilage contained in the cells of the mesophyll is stained red with *alkaline corallin TS* but produces no red colour with *ruthenium red TS* and no purple colour with 0.01 M *iodine*.

Acid-insoluble ash Not more than 1.5 per cent w/w (Appendix 7.6).

Ethanol (60 per cent)-soluble extractive Not less than 68.0 per cent w/w (Appendix 6.5). Use the powdered drug, previously dried at 105° for 1 hour.

SQUILL, POWDERED

Complies with the requirements for Identification, Acid-insoluble ash and Ethanol (60 per cent)-soluble extractive described under Squill and with the following requirements.

Description White or yellowish white; very hygroscopic. Diagnostic structures: abundant, acicular crystals of calcium oxalate, the larger ones frequently broken, some embedded in mucilage which is stained red with *alkaline corallin TS* but produces no colour with 0.01 M *iodine*; lignified vessels with spiral and anular thickening; fragments of epidermis with very infrequent stomata.

Packaging and storage Powdered Squill shall be kept in tightly closed containers with suitable desiccants, protected from light, and stored at a temperature not exceeding 25°.

INDIAN SQUILL

Urginea

Category Expectorant.

Indian Squill is the bulb of *Drimia indica* (Roxb.)

Jessop [*Urginea indica* (Roxb.) Kunth] (Family Asparagaceae), collected soon after the plant has flowered, divested of dry, outer membranous coats and usually cut longitudinally into slices and dried.

Origin of plant Indian Squill is native to India, particularly Western Himalayas, Bihar, Konkan, and along the Coromandel Coast.

Constituents Indian Squill contains steroid glycosides such as scillarens A and B. It also contains flavonoids, sterols, alcohols, fatty acids, and mucilage.

Description Odourless or almost odourless.

Macroscopical Curved or irregularly shaped strips, 10 to 50 mm long, 3 to 10 mm wide, 1 to 3 mm thick, often tapering towards each end, occasionally grouped tree or four to a portion of the axis; ridged in the direction of their length and varying in colour, from pale yellowish brown to buff; brittle when dry, but tough and flexible when exposed to air.

Microscopical Powdered drug of Indian Squill shows epidermis, mesophyll and vascular bundles. Epidermis: thin-walled tetrahedral to hexahedral cells, three to five times longer than wide; cuticle striated, thick; stomata rare, anomocytic, circular in outline. Mesophyll: thin-walled polygonal cells containing mucilage and some containing bundles of acicular crystals of calcium oxalate. Vascular bundles: collateral, scattered throughout the mesophyll; xylem vessels with spiral and annular wall thickening; trichomes and starch absent.

Other relevant information

It is contra-indicated in patients who are on digitalis glycosides.

Consuming in large doses may cause nausea and vomiting.

Packaging and storage Indian Squill shall be stored in a dry place at a temperature not exceeding 25°.

Identification The mucilage contained in the cells of the mesophyll is stained red with *alkaline corallin TS* and reddish purple with 0.01 M *iodine*.

Total ash Not more than 6.0 per cent w/w (Appendix 7.7).

SQUILL, INDIAN, POWDERED

Complies with the requirements for Identification and Total ash described under Indian Squill and with the following requirements.

Description Buff coloured; very hygroscopic. Diagnostic structures: numeours acicular crystals of calcium oxalate, the larger ones frequently broken; abundant cells containing mucilage, some with embedded calcium oxalate crystals; the mucilage is stained red with *alkaline corallin TS* and reddish purple with 0.01 M *iodine*; lignified vessels with spiral and annular thickening; fragments of epidermis with very infrequent stomata.

Packaging and storage Powdered Indian Squill shall be kept in tightly closed containers with suitable desiccants, protected from light, and stored at a temperature not exceeding 25°.

SQUILL TINCTURE

Category Expectorant.

Squill Tincture may be prepared as follows.

Squill, brused	100 g
Ethanol (60 Per Cent)	1000 mL

Prepare by *maceration* (Appendix 7.18).

Dose 0.3 to 2 mL.

Warning Large doses produce nausea, vomiting, and diarrhea.

Additional information Squill has a digitalis-like action on the heart.

Weight per millilitre 0.925 to 0.945 g (Appendix 4.9).

Ethanol content 52.0 to 57.0 per cent v/v (Appendix 6.5).

Total solids 4.5 to 6.5 per cent w/v (Appendix 7.21); use 5 mL.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

STORAX

Category Expectorant; antiseptic; pharmaceutic aid.

Storax is a balsam obtained from the trunk of *Liquidambar orientalis* Mill., known in commerce as Levant Storax, or of *Liquidambar styraciflua* L. known in commerce as American Storax (Family Altingiaceae).

Origin of plant Levant Storax-yielding plants are native to East Aegean Islands to west and southwest Turkey. An American Storax-yielding plant is native to North America, Central America and northern South America.

Constituents Levant Storax contains styrene, cinnamic acid and its esters such as cinnamyl cinnamate or styracin. It also contains triterpene acids, vanillin, and aromatic alcohols. American Storax contains styresinol, both free and as cinnamic acid esters, free cinnamic acid, styracin, vanillin, styrol, etc.

Description Odour balsamic, characteristic; taste acrid.

Levant Storax: semiliquid, greyish to greyish brown, opaque mass depositing on a heavy dark brown layer. American Storax: semisolid or sometimes solid mass, softened when gently warmed.

Solubility Insoluble in *water*. Soluble, usually incompletely, in an equal weight of warm *ethanol*. Soluble in *acetone*, in *carbon disulfide*, and in *ether*, some insoluble residue usually remaining.

Packaging and storage Storax shall be kept in well-closed containers, at a temperature not exceeding 25°.

Loss on drying Not more than 20.0 per cent w/w after drying 2 g at 105° for 2 hours (Appendix 4.15).

Ethanol-insoluble substances Not more than 5.0 per cent w/w, determined by the following method. Accurately weigh about 10 g of mixed Storax in a beaker, heat at 105° for 30 minutes, take up the residue in 100 mL of hot *ethanol*, filter through counter-balanced filters or a tared filter crucible, and wash the residue with small portions of hot *ethanol* until the last washing is colourless or practically so. Dry the residue at 105° for 1 hour and weigh.

Ethanol-soluble substances Not less than 70.0 per cent w/w, determined by the following method. Evaporate the combined ethanol filtrate and washings obtained in the test for *Ethanol-insoluble substances* at a temperature not exceeding 60°. Dry the residue at 105° for 1 hour and weigh the yellow to brown residue of purified Storax so obtained.

Acid value, Saponification value, Cinnamic acid The purified Storax obtained in the test for *Ethanol-soluble substances* meets the requirements of the following tests.

Acid value 50 to 85 for Levant Storax and 36 to 85 for American Storax, determined by the following method. Dissolve about 1 g of the purified Storax, accurately weighed, in 50 mL of *neutralized ethanol*, add 0.5 mL of *phenolphthalein TS*, and titrate with 0.5M *sodium hydroxide VS*.

Saponification value 160 to 200, determined by the following method. Place about 2 g of the purified Storax, accurately weighed, in a 250-mL flask, mix it with 50 mL of *petroleum ether (boiling range, 40° to 60°)*, add 25.0 mL of 0.5 M *ethanolic potassium hydroxide VS*, and allow the mixture to stand for 24 hours with frequent agitation. Then add 0.5 mL of *phenolphthalein TS* and titrate the excess alkali with 0.5 M *hydrochloric acid VS*.

Cinnamic acid Not less than 25 per cent of the weight of purified Storax taken, determined by the following method. Add about 2 g of the purified Storax, accurately weighed, to 25 mL of 0.5 M *ethanolic potassium hydroxide*, and boil the mixture for 1 hour under a reflux condenser. Add 0.5 mL of *phenolphthalein TS*, neutralize with 0.25 M *sulfuric acid*, and evaporate the ethanol on a water-bath. Dissolve the residue in 50 mL of *water*, and shake the solution with 20 mL of *ether*. Shake the separated ether with 5 mL of *water*, adding the washing to the aqueous solution, and reject the ether extract. Add to the aqueous solution 10 mL of *dilute sulfuric acid*, and shake with four 20-mL portions of *ether*. Wash the combined ether extracts with 5 mL of *water*, rejecting the aqueous washing, transfer to a flask, and distil off the ether. Add to the residue 100 mL of *water* and boil the mixture vigorously for 15 minutes under a reflux condenser. Filter while hot and allow the filtrate to cool to about 25°: white crystals of cinnamic acid separate. Collect and dry the cinnamic acid by vacuum filtration. Repeat the extraction of the residue twice by boiling each time under a reflux condenser, as previously described, with the filtrate from the preceding crystallization, and collect the additional cinnamic acid in the same crucible. Finally wash the cinnamic acid with two 10-mL portions of ice-cold *water*, dry at 80°, and weigh. A portion of the acid recrystallized from hot water melts between 134° and 135°.

To about 50 mg of the cinnamic acid obtained as directed above add 5 mL of 1 M *sulfuric acid*, heat, and add *potassium permanganate TS*: the odour of benzaldehyde is perceptible.

STRAMONIUM

Thai name ลำโพงฝรั่ง (LAMPHONG FA-RANG)

Category Anticholinergic; antispasmodic.

Stramonium consists of the dried leaf or the dried leaf, flowering tops and occasionally fruits of *Datura stramonium* L. and its varieties (Family Solanaceae). It contains not less than 0.25 per cent of total alkaloids, calculated as hyoscyamine with reference to the material dried to constant weight at 100° to 105°. The alkaloids consist mainly of those of the hyoscyamine-atropine group together with hyoscine.

Origin of plant Stramonium is native to Central America and the Caribbean.

Constituents Stramonium contains alkaloids, predominantly hyoscyamine, hyoscine and atropine. It also contains flavonoids, coumarins, tannins, and glycosides.

Description Odour unpleasant; taste bitter.

Macroscopical Leaf dark brownish green or dark greyish green, ovate or triangular-ovate, apex acuminate, base often unequal, margin dentate, pubescent along veins when young, becoming nearly glabrous with aged, much twisted and shrunken during drying, thin and brittle; petiole short. Stem green or purplish green, slender, curved and twisted, longitudinally wrinkled,

sometimes transversely wrinkled, dichasially branched, with a single flower or an immature fruit in the fork. Flower: calyx 5-lobed, gamosepalous, 5-lobed; corolla brownish white or purplish, trumpet-shaped; pedicel short. Fruit a capsule, usually covered with numerous short, stiff emergences; seeds brown or black with minutely pitted testa.

Microscopical Powdered drug of the leaf shows fragments of upper and lower epidermises in surface view, anisocytic and anomocytic stomata, fragments of covering trichomes, glandular trichomes, mesophyll, fragments of spongy parenchyma, fibres and reticulate vessels of stems, pollen grains, fragments of corolla, and fragments of seeds. Fragments of upper and lower epidermises in surface view: slightly wavy anticlinal-walled cells and smooth cuticle accompanied by palisade and spongy parenchyma. Anisocytic and anomocytic stomata present more frequently in lower epidermis. Fragments of covering trichomes conical, uniseriate, with 3 to 5 warty-walled cells, some of which collapsed. Glandular trichomes short and clavate, with 2- to 7-celled heads. Mesophyll, in transverse view, dorsiventral, consisting of a single layer of palisade cells and a spongy parenchyma containing cluster of calcium oxalate crystals. Fragments of spongy parenchyma, some containing small clusters of calcium oxalate crystals associated with annularly and spirally thickened vessels, in surface view. Pollen grains, subspherical. Fragments of corolla: wavy-walled cells and underlying mesophyll, some containing prismatic or cluster crystals of calcium oxalate. Fragments of seeds containing yellowish-brown sinuous, thick-walled sclereids of testa and, occasionally, prismatic and microsphenoidal crystals of calcium oxalate.

Other relevant information It should be used with caution in patients with CNS depressants.

Packaging and storage Stramonium shall be kept in well-closed containers, protected from light.

Identification

A. Shake 1 g of powdered drug with 10 mL of 0.05 M *sulfuric acid* for 2 minutes, filter, add 1 mL of *strong ammonia solution* and 5 mL of *water* to the filtrate, extract cautiously with 15 mL of *peroxide-free ether* to avoid the formation of an emulsion, dry the ether layer over *anhydrous sodium sulfate*, and filter. Evaporate the ether in a porcelain dish, add 0.5 mL of *nitric acid* and evaporate to dryness on a water-bath. Add 10 mL of *acetone* and, dropwise, a 3 per cent w/v solution of *potassium hydroxide* in *ethanol*: a deep violet colour is produced.

B. Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 50 mg of *hyoscyamine sulfate* in 9 mL of *methanol* (solution A) and dissolve 15 mg of *hyoscine hydrobromide* in 10 mL of *methanol* (solution B); mix 3.8 mL of solution A with 4.2 mL of solution B and dilute to 10 mL with *methanol*.

Test solution To 1.0 g of the powdered drug, add 10 mL of 0.05 M *sulfuric acid*, shake for 15 minutes, and filter. Wash the filter with 0.05 M *sulfuric acid* until 25 mL of filtrate is obtained. To the filtrate add 1 mL of *strong ammonia solution* and shake with two 10-mL portions of *peroxide-free ether*. If necessary, separate by centrifugation. Dry the combined ether layers over *anhydrous sodium sulfate*, filter, and evaporate to dryness on a water-bath. Dissolve the residue in 0.5 mL of *methanol*.

Adsorbent *Silica gel G*.

Mobile phase *Strong ammonia solution, water, acetone* (3:7:90).

Application Apply 10 µL and 20 µL each of *Standard solution* and *Test solution* as bands of 2 cm long and 0.3 cm wide, leaving 1 cm between the bands.

Development and drying Allow the solvent front to ascend 10 cm above the line of application. Dry the developed plate at 105° for 15 minutes; allow to cool.

Detection A Spray the plate with *acetic potassium iodobismuthate TS*, using about 10 mL for a plate 20 cm square, until the orange or brown zones become visible against a yellow background. Observe the result. Examine the plate in daylight.

Results A The test solution shows a band due to hyoscyamine in the lower third and a band due to hyoscine in the upper third of the chromatograms, corresponding in size, colour and R_f to the bands shown by the standard solution of the same volume applied. Faint secondary bands may appear particularly in the middle of the chromatogram obtained from 20 µL of the test solution or near the point of application in the chromatogram obtained from 10 µL of the test solution.

Detection B Spray the plate with *sodium nitrite TS* until transparent. Observe the result after 15 minutes. Examine the plate in daylight.

Results B The bands due to hyoscyamine in the chromatogram obtained from the test solution change from brown to reddish brown but not to greyish blue (atropine), and any secondary bands disappear.

Foreign matter Not more than 3.0 per cent w/w of stem with a diameter exceeding 5 mm (Appendix 7.2).

Acid-insoluble ash Not more than 4.0 per cent w/w (Appendix 7.6).

Total ash Not more than 20.0 per cent w/w (Appendix 7.7).

Assay Reduce 50 g, selected from a well-mixed sample, to *fine powder* and determine the moisture content of 2 g

by drying to constant weight at 105°. Moisten 10 g, accurately weighed, with a mixture of 5 mL of 10 M *ammonia*, 10 mL of *ethanol* and 30 mL of *peroxide-free ether* and mix thoroughly. Transfer the mixture to a small percolator with the aid of the extracting mixture if necessary, allow to macerate for 4 hours, and then percolate with a mixture of 1 volume of *chloroform* and 3 volumes of *peroxide-free ether* until complete extraction of the alkaloids is effected (Appendix 7.4). Concentrate the percolate to about 50 mL by distillation on a water-bath and transfer to a separator with the aid of *peroxide-free ether*. Add a quantity of *peroxide-free ether* at least 2.1 times the volume of the percolate to produce a liquid considerably less dense than water. Extract the solution with not less than three 20-mL portions of 0.25 M *sulfuric acid*, separating the layers by centrifugation if necessary, and transfer each acid extract to a second separator. Make the combined extracts alkaline with 10 M *ammonia* and extract with three 30-mL portions of *chloroform*. Combine the chloroform extracts, add 4 g of *anhydrous sodium sulfate* and allow to stand for 30 minutes, shaking occasionally. Decant the chloroform and wash the sodium sulfate with three 10-mL portions of *chloroform*. Evaporate the combined chloroform extracts and washings to dryness on a water-bath and heat the residue at 100° to 105° for 15 minutes. Dissolve the residue in several mL of *chloroform*, add 20.0 mL of 0.01 M *sulfuric acid VS*, remove the chloroform by evaporation on a water-bath, and titrate the excess acid with 0.02 M *sodium hydroxide VS* using *methyl red TS* as indicator. Each mL of 0.01 M *sulfuric acid VS* is equivalent to 5.788 mg of total alkaloids, calculated as hyoscyamine.

STRAMONIUM TINCTURE

Category Antispasmodic.

Stramonium Tincture contains not less than 0.0225 per cent w/v and not more than 0.0275 per cent w/v of the alkaloids of Stramonium, calculated as hyoscyamine.

Stramonium Tincture may be prepared as follows.

Stramonium, in *moderately coarse powder*	100 g
Ethanol (45 Per Cent)	a sufficient quantity

Prepare about 900 mL of a tincture by percolation (Appendix 7.18). Determine the proportion of alkaloids in this tincture by the Assay, and add, if necessary, sufficient Ethanol (45 Per Cent) to produce a Stramonium Tincture of the required strength.

Dose Up to 6 mL daily, in divided doses.

Ethanol content 40.0 to 45.0 per cent v/v (Appendix 6.5).

Assay Evaporate 100.0 mL on a water-bath to about 10 mL, and transfer to a separator with the aid of 25 mL of *chloroform* and a mixture of 10 mL of *water* and 3 mL of 5 M *ammonia*. Shake well, allow to separate, and filter the chloroform layer into a second separator through a plug of *absorbent cotton* previously moistened with *chloroform*. Continue the extraction with further 25-mL portions of *chloroform* until complete extraction of the alkaloids is effected (Appendix 7.4), running each chloroform solution through the same plug of cotton. Extract the combined chloroform solutions with successive portions of a mixture of 3 volumes of 0.1 M *sulfuric acid* and 1 volume of *ethanol* until complete extraction of the alkaloids is effected, filtering each extract through a plug of cotton previously moistened with *water*. Wash the mixed acid solutions with 10, 5, and 5 mL of *chloroform*, extracting each chloroform solution with the same 20 mL of 0.05 M *sulfuric acid* and rejecting the chloroform. Combine the acid solutions, neutralize with 5 M *ammonia*, add 5 mL in excess, and shake with successive 25-mL portions of *chloroform* until complete extraction of the alkaloids is effected, washing each chloroform solution with the same 10 mL of *water* and filtering into a flask through a plug of *absorbent cotton* previously moistened with *chloroform*. Distil most of the chloroform from the combined extracts and transfer the remainder of the solution to a shallow open dish. Evaporate the reminder of the chloroform without the aid a current of air, heat the residue in an oven at 100° for 15 minutes, and dissolve in a little *chloroform*. Evaporate to dryness without the aid of a current of air, and again heat in an oven at 100° for 15 minutes. Dissolve the residue in 2 mL of *chloroform*, add 5.0 mL of 0.025 M *sulfuric acid VS*, warm to remove the chloroform, cool, and titrate the excess of acid with 0.05 M *sodium hydroxide VS*, using *methyl red TS* as indicator. Perform a blank determination (Residual Titrations, Appendix 6.17). Each mL of 0.025 M *sulfuric acid VS* is equivalent to 14.47 mg of alkaloids, calculated as hyoscyamine.

Other requirements Complies with the requirements described under "Tinctures" (Appendix 1.16).

SWEET ORANGE OIL

Thai name น้ำมันผิวส้มหวาน (NAMMAN PHIU SOM WAN)

Category Pharmaceutic aid.

Sweet Orange Oil is the volatile oil without heating, by suitable mechanical treatment from the fresh peel of the fruit of *Citrus* × *sinensis* (L.) Osbeck (Family Rutaceae). It contains not less than 1.0 per cent w/w of aldehydes, calculated as decanal, $C_{10}H_{20}O$.

Origin of plant; Constituents See under *Orange Oil*.

Description Clear, pale yellow or orange, mobile liquid, which may become cloudy when chilled.

Packaging and storage Sweet Orange Oil shall be kept in well-filled, tightly closed containers, protected from light, and stored at a temperature not exceeding 25°.

Identification Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 2 mg of *bergapten*, 10 µL of *linalool*, and 20 µL of *linalyl acetate* in 10 mL of *ethanol*.

Test solution Dilute 0.2 mL of the sample in 1 mL of *ethanol*.

Adsorbent *Silica gel G*.

Mobile phase *Ethyl acetate* and *toluene* (15:85).

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (366 nm).

Results A When examined under ultraviolet light (366 nm), the test solution shows many blue fluorescent bands in the lower third of the chromatogram. No greenish yellow fluorescent band corresponding to the bergapten band obtained from the standard solution appears.

A greenish yellow fluorescent band corresponding to the bergapten band obtained from the standard solution appears also in the middle of the chromatogram.

Detection B Spray the plate with *anisaldehyde TS*, heat at 105° for 10 minutes, and examine under ultraviolet light (366 nm). Observe the result.

Results B When examined under ultraviolet light (366 nm), the test solution shows a brownish orange fluorescent band due to linalool in the middle of the chromatogram and a faint brownish orange fluorescent band due to linalyl acetate in the upper third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution. Many blue fluorescent bands in the lower third of the chromatogram, many brownish orange fluorescent bands and many orange fluorescent bands in the middle of the chromatogram, and a brown fluorescent band in the upper third of the chromatogram are present. No faint greenish yellow fluorescent band corresponding to the bergapten band obtained from the standard solution appears.

Test for Bergapten Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 2 mg of *bergapten*, 10 µL of *linalool* and 20 µL of *linalyl acetate* in 10 mL of *ethanol*.

Test solution Dilute 0.2 mL of the sample in 1 mL of *ethanol*.

Adsorbent *Silica gel G*.

Mobile phase *Ethyl acetate* and *toluene* (15:85).

Application Apply 10 µL each of *Standard solution* and *Test solution* as 10-mm bands.

Development and drying Dry the developed plate in air.

Detection A Examine the plate under ultraviolet light (366 nm).

Results A When examined under ultraviolet light (366 nm), the test solution shows no greenish-yellow fluorescent band corresponding to the bergapten band obtained from the standard solution.

Detection B Spray the plate with *anisaldehyde TS*, heat at 105° for 10 minutes and examine under ultraviolet light (366 nm). Observe the result.

Results B When examined under ultraviolet light (366 nm), the test solution shows no faint greenish yellow fluorescent band corresponding to the bergapten band obtained from the standard solution.

Relative density 0.842 to 0.850 g (Appendix 4.9).

Refractive index 1.470 to 1.476, at 20° (Appendix 4.7).

Optical rotation +94° to +99° (Appendix 4.8).

Peroxide Value Not more than 20 (Appendix 5.12).

Residue on evaporation 1.0 to 5.0 per cent w/w when determined by the method for "Residue on Evaporation of Volatile Oils" (Appendix 7.13). Use 5 g and heat for 4 hours.

Assay Carry out the method for the "Determination of Aldehydes" (Appendix 7.9), using 10 g, omitting the *toluene* and using a volume, not less than 7 mL, of *hydroxylamine in ethanol (60 per cent) TS* that exceeds by 1 to 2 mL the volume of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* required. Each mL of 0.5 M *potassium hydroxide in ethanol (60 per cent) VS* is equivalent to 78.76 mg of $C_{10}H_{20}O$.

TURPENTINE LINIMENT

Category Counter-irritant; rubefacient.

Turpentine Liniment yield, on distillation in steam, not less than 72.0 per cent v/w and not more than 79.0 per cent v/w of volatile oil and camphor.

Turpentine Liniment may be prepared as follows.

Soft Soap	75	g
Camphor, Racemic or Natural	50	g
Turpentine Oil	650	mL
Purified Water, freshly boiled and cooled	225	mL

Triturate Camphor with Soft Soap until thoroughly mixed and gradually add Turpentine Oil,

triturating well after each addition. Transfer the mixture to a bottle using Purified Water and shake thoroughly until a creamy emulsion is formed.

Strength available 65 per cent v/v of turpentine oil and 5 per cent w/v of camphor.

Dose Adults: *Topical*, to the affected area, not more than three or four times a day.

Refractive index Of the liquid obtained in the Assay, 1.465 to 1.477 (Appendix 4.7).

Assay Acidify about 50 g of Turpentine Liniment, accurately weighed, with 1M *sulfuric acid* using *methyl orange TS* as indicator, distil in steam and collect the distillate in separator. Occasionally separate the aqueous portion of the distillate and return to the flask. When all the volatile matter has been distilled, remove the aqueous portion of the distillate and measure the volume of the oily liquid.

Other requirements Complies with the requirements described under "Liniments" (Appendix 1.16).

UNCARIA HOOK

Gambir Plant; Gouteng; Uncaria Stem with Hooks.

Category Antipyretic.

Uncaria Hook is the dried hook-bearing branch of *Uncaria rhynchophylla* (Miq.) Miq. ex Havil. and possibly other species of *Uncaria* (Family Rubiaceae). It contains not less than 0.2 per cent of total alkaloids calculated as isorhynchophylline ($C_{22}H_{28}N_2O_4$), on the dried basis.

Origin of plant Uncaria Hook is distributed throughout East Asia from South China to Vietnam, Taiwan, and Japan.

Consituents Uncaria Hook contains indole alkaloids such as isorhynchophylline and rhynchophylline. It also contains flavonoids, quinic acids and tannins.

Description Odour mild; taste, bland.

Macroscopical Fragments of stem bearing paired hooks; stem cylindrical or sub-square, 2 to 3 cm long, 2 to 5 mm wide, externally reddish brown and glabrous, longitudinally finely striated, petiole scars and arc-shaped stipule scars usually present; hook slightly flattened or rounded and curved downwards, 1 to 5 cm long, apex acute, base broad, mostly occur in paired, opposite to each other along the stem, occasionally single.

Microscopical Transverse section of the stem shows a central cavity or a spongy, whitish pith.

Transverse section of the hook shows vascular bundles arranged in a ring and unevenly distributed throughout the cortex; and parenchyma containing sandy crystals of calcium oxalate in the secondary cortex.

Powdered drug of Uncaria Hook shows dermal tissues, phloem parenchyma, pericyclic fibres, sclereids of cortical parenchyma, xylems, medullary rays, and pith. Dermal tissue: irregular polygonal epidermal cells covered with thick cuticle, and collenchyma. Phloem parenchyma, some containing sandy crystals of calcium oxalate. Pericyclic fibres, numerous, with thick and unchannelled walls. Sclereids of cortical parenchyma, slightly thickening walled and obliquely pitted cells varying greatly in shape and size, isolated or in small groups. Xylem: large bordered-pitted vessels, bordered-pitted tracheids and annular vessels, all of which often associated with the outer layer of pith with channel-walled cells. Medullary rays, numerous bordered pitted thick-walled rectangular cells. Pith, rounded to slightly pitted-walled ovoid cells.

Packaging and storage Uncaria Hook shall be kept in well-closed containers and stored in a dry place.

Identification

A. To 1 g of the powdered drug add 20 mL of *methanol*, boil under a reflux condenser on a water-bath for 5 minutes, and filter. Evaporate the filtrate to dryness, add 5 mL of *dilute acetic acid* to the residue, warm the mixture on a water-bath for 1 minute, and filter after cooling. Spot one drop of the filtrate on a filter paper, air-dry, spray Dragendorff's TS for spraying on it, and allow to stand for a while: a yellow-red colour develops.

Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 1 mg of *isorhynchophylline* and 1 mg of *rhynchophylline* in 2 mL of *methanol*.

Test solution To 1.0 g of the powdered drug add 1 mL of *strong ammonia solution*. After 30 minutes, add 25 mL of *ethyl acetate* and sonicate for 20 minutes. Filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in 1 mL of *methanol*, centrifuge and use the supernatant.

Adsorbent *Silica gel F254* (HPTLC plate)

Mobile phase *Water*, *methanol*, and *dichloromethane* (1:15:125).

Application Apply 5 µL each of *Standard solution* and *Test solution* as 8-mm bands.

Development and drying Allow the solvent front to ascend 6 cm above the line of application. Dry the developed plate in air.

Detection Examine the plate under ultraviolet light (254 nm).

Results When examined under ultraviolet light

(254 nm), the test solution shows a quenching band due to isorhynchophylline at about the middle and a quenching band due to rhynchophylline in the lower third of the chromatogram, corresponding in R_f value to the bands shown by the standard solution.

Loss on drying Not more than 10.0 per cent w/w after drying at 105° for 2 hours (Appendix 4.15).

Total ash Not more than 3.0 per cent w/w (Appendix 7.7).

Assay Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5). (**Note** Use freshly prepared solutions. Store and inject them at 5°, using a cooled autosampler.)

Standard preparation (a) Dissolve about 5 mg of Isorhynchophylline RS, accurately weighed, in a mixture of equal volumes of *methanol* and *dichloromethane* and dilute to 25.0 mL with the same mixture of solvents. Dilute 1.0 mL of the solution to 10.0 mL with *methanol*.

Standard preparation (b) Dissolve about 5 mg of Uncaria Stem with Hooks Dry Extract for System Suitability RS, accurately weighed, in 1.0 mL of *methanol*. Filter through a 0.45-µm membrane filter.

Assay preparation Weigh accurately about 500 mg of the powdered Uncaria Hook and add 3.0 mL of *strong ammonia solution*. After 30 minutes, add 50.0 mL of a mixture of equal volumes of *methanol* and *dichloromethane*. Sonicate for 30 minutes. Filter and evaporate the filtrate to dryness under reduced pressure. Dissolve the residue in *methanol* and dilute to 10.0 mL with the same solvent. Filter through a 0.45-µm membrane filter.

Mobile Phase:

MOBILE PHASE A Dilute 0.15 mL of *diethylamine* to 1000 mL with *water*.

MOBILE PHASE B *Acetonitrile*.

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0 - 3	75	25
3 - 15	75 → 70	25 → 30
15 - 25	70 → 55	30 → 45
25 - 35	55	45
35 - 40	55 → 10	45 → 90

Chromatographic system

DETECTOR Ultraviolet light (245 nm)

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with octadecylsilyl silica gel for chromatography with extended pH range (5 µm), end-capped.

TEMPERATURE

Column 5°

FLOW RATE 1.0 mL per minute

System suitability

SAMPLE *Standard preparation (b)*

Suitability requirements

RESOLUTION Not less than 1.5 between alkaloid and isorhynchophylline peaks.

RELATIVE STANDARD DEVIATION Not more than 0.2 per cent for total alkaloids.

Procedure Inject a portion (about 10 mL) of *Standard preparation (a)*, *Standard preparation (b)* and *Assay preparation* into the chromatograph. Measure the areas of the principal peaks in each chromatogram. Identify the peaks due to alkaloids 1, 2, 3, 4, 6, 7, 8, and 9 and isorhynchophylline in the assay preparation using the retention time of isorhynchophylline in the standard preparation (a) and the approximate relative retention times of 0.50, 0.58, 0.94, 0.97, 1.05, 1.13, 1.22, and 1.27 for alkaloids 1, 2, 3, 4, 6, 7, 8, and 9 in the standard preparation (b), respectively, with reference to the isorhynchophylline peak.

Calculation Calculate the percentage content of total alkaloids, calculated as isorhynchophylline, in the portion of the Uncaria Hook taken by the expression,

$$A(WP)/25aw$$

in which A is the sum of the areas of the peaks due to alkaloids 1, 2, 3, 4, 6, 7, 8, and 9 and the peak due to isorhynchophylline obtained from *Assay preparation; W* is the weight, in g, of Isorhynchophylline RS in *Standard preparation (a)*; P is the declared content of $C_{22}H_{28}N_2O_4$ in Isorhynchophylline RS; a is the peak area due to isorhynchophylline obtained from *Standard preparation (a)*; and w is the weight, in g, of Uncaria Hook in *Assay preparation*.

VALERIAN

Category Sedative.

Valerian is a dried, whole or fragmented underground parts of *Valeriana officinalis* L. (Family Valerianaceae), carefully dried at a temperature below 40°, including the rhizome surrounded by the roots and stolons. It yields not less than 0.4 mL of volatile oil from each 100 g of drug and contains not less than 0.17 percent w/w of sesquiterpenic acids, calculated as valerenic acid, $C_{15}H_{22}O_2$, on the dried basis.

Origin of plant Valerian is native to Eurasia, especially in damp woods, ditches and along the streams in Europe.

Constituents Valerian contains valepotriates and sesquiterpenes of the volatile oil, such as acetoxyvalerenic acid, valerenic acid and valerenal.

Description Odour, characteristic, penetrating, resembling that of valeric acid and camphor; taste, initially sweetish then camphoraceous and slightly bitter.

Macroscopical Rhizome: entire or longitudinally cut , 2 to 5 cm long, 1 to 3 cm in diameter, yellowish grey to greyish brown; base elongated or compressed, covered by and merging with numerous roots; apices usually bearing cup-shaped scars from aerial parts; stem base rarely present; fracture revealing pith with a central cavity; longitudinal cut showing transverse septum. Roots, numerous, yellowish grey to greyish brown with longitudinal stripes, slender, cylindrical, about 10 cm long and up to 3 mm in diameter, with a few filiform, fragile secondary root; fracture, short. Stolon pale yellowish grey, showing prominent nodes separated by longitudinally striated internodes, each of which 2 to 5 cm long; fracture fibrous.

Microscopical Transverse section of the rhizome shows epidermis, cork, cortex, endodermis, pericycle, vascular bundles, and xylem. Epidermis: slightly thick-walled polygonal cells. Cork: up to 7 layers of slightly suberized, large, polygonal, brownish cells. Cortex: thick-walled parenchyma containing numerous starch granules and traversed by numerous root traces. Endodermis: a single layer of tangentially elongated cells containing volatile oil globules. Pericycle, parenchymatous. Vascular bundle, collateral, in a ring and surrounding a very large parenchymatous pith, containing starch granules and sometimes scattered groups of pitted, thick-walled sclereids with a narrow lumen. Xylem, with few slender, annular, spiral and pitted vessels.

Transverse section of the branch shows components similar to those of the rhizomes, but with a prominent endodermis and a well-defined ring of vascular bundles showing secondary thickening.

Transverse section of the root shows a piliferous layer of papillose cells, exodermis, cortex, endodermis, and primary xylem. Piliferous layer of papillosed cells, some developed into root hairs. Exodermis: a single layer of suberized-walled quadrangular to polygonal cells containing volatile oil globules. Cortex: parenchyma cells with numerous starch granules; the outermost cells containing volatile oil globules. Endodermis: a layer of radially thick-walled cells. Primary xylem: 3 to 11 arches surrounding a small central parenchymatous pith containing starch granules, sometimes showing a cleft or stellate hilum.

Powdered drug of Valerian Root possesses the microscopical characteristics of the unground drug with abundant simple or 2- to 6-compound starch granules characterized by an indistinct cleft or a radiate hilum.

Packaging and storage Valerian shall be kept in well-closed containers, protected from light and stored in a cool and dry place.

Identification

Carry out the test as described in the "Thin-Layer Chromatography" (Appendix 3.1).

Standard solution Dissolve 5 mg of *acetoxyvalerenic acid* and 5 mg of *valerenic acid* in 20 mL of *methanol*.

Test solution Suspend 1 g of the powdered drug in 10 mL of *methanol* and sonicate for 10 minutes. Filter the supernatant through a 0.45-µm membrane filter. Use the filtrate.

Adsorbent *Silica gel G* (TLC or HPTLC plate).

Mobile phase *Glacial acetic acid*, *ethyl acetate*, and *cyclohexane* (2:38:60).

Application Apply 20 µL (or 5 µL) each of *Standard solution* and *Test solution* as 10-mm (or 8-mm) bands.

Development and drying Allow the solvent front to ascend 10 cm (or 6 cm) above the line of application. Dry the developed plate in air.

Detection Spray the plate with *anisaldehyde TS*, heat at 105° for 5 to 10 minutes and examine immediately under visible light. Observe the result.

Results When examined under visible light, the test solution shows a violet band due to acetoxyvalerenic acid in the lower third and a violet band due to valerenic acid in the upper third of the chromatogram, corresponding in colour and R_f to the bands shown by the standard solution.

Loss on drying Not more than 12.0 per cent w/w after drying at 105° for 2 hours (Appendix 4.15).

Acid-insoluble ash Not more than 5.0 per cent w/w (Method II, Appendix 7.6).

Total ash Not more than 12.0 per cent w/w (Appendix 7.7).

Assay

FOR VOLATILE OIL Carry out the method for the "Determination of Volatile Oil" (Appendix 7.3), using 40 g, in *No.500 powder*, freshly prepared and accurately weighed.

Use 500 mL of *water* as the distillation liquid and a 2000-mL flask. Distil at a rate of 3 to 4 mL per minute for 4 hours. Use 0.50 mL of *xylene* in the graduated tube.

FOR SESQUITERPENIC ACIDS Carry out the determination as described in the "Liquid Chromatography" (Appendix 3.5).

Standard preparation Dissolve Valerian Dry Extract RS in *methanol* to contain 1.0 mg of valerenic acid and dilute to 10.0 mL with the same solvent. Sonicate for 10 minutes and filter through a 0.45-µm membrane filter.

Assay preparation Transfer about 1.5 g of Valerian, in *No. 710 powder*, accurately weighed, to a 100-mL round-bottomed flask with a ground-glass neck. Add 20 mL of *methanol*. Mix and heat on a water-bath

under a reflux condenser for 30 minutes. Allow to cool and filter. Transfer the filter with the residue to the 100-mL round-bottomed flask. Add 20 mL of *methanol* and heat on a water-bath under the reflux condenser for 15 minutes. Allow to cool and filter. Combine the filtrates and dilute to 50.0 mL with *methanol*, rinsing the round-bottomed flask and the filter.

Mobile phase

MOBILE PHASE A *Acetonitrile for chromatography* and a 0.5 per cent w/v solution of *phosphoric acid* (20:80).

MOBILE PHASE B *Acetonitrile for chromatography* and a 0.5 per cent w/v solution of *phosphoric acid* (80:20).

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0 - 5	55	45
5 - 18	55 → 20	45 → 80
18 - 22	20	80

Chromatographic System

DETECTOR Ultraviolet light (220 nm)

COLUMN A stainless steel column (25 cm × 4.6 mm), packed with octadecylsilyl bonded to porous silica or ceramic microparticles (5 µm).

COLUMN TEMPERATURE 35°

FLOW RATE 1.5 mL per minute

System Suitability

SAMPLE *Standard preparation*

(**Note** The relative retention with reference to valerenic acid for acetoxyvalerenic acid is about 0.5, where the retention time for valerenic acid is about 19 minutes.)

Procedure Separately inject equal volumes (about 20 mL) of *Standard preparation* and *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Identify the peaks due to acetoxyvalerenic acid and valerenic acid in the assay preparation using the retention time of acetoxyvalerenic acid and valerenic acid in the standard preparation.

Calculation Calculate the contents of sesquiterpenic acids, calculated as valerenic acid, using the following expression:

$$\frac{(A_{u1}+A_{u2})WV(5P)}{A_s w},$$

in which A_{u1} and A_{u2} are the areas of the peak due to acetoxyvalerenic acid and to valerenic acid obtained from *Assay preparation*; A_s is the area of the peak due to valerenic acid obtained from *Standard preparation*; w is the weight, in g, of Valerian in *Assay preparation*, W is the weight, in g, of Valerian Dry Extract RS in *Standard preparation*; P is the declared content of $C_{15}H_{22}O_2$ in Valerian Dry Extract RS.

VALERIAN, POWDERED

Complies with the requirements for Contents of volatile oil and valerenic acid, Packaging and storage, Identification, Loss on drying, Acid-insoluble ash, and Total ash stated under Valerian and with the following requirement.

Description Light brown. Diagnostic structures: numerous fragments of parenchyma composed of rounded or elongated cells containing simple or 2- to 6-compound starch granules; occasional cells filled with light brown resin; rectangular sclereids with pitted walls, 5 to 15 µm thick; xylem vessels isolated or in small groups, 10 to 50 µm in diameter; root hairs and fragments of cork also present.

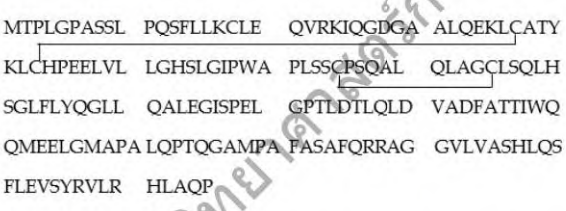
MONOGRAPHS

BIOLOGICAL PRODUCTS

BIOPHARMACEUTICAL PRODUCTS

กรมวิทยาศาสตร์การแพทย์

FILGRASTIM CONCENTRATED SOLUTION



$C_{845}H_{1339}N_{223}O_{243}S_9$ 18799 *121181-53-1*

Category Granulocyte-colony stimulating agent.

Filgrastim Concentrated Solution is a solution of a protein, produced by a method based on recombinant DNA (rDNA) technology, containing the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated. huG-CSF is produced and secreted by endothelial cells, monocytes and other immune cells. The protein stimulates the differentiation and proliferation of leucocyte stem cells into mature granulocytes. It contains not less than 0.9 mg of the protein per mL.

The tests for host-cell-derived proteins and host-cell- or vector-derived DNA are carried out on each batch of filgrastim concentrated solution, unless exemption has been granted by a competent authority. The limit is established by each laboratory and approved by the competent authority.

Strength available Not less than 0.9×10^8 IU per mg of protein.

Description Clear, colourless or slightly yellowish liquid.

Storage Filgrastim Concentrated Solution shall be stored according to the conditions approved by the competent authority.

Labelling Complies with the "General Information for Biological Products". In addition, the label on the container states (1) the content, in mg of protein per mL; (2) the potency, in IU per mg of protein; (3) the storage condition.

Identification

A. The assay or, where applicable, the biological activity may serve as an identification test.

B. Examine the electropherograms obtained in the test for Impurities with charges differing from that of filgrastim: the principal band in the electropherogram obtained from the test solution is similar in position to the principal band in the electropherogram obtained from reference solution (a).

C. Examine the chromatograms obtained in the test for Impurities with molecular weights higher than that of filgrastim: the principal peak in the chromatogram obtained from the test solution is similar in retention time to the principal peak in the chromatogram obtained from the reference solution.

D. Examine the electropherograms obtained under both reducing and non-reducing conditions in the test for Impurities with molecular weights differing from that of filgrastim: the principal band in the electropherogram obtained from test solution (a) is similar in position to the principal band in the electropherogram obtained from reference solution (b).

E. Examine the chromatograms obtained in the test for Related proteins: the principal peak in the chromatogram obtained from the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained from the reference solution.

F. **Peptide mapping** Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

(a) Selective cleavage of the peptide bonds

Standard solution Transfer about 25 µg of Filgrastim RS, accurately weighed, into a polypropylene tube. Add 25.0 µL of a 0.1 mg per mL solution of *glutamyl endopeptidase for peptide mapping*. Dilute to 100.0 µL with *0.02 M sodium phosphate buffer solution pH 8.0*, stopper the tube and incubate at about 37° for 17 hours. Cool to 2° to 8° until analysis.

Test solution Transfer an accurately measured volume of the sample containing about 25 µg of protein into a polypropylene tube. Prepare at the same time and in the same manner as for the standard solution but using the sample instead of Filgrastim RS.

(b) Separation by chromatography

CHROMATOGRAPHY

Mobile phase

MOBILE PHASE A *Trifluoroacetic acid, water, acetonitrile* (0.5:950:50)

MOBILE PHASE B *Trifluoroacetic acid, water, acetonitrile* (0.5:50:950)

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0 – 8	97 → 94	3 → 6
8 - 25	94 → 66	6 → 34
25 - 40	66 → 10	34 → 90
40 - 45	10	90

Chromatographic system

DETECTOR Ultraviolet light (215 nm).

COLUMN A stainless steel column (10 cm × 2.1 mm), packed with octadecylsilyl bonded to porous

silica microparticles, compatible with 100 per cent aqueous mobile phases (5 µm) with a pore size of 20 nm, end-capped.

COLUMN TEMPERATURE 60°

FLOW RATE 0.2 mL per minute

System suitability

SAMPLE *Standard solution*

Suitability requirements The chromatogram obtained from the standard solution is qualitatively similar to the chromatogram of filgrastim digest supplied with Filgrastim RS.

Procedure Inject a portion (about 10 mL) of *Standard solution* and *Test solution* into the chromatograph. Measure the areas of the principal peaks in each chromatogram. Identify the peaks due to filgrastim in the test solution using the retention time of filgrastim in the standard solution.

Results The profile of the chromatogram obtained from the test solution corresponds to that of the chromatogram obtained from the standard solution.

Impurities with molecular weights higher than that of filgrastim Carry out the test as described in the "Size-Exclusion Chromatography" (Appendix 3.6), use the normalization procedure.

Diluent Dissolve 4.1 g of *sodium acetate* in 400 mL of *water*, adjust to pH 4.0 with *acetic acid* and dilute to 500 mL with *water*.

Standard solution Dilute Filgrastim RS with *Diluent* to obtain a concentration of 0.4 mg per mL.

Test solution Dilute Filgrastim Concentrated Solution with *Diluent* to obtain a concentration of 0.4 mg per mL.

Resolution solution Mix a suitable portion of *Standard solution* for about 30 seconds using a vortex mixer.

Mobile phase Dissolve 7.9 g of *ammonium hydrogen carbonate* in 1000 mL of *water* and adjust to pH 7.0 with *phosphoric acid*; dilute to 2000 mL with *water*.

Chromatographic system

DETECTOR Ultraviolet light (215 nm)

COLUMN A column (30 cm × 7.8 mm), packed with spherical silica (5 mm) with hydrophilic coating (suitable for fractionation of globular proteins in the relative molecular mass range of 10,000 to 500,000), equipped with a similarly packed guard column of a suitable size.

COLUMN TEMPERATURE 30°

FLOW RATE 0.5 mL per minute

System suitability

SAMPLE *Resolution solution*

(**Note** The relative retention with reference to the filgrastim monomer: aggregates, filgrastim oligomer 1, filgrastim oligomer 2, and filgrastim dimer are about 0.60, 0.75, 0.80, and 0.85, respectively, where the retention time for filgrastim monomer is about 19 minutes.)

Suitability requirements

RETENTION TIME 17 to 20 minutes for filgrastim monomer.

RESOLUTION Not less than 3 between filgrastim dimer and filgrastim monomer peaks.

Procedure Separately inject equal volumes (about 20 µL) of *Standard solution* and *Test solution* into the chromatograph, record the chromatograms and measure the responses for the major peaks.

Calculation Calculate the percentage content of the dimer, oligomers and aggregates.

Limits

— impurities with molecular weights higher than that of filgrastim, other than the dimer: not more than 0.5 per cent;

— total of impurities with molecular weights higher than that of filgrastim: not more than 2 per cent.

Impurities with molecular weights differing from that of filgrastim Carry out the test as described in the "Electrophoresis" (Appendix 3.7) under both reducing and non-reducing conditions.

Sample buffer (non-reducing conditions) Mix equal volumes of *water* and *concentrated SDS-PAGE sample buffer*.

Sample buffer (reducing conditions) Mix equal volumes of *water* and *concentrated SDS-PAGE sample buffer for reducing conditions* containing *2-mercaptoethanol* as the reducing agent.

Reference solution (a) Solution of molecular weight markers suitable for calibrating SDS-polyacrylamide gels in the range of 14.4 to 94 kDa.

Reference solution (b) Dilute Filgrastim RS with *Sample buffer* to obtain a concentration of 100 µg per mL.

Test solution (a) Dilute Filgrastim Concentrated Solution with *Sample buffer* to obtain a concentration of 100 µg per mL.

Test solution (b) To 200 µL of *Test solution (a)* add 200 µL of *Sample buffer*.

Test solution (c) Dilute 200 µL of *Test solution (b)* to 1.0 mL with *Sample buffer*.

Test solution (d) Dilute 200 µL of *Test solution (c)* to 1.0 mL with *Sample buffer*.

Test solution (e) To 200 µL of *Test solution (d)* add 200 µL of *Sample buffer*.

Electrophoresis System

SAMPLE TREATMENT Boil for 5 minutes.

GEL DIMENSIONS 1 mm thick.

RESOLVING GEL 13 per cent *acrylamide*.

APPLICATION 20 µL.

DETECTION Silver staining.

System suitability

SAMPLE *Reference solution (a)* the validation criteria are met;

SUITABILITY REQUIREMENTS

— a band is seen in the electropherogram obtained from the test solution (e);

— a gradation of intensity of staining is seen in the electropherograms obtained from the test solutions (a) to (e).

Limit *Test solution (a)*

— impurities with molecular weights lower or higher than that of filgrastim: no band is more intense than the principal band in the electropherogram obtained from *Test solution (d)* (2.0 per cent).

Impurities with charges differing from that of filgrastim Carry out the test as described in the "Isoelectric Focusing" (Appendix ==).

Reference solution (a) Dilute Filgrastim RS with *water* to obtain a concentration of 0.3 mg per mL.

Reference solution (b) Dilute Filgrastim RS with *water* to obtain a concentration of 30 µg per mL.

Reference solution (c) Use an isoelectric point (pI) calibration solution, in the pI range of 2.5 to 6.5, prepared according to the manufacturer's instructions.

Test solution Dilute Filgrastim Concentrated Solution with *water* to obtain a concentration of 0.3 mg per mL.

Focusing system

pH GRADIENT 4.5 to 8.0

CATHOLYTE 1 M *sodium hydroxide*

ANOLYTE 0.04 M *glutamic acid* in a 0.0025 per cent v/v solution of *phosphoric acid*

APPLICATION 20 µL

Detection as described in the "Isoelectric Focusing" (Appendix ==).

System suitability

SAMPLE *Reference solutions (a)* and *(c)*

SUITABILITY REQUIREMENTS

— in the electropherogram obtained from reference solution (c), the relevant isoelectric point markers are distributed along the entire length of the gel

— in the electropherogram obtained from reference solution (a), the pI of the principal band is 5.7 to 6.3.

Limit

— any impurity: no band is more intense than the principal band in the electropherogram obtained from reference solution (b) (10 per cent).

Bacterial endotoxins When tested as described in the "Test for Bacterial Endotoxins" (Appendix 8.5), it contains not more than 2 Endotoxin Units in the volume that contains 1.0 mg of protein.

Related proteins Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5), use the normalization procedure.

Reference solution (a) Dilute the content of a vial of Filgrastim RS with *water* to obtain a concentration of 0.5 mg per mL.

Reference solution (b) To 250 µL of *Reference solution (a)*, add 2.5 µL of a 4.5 g per L solution of *hydrogen peroxide*. Mix and incubate at 25°±2° for 30 minutes, then add 1.9 mg of *L-methionine*.

Reference solution (c) To 250 µL of *Reference solution (a)*, add 0.25 mg of *dithiothreitol*. Mix and incubate at 35°±2° for 60 minutes.

Test solution Dilute Filgrastim Concentrated Solution with *water* to obtain a concentration of 0.5 mg per mL.

Mobile phase

MOBILE PHASE A *Trifluoroacetic acid* and *water* (1:1000)

MOBILE PHASE B *Trifluoroacetic acid*, *water*, *acetonitrile for chromatography* (1:100:900)

The step gradient of mobile phases is as follows:

Time (Minutes)	*Mobile Phase A* (Per Cent V/V)	*Mobile Phase B* (Per Cent V/V)
0 – 30	60 → 20	40 → 80
30 - 35	20	80
35 - 45	20 → 60	80 → 40

Chromatographic system

DETECTOR Ultraviolet light (215 nm).

COLUMN A stainless steel column (15 cm × 4.6 mm), packed with butylsilyl chemically bonded to porous silica or ceramic microparticles (5 µm) with a pore size of 30 nm, end-capped.

COLUMN TEMPERATURE 60°

FLOW RATE 0.8 mL per minute

Procedure Separately inject equal volumes (about 50 µL) of *Reference solutions (b)* and *(c)* and *Test solution* into the chromatograph, record the chromatograms and measure the responses for the major peaks.

System suitability

SAMPLE *Reference solution (b)*

(**Note** The relative retention with reference to filgrastim: oxidized filgrastim (form 1), oxidized filgrastim (form 2), and reduced filgrastim are about 0.84, 0.98 and 1.04, respectively, where the retention time for filgrastim is about 23 minutes.)

SUITABILITY REQUIREMENTS

Symmetry factor Not more than 1.8 for the peak due to filgrastim;

Peak-to-valley ratio Not less than 2.0, where H_p = height above the baseline of oxidized filgrastim (form 2) peak and H_v = height above the baseline of the lowest point of curve separating this peak from the filgrastim peak.

System suitability

SAMPLE *Reference solution (c)*

SUITABILITY REQUIREMENTS

Resolution Not less than 1.5 between the filgrastim and reduced filgrastim peaks.

Symmetry factor Not more than 1.8 for the filgrastim peak.

Limits

— any impurity: for each impurity, not more than 1.0 per cent;

— total: not more than 2.0 per cent.

Assay

FOR PROTEIN Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Reference solution (a), Test solution, Mobile phase, Chromatographic system, Procedure and **System suitability** Proceed as directed in the test for Related proteins.

Calculation Calculate the content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in the Solution taken, using the declared content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in Filgrastim RS.

FOR POTENCY The potency of the Solution is determined by comparison of the dilutions for the test solution with the dilutions of the International Standard of filgrastim or with a reference solution calibrated in International Units.

The International Units (IU) is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Carry out the assay by using a suitable method such as the following, which uses the conversion of a tetrazolium salt (MTS) as a staining method. Alternative methods of quantifying cell proliferation, such as measurement of intracellular ATP by luciferase bioluminescence, have also been found suitable, and may be used as the assay readout, subject to appropriate validation. The assay conditions (for example, cell concentration, incubation time and dilution steps) are then adapted accordingly.

Use an established cell line responsive to filgrastim. M-NFS-60 cells (ATCC No. CRL-1838) that have been made sensitive to G-CSF have been found suitable. Incubate with varying dilutions of test and reference solutions of filgrastim. Then incubate with a solution of *tetrazolium salt*. This cytochemical stain is converted by cellular dehydrogenases to a coloured formazan product. The formazan is then measured spectrophotometrically.

Add 50 µL of dilution medium to all wells of a 96-well microtitre plate. Add an additional 50 µL of this solution to the wells designed for the blanks. Add 50 µL of each solution to be tested in triplicate (test solution and reference solution at a concentration of about 800 IU per mL, plus a series of 10 twofold dilutions to obtain a standard curve). Prepare a suspension of M-NFS-60 cells containing 7×10^5 cells per mL. Immediately before use, add *2-mercaptoethanol* to a final concentration of 0.1 mM, and add 50 µL of the prepared cell suspension to each well, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0° to 38.0° for 44 to 48 hours in a humidified incubator using 6±1 per cent CO_2. Add 20 µL of a 0.5 per cent w/v sterile solution of *tetrazolium salt* to each well and re-incubate for 4 hours. Estimate the quantity of formazan produced using a microtitre well plate reader at 490 nm.

Calculation Calculate the potency of the Solution using a suitable statistical method as described in the "Statistical Analysis of Results of Biological Assays and Tests" (Appendix 9), for example the parallel line assay.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

FILGRASTIM INJECTION

Category (Thai FDA) Granulocyte-colony stimulating agent.

Filgrastim Injection is a sterile solution for injection of a protein prepared from Filgrastim concentrated solution having the primary structure of the 174-amino-acid isoform of human granulocyte colony-stimulating factor (huG-CSF) plus 1 additional amino acid, an *N*-terminal methionine. In contrast to its natural counterpart, the protein is not glycosylated. It contains not less than 90.0 per cent and not more than 110.0 per cent of the amount of filgrastim stated on the label. Its potency is not less than 80 per cent and not more than 125 per cent of the potency stated on the label when determined using the conditions described under Assay.

Description Clear, colourless or slightly yellowish liquid.

Strengths available (Drug Information Handbook 28th edition) 300 µg per 0.5 mL, 300 µg per mL, 480 µg per 0.8 mL, and 480 µg per 1.6 mL.

Dose *Intravenous, intramuscular* or *subcutaneous*, as directed by a physician.

Other relevant information

1. It may cause cough, trouble breathing; nosebleeds; bone pain, muscle or joint pain; diarrhea; headache; numbness; or rash, thinning hair.

2. It should not be diluted with 0.9 per cent w/v sodium chloride injection because precipitation may

occur.

3. Caution should be exercised if it is to be used in children, pregnant and nursing women.

Packaging and storage Filgrastim Injection shall be stored in a sterile, tightly closed, tamper-evident container, protected from light, at a temperature between 2° and 8°.

Identification

A. The assay or, where applicable, the biological activity, may serve as an identification test.

B. Examine the chromatograms obtained in the test for Related proteins: the principal peak in the chromatogram obtained from the test solution is similar in retention time and shape to the principal peak in the chromatogram obtained from reference solution (a).

pH As approved by the competent authority (Appendix 4.11).

Impurities with molecular weights higher than that of filgrastim Carry out the test as described in the "Size-Exclusion Chromatography" (Appendix 3.6). Use the normalization procedure.

Diluent, Standard solution, Resolution solution and **Mobile phase, Chromatographic system, Procedure, System suitability,** and **Calculation** Proceed as directed in the test for Impurities with molecular weights higher than that of filgrastim under *Filgrastim Concentrated Solution*.

Test solution Dilute Filgrastim Injection with *Diluent* to obtain a concentration of 0.4 mg per mL.

Limits

– impurities with molecular weights higher than that of filgrastim, other than the dimer: not more than 0.5 per cent;

– total of impurities with molecular weights higher than that of filgrastim: not more than 1.0 per cent.

Impurities with charges differing from that of filgrastim Carry out the test as described in the "Isoelectric Focusing" (Appendix ==), with the following modifications. (**Note** Prepare reference solutions (a), (b) and (c) immediately before loading on the gel.)

Reference solution (a) Dilute Filgrastim RS with *water* to obtain a concentration of 0.6 mg per mL.

Reference solution (b) To 10 µL of *Reference solution (a)* add 190 µL of *water* to obtain a concentration of 30 µg per mL.

Reference solution (c) To 10 µL of *Reference solution (a)* add 490 µL of *water* to obtain a concentration of 12 µg per mL.

Reference solution (d) Use an isoelectric point (pI) calibration solution, in the pI range of 3 to 10, prepared according to the manufacturer's instructions.

Test solution Dilute the Filgrastim Injection with *water* to obtain a concentration of 0.6 mg per mL.

Gel concentration 5 per cent; commercially available gels may be used.

Focusing system

pH GRADIENT 5.0 to 7.0

CATHOLYTE a 2 per cent w/v solution of *sodium hydroxide*

ANOLYTE a 3 per cent w/v solution of *glacial acetic acid*

APPLICATION 15 µL

Procedure Proceed with the isoelectric focusing by applying the electrical parameters according to the manufacturer's instructions. The following parameters have been found suitable for gels of dimensions 250 mm × 110 mm × 0.43 mm:

PRE-FOCUSING 700 V, 12 mA, 8 W, 20 minutes;

POST-APPLICATION 500 V, 8 mA, 8 W, 20 minutes;

FOCUSING 2000 V, 14 mA, 14 W, 90 minutes;

FINAL FOCUSING 2500 V, 14 mA, 14 W, 10 minutes.

Detection Silver staining

Fixing solution A solution containing 3.6 per cent w/v solution of *sulfosalicylic acid* and 11.6 per cent w/v solution of *trichloroacetic acid*.

Sensitizing solution Dissolve 17 g of *sodium acetate* in about 100 mL of *water*. Add 75 mL of *ethanol*, 10 mL of a 5 per cent w/v solution of *anhydrous sodium thiosulfate* and 1.25 mL of a 25 per cent w/v solution of *glutaraldehyde* and dilute to 250 mL with *water*.

Silver solution Mix 100 µL of *formaldehyde solution* and 25 mL of a 2.5 per cent w/v solution of *silver nitrate* and dilute to 250 mL with *water*.

Developing solution Dissolve 6.25 g of *sodium carbonate* in about 200 mL of *water*, add 50 µL of *formaldehyde solution* and dilute to 250 mL with *water*.

Stopping solution Dissolve 3.65 g of *sodium edetate* in 250 mL of *water*. Alternatively, commercially available staining kits may be used.

System suitability

SAMPLE *Reference solutions (c)* and *(d)*

SUITABILITY REQUIREMENTS

– in the electropherogram obtained from reference solution (d), the relevant isoelectric point markers are distributed along the entire length of the gel

– in the electropherogram obtained from reference solution (c), the principal band is clearly visible.

Limits

– maximum seven bands of an intensity between that of the principal band in the electropherogram obtained from reference solution (c) (2 per cent) and that of the principal band in the electropherogram obtained from reference solution (b) (5 per cent) are present;

– no band has an intensity greater than that of the principal band in the electropherogram obtained from

reference solution (b) (5 per cent).

Bacterial endotoxins When tested as described in the "Test for Bacterial Endotoxins" (Appendix 8.5), it contains not more than 10 Endotoxin Units in the volume that contains 1.0 mg of protein.

Osmolality As approved by the competent authority (Appendix 4.35).

Related proteins Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5), use the normalization procedure.

Reference solution (a), Reference solution (b), Reference solution (c), Mobile phase Proceed as directed in the Related proteins under *Filgrastim Concentrated Solution*.

Chromatographic system, Procedure and **System suitability** Proceed as directed in the Related proteins under *Filgrastim Concentrated Solution*.

Test solution Dilute the Injection with *water* to obtain a concentration of 0.5 mg per mL.

Limits

— any impurity: for each impurity, not more than 3.0 per cent;

— total: not more than 6.5 per cent.

Assay

FOR PROTEIN Carry out the test as described in the "Liquid Chromatography" (Appendix 3.5).

Reference solution (a), Test solution, Mobile phase, Chromatographic system, Procedure and **System suitability** Proceed as directed in the Related proteins.

Calculation Calculate the content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in the Injection taken, using the declared content of $C_{845}H_{1339}N_{223}O_{243}S_9$ in Filgrastim RS.

FOR POTENCY The potency of the Injection is determined by comparison of the dilutions for the test solution with the dilutions of the International Standard of filgrastim or with a reference solution calibrated in International Units.

The International Units (IU) is the activity contained in a stated amount of the appropriate International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organization.

Carry out the assay by using a suitable method such as the following, which uses the conversion of a tetrazolium salt (MTS) as a staining method. Alternative methods of quantifying cell proliferation, such as measurement of intracellular ATP by luciferase bioluminescence, have also been found suitable, and may be used as the assay readout, subject to appropriate validation. The assay conditions (for example, cell concentration, incubation time and dilution steps) are then adapted accordingly.

Use an established cell line responsive to filgrastim. M-NFS-60 cells (ATCC No. CRL-1838) that have been made sensitive to G-CSF have been found suitable. Incubate with varying dilutions of test and reference solutions of filgrastim. Then incubate with a solution of *tetrazolium salt*. This cytochemical stain is converted by cellular dehydrogenase to a coloured formazan product. The formazan is then measured spectrophotometrically.

Add 50 µL of dilution medium to all wells of a 96-well microtitre plate. Add an additional 50 µL of this solution to the wells designed for the blanks. Add 50 µL of each solution to be tested in triplicate (test solution and reference solution at a concentration of about 800 IU per mL, plus a series of 10 twofold dilutions to obtain a standard curve). Prepare a suspension of M-NFS-60 cells containing 7×10^5 cells per mL. Immediately before use, add *2-mercaptoethanol* to a final concentration of 0.1 mM, and add 50 µL of the prepared cell suspension to each well, maintaining the cells in a uniform suspension during addition.

Incubate the plate at 36.0° to 38.0° for 44 to 48 hours in a humidified incubator using 6±1 per cent CO_2. Add 20 µL of a 0.5 per cent w/v sterile solution of *tetrazolium salt* to each well and re-incubate for 4 hours. Estimate the quantity of formazan produced using a microtitre well plate reader at 490 nm.

Calculation Calculate the potency of the Injection using a suitable statistical method as described in the "Statistical Analysis of Results of Biological Assays and Tests" (Appendix 9), for example the parallel line assay.

The estimated potency is not less than 80 per cent and not more than 125 per cent of the stated potency. The confidence limits (P = 0.95) are not less than 74 per cent and not more than 136 per cent of the estimated potency.

Other requirements Complies with the requirements described under "Parenteral Preparations" (Appendix 1.16).

ADVANCED THERAPY MEDICINAL PRODUCTS

CELL-BASED AND GENE THERAPY MEDICINAL PRODUCTS: RAW MATERIALS OF BIOLOGICAL ORIGIN FOR THEIR PRODUCTION

GENERAL INFORMATION

The purpose of this general information is to provide information and guidance on the development of appropriate qualification strategies for raw materials, or ancillary materials (according to the United States Pharmacopeia), used in the manufacture of cell-based/gene therapy (gene transfer) medicinal products. Due to the diverse nature of the raw materials that may require special considerations, the provisions of the chapter are extensive and do not exclude the use of different production and control methods. The manufacturer of a raw material shall take responsibility in qualifying (proving to be suitable for the intended use) the raw material in accordance with the requirements given herein. Nevertheless, the user of a raw material plays an ultimate role in ensuring that the material is of suitable quality for the specific use.

The quality of the raw material may be considered according to the stage of development of the cell-based/gene therapy medicinal product, thereby acknowledging the inherent evolution of the quality profile of the product during its pharmaceutical and clinical development. Typically, the extent of control increases as clinical development progresses. The patient's safety needs to be ensured in early phases of clinical development. When assessing the impact of the raw material on the quality, safety and efficacy of the cell-based/gene therapy medicinal product, a risk-based approach is employed. To minimize risks, the use of raw materials free from human or animal substances is preferred.

Due to its biological nature, the quality of a raw material used for manufacturing a cell-based/gene therapy medicinal product may impact the safety, potency, and purity of the final product. Thus, the quality requirements for this type of material are to be established. Examples of the critical quality attributes specific to each type of raw material are addressed in this general information.

It should be noted that changes in raw materials during the life cycle of the cell-based/gene therapy medicinal product may affect the quality of the medicinal product and thus require additional studies to demonstrate comparability.

INTRODUCTION

In addition to chemical medicinal products, rapid development in biology, biotechnology, and medical sciences has brought to light an Advanced Therapy Medicinal Product (ATMP): a medicinal product that is gene-, tissue-, or cell-based. This new type of medicinal product shows a high potential in the treatment of various diseases, especially those rare or serious ones where there is a previously unmet medical need.

Cells used in cell-based medicinal products may be self-renewing stem cells, more committed progenitor cells, or terminally differentiated cells exerting a specific defined physiological function. They may be autologous or of allogeneic origin.

Gene therapy or gene transfer medicinal products for human use contain genes that lead to a therapeutic, prophylactic or diagnostic effect by inserting 'recombinant' genes into the body to modify or manipulate the expression of the body's genes or to alter the biological properties of living cells. Similar to cell-based medicinal products, this type of product can treat a wide range of diseases, including genetic disorders, cancer, or long-term diseases.

Since these products are not usually amenable to extensive purification, filtration, and terminal sterilization, procedures, reagents, and materials qualification are critically important to ensuring the products' quality.

The term "raw materials" refers to materials that come into contact with the cellular starting material, product intermediate, or final product manufacturing, but are not intended to be present in the final product.

SCOPE

This chapter applies to raw materials of biological origin used for the production of cell-based/gene therapy medicinal products. The raw materials used in the manufacture of active substances are not intended to form part of the active substance. The raw materials can be extracted from various biological sources or produced by recombinant DNA technology.

Classification of raw materials cell-based/gene therapy medicinal products Raw materials cell-based/gene therapy medicinal products to be included in this Pharmacopoeia are classified as follows:

– Sera and serum replacements;

– Proteins produced by recombinant DNA technology such as growth factors, cytokines, hormones, enzymes, and monoclonal antibodies;

– Proteins extracted from biological material such as enzymes and polyclonal antibodies;

– Vectors.

The principles of this chapter may also be applied to other classes of biological rawmaterials where appropriate.

Medical devices, plastics and chemically synthesized

raw materials, such as basal media (purely composed of chemicals), synthetic peptides, or synthetic polynucleotides, are not within the scope of this chapter.

RISK ASSESSMENT

Evaluation of the impact of the raw material on the quality, safety, and efficacy of cell-based/gene therapy medicinal products must be performed by the user of the raw material. No single measure or combination of measures can guarantee the quality, functionality, and safety of a raw material for its intended use. Therefore, a risk assessment must consider the biological origin and traceability of the raw material, the production steps applied to it, and the ability of the drug product manufacturing process to control or remove the raw material from the final medicinal product.

Any risk factor must be evaluated in relation to the clinical benefit/risk of the cell-based or gene therapy medicinal product. The evaluation of risk should be based on scientific knowledge, with the ultimate goal of patient protection. When evaluating the risk posed by the raw material to the final medicinal product, the exposure of a patient to residual amounts of raw material with potential harmful effects (e.g., adverse immune reactions) should be considered in relation to the clinical benefit/risk of the cell-based or gene therapy medicinal product.

GENERAL REQUIREMENTS

Origin

The origin of the raw material and if relevant any biological substances used for the production of the raw material must be known. Special attention must be paid to risks related to the sourcing (including pooling) of the substances used for the production of the raw material. Depending on the source of the raw material and the substances used in its production, raw materials can be divided into three categories:

1) raw materials of human or animal origin;
2) raw materials produced using substances of human or animal origin;
3) raw materials free from substances of human or animal origin.

Traceability of all raw materials is required, with particular attention to those materials with an inherent safety concern, i.e., those of human or animal origin.

Due to the inherent risk of transmitting adventitious agents, it is recommended to minimize, wherever possible, the use of raw materials of human or animal origin. If such raw materials are required for the production of cell-based/gene therapy medicinal products, appropriate measures are taken to minimize the risks of transmitting adventitious agents such as viruses, prions, bacteria, and protozoa.

For human blood and tissue-derived materials, only carefully evaluated donors who have been adequately tested for infectious measures enable each donation to be followed from the donation to the raw material and to the final product, and vice-versa.

When raw materials of animal origin are used, these animals fulfil specific health requirements and should be fit for human consumption and reared under controlled conditions, when applicable. If the origin of the animals is not fully traceable (e.g., animals collected from the wild), information on their geographic location at the time of sourcing should be considered.

When vectors or proteins produced by recombinant DNA technology are used as raw materials, traceability to the master cell bank/virus seed lot is required.

For all raw materials of human or animal origin, or raw materials produced using substances of human or animal origin, a viral risk assessment is performed according to the requirements of "Viral Safety" (Appendix ==). The extent of viral safety testing is dependent on the results of the initial risk assessment. In addition, a risk assessment with respect to transmissible spongiform encephalopathies is carried out and suitable measures are taken to minimize such risks as described in "Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products" Appendix==).

Production

All raw materials are produced within a suitable quality management system and production facilities.

Suitable in-process controls are in place to ensure that the production process is under control and consistently produces raw materials of defined quality.

Quality attributes for raw materials include identity, purity and biological activity where applicable, and they are to be demonstrated using appropriate, qualified control methods. Relevant specifications in terms of identity, purity/impurity profile and assays are to be established.

The production process is optimized to consistently minimize and/or remove adventitious agents and harmful impurities, whilst retaining the quality of the raw material. This can be achieved using one or a combination of the following measures:

– using validated inactivation/removal procedures such as gamma sterilization or low pH during chromatography, where possible;
– demonstrating the ability of a production process to minimize, remove, or inactivate adventitious agents or harmful impurities;
– testing for adventitious agents or harmful impurities.

A raw material is sterile and produced under aseptic

conditions and/or subject to terminal sterilization, unless otherwise justified. If the raw material is not sterile, the level of microbial contamination must be known.

Additives, such as stabilizers, may be added to the raw material. In cases where antibiotics and stabilizers of biological origin are used in the production of the raw material, their presence is justified and careful consideration is given to their selection, use, quality, and concentration in the raw material, as well as their impact on the actual raw material itself.

General Quality Requirements

Raw materials must meet pre-defined quality requirements for identity, purity, and biological activity. In order to ensure the function of the raw material, it is subject to testing using appropriately qualified methods. The identity test must reflect the uniqueness of the raw material and distinguish it from other related or similar substances. Impurities include both process-related substances (e.g., in the case of recombinant proteins: host-cell-derived proteins (HCP), host-cell-derived DNA and vector-derived DNA (residual DNA), other biological or chemical substances), and product-related substances (e.g., aggregates and degradation products). The content of a raw material may be expressed either in absolute or relative terms. The assay for determination of biological activity may be used to establish the content.

IDENTIFICATION

The identity tests are specific for the particular raw material and address the molecular structure/composition or other relevant physico-chemical, biological or immunochemical properties. Methods used in the determination of biological activity and purity may also serve to identify the raw material. Identification may be carried out by comparison with a defined reference material or a representative batch of the raw material.

GENERAL TESTING METHODS

Tests that may be applicable to raw materials include the followings (see also the sections below that are for specific raw materials, i.e., Sera and serum replacements, Vectors):

Appearance Liquid or reconstituted freeze-dried raw materials comply with the limits defined for the particular raw material with regard to "Clarity of Solution" (Appendix 4.1) and "Colour of Solution" (Appendix 4.2).

Solubility Freeze-dried raw materials dissolve completely in the prescribed volume of reconstituting liquid within a specified time, at a specified temperature, as defined for the particular raw material.

Osmolality Within the limits defined for the particular raw material (Appendix 4.35).

pH Within the limits defined for the particular raw material (Appendix 4.11).

Elemental impurities Within the limits defined for the particular raw material.

Total protein Within the limits defined for the particular raw material (Appendix ==).

Related substances The content of product-related substances is within the limits defined for the particular raw material.

Microbiological control Depending on the raw material concerned, it complies with the "Sterility Test" (Appendix 10.1) or the "Limits for Microbial Contamination" (Appendix 10.5).

Viral contaminants Depending on the raw material concerned, relevant virus contamination is determined.

Bacterial endotoxins Less than the limit defined for the particular raw material (Appendix 8.5).

Mycoplasmas Raw materials are free from mycoplasmas (Appendix ==).

Stabilizer Where applicable, it complies with the limits defined for the particular raw material.

Water Freeze-dried raw materials comply with the limits defined for the particular raw material (Appendix 4.12).

ASSAY

Content The content (e.g., protein content)/composition of the raw material is determined by an appropriate qualified method.

Biological activity Where relevant, the biological activity is determined by a suitable assay. Where relevant (e.g., for enzymes), the biological activity is expressed per milligram of total protein (specific activity).

REFERENCE MATERIAL OR REFERENCE BATCH

An appropriate reference material or a representative batch of the raw material is used to perform the above-mentioned identification, tests, and assay. Where available, the use of established reference substances or reference standards, such as European Pharmacopoeia reference standards or WHO International Standards, is recommended.

Storage

The shelf life and storage conditions are defined.

Labelling

The label states the expiration date, conditions for storage and use, and any code that may be required for traceability including the biological origin of the raw material.

Statements required to appear on the "label on the

container" may appear on a label fastened to the immediate container, or in indelible writing in or on the body of the container itself. They should also be demonstrated on the label on the package.

SERA AND SERUM REPLACEMENTS

DEFINITION

Sera from human or animal sources and serum replacements (including platelet lysates and other undefined growth additives, conditioned media, blood and other cellular components) are used as growth additives for cell culture. Sera and serum replacements used to promote cellular growth are typically complex biological mixtures, whose exact composition is not always possible to define. Due to this complex nature, special attention is given to verifying the consistency and performance of every batch.

Bovine serum If bovine serum is used, use *bovine serum*.

Human serum and platelet lysates Human serum and platelet lysates used as raw materials for the production of cell-based/gene therapy medicinal products are human blood-derived materials, which can originate from the recipient (autologous) or from another individual (allogeneic).

Conditioned media Conditioned media, isolated and purified from cultured cell supernatant, may also be used to enhance cell proliferation due to various growth factors and cytokines secreted by the cells into the medium.

Other growth additives with undefined composition Cell and/or tissue lysates may be used as growth additives.

Composite media Composite media contain growth additives such as bovine serum, growth factors, etc. The principles described in this section of the appendix apply to individual ingredients of biological origin and/or biologically active ingredients of the composite media.

PRODUCTION

Due to potential differences in quality between batches of serum, cell, or tissue lysate, suitable measures are implemented to verify the consistency of each batch before using them as raw materials for the production of cell-based/gene therapy medicinal products.

Because of the inherent risk of transmitting infectious agents from pooled plasma, pooled sera, or other derivatives from pooled allogeneic human blood or plasma, consideration is given to limit the number of donations which are pooled, unless sufficient methods for inactivation/removal of viruses are applied during production, where applicable.

For conditioned media, a cell bank system is preferred. The removal of the cells from the media must be ensured and potential impurities originating from these cells determined if possible.

IDENTIFICATION

It is recognized that the exact qualitative composition of sera and serum replacements may be difficult to determine. However, the approximate protein composition in both cases may be determined by, for example, protein electrophoresis. Where relevant, tests for total protein content or any chemical additives are performed. For human serum, the electrophoretic pattern corresponds to that of an appropriate serum reference batch. Alternatively, identity may be determined by comparison of albumin content with an appropriate serum reference batch. For serum replacements, the electrophoretic pattern or the use of markers secreted by cells/platelets may be used. Human origin is determined by a suitable "Immunochemical Methods" (Appendix 14.5), unless otherwise justified.

TESTS

See under General Testing Methods.

Haemoglobin Where relevant, within the limits defined for the particular raw material.

Cell-derived impurities Where relevant, within the limits defined for the particular raw material.

Specific tests for viral contaminants For bovine serum, the tests for viral contaminants specified in *bovine serum* apply. For human serum, the tests for viral safety specified in the *Plasma for Fractionation* apply.

ASSAY

The serum or serum replacement must show cell growth promoting properties that are within the limits defined for the particular raw material. More than one type of assay may be necessary to show suitability for the intended use.

PROTEINS PRODUCED BY RECOMBINANT DNA TECHNOLOGY

DEFINITION

Proteins and peptides produced by recombinant DNA technology, which are used as raw materials, include growth factors, cytokines, hormones, enzymes, and monoclonal antibodies.

Growth factors, cytokines and hormones They are substances typically used for stimulation or inactivation, growth promotion, or differentiation of cells in cell culture systems.

Other proteins Enzymes (e.g., collagenases), as raw materials, may be used for extraction of active substances from tissues and/or fluids. Other proteins (e.g., fibronectin) may be used as culture supports or media components.

Monoclonal antibodies Used as raw materials, they

include immunoglobulins and fragments of an immunoglobulin with defined specificity. Antibodies can either be conjugated (chemically modified) or non-conjugated. Typical chemical modifications include fluorescent labelling and conjugation to magnetic beads. Antibodies, as raw materials, may be used for selection, activation/stimulation, isolation or purification of cells in cell culture.

PRODUCTION

Production of proteins using recombinant DNA technology is based on a well-characterized host-vector system, using a master cell bank and, if applicable, a working cell bank derived from the master cell bank. The expressed protein is extracted and purified using a variety of techniques, such as extraction, precipitation, centrifugation, concentration, filtration and/or chromatography.

During protein production using recombinant DNA technology, process-related impurities including residual host-cell or vector DNA and host-cell proteins must be reduced to acceptable levels. Particular attention must also be given to product-related impurities.

IDENTIFICATION

Identity is established by appropriate, qualified methods, such as "Electrophoresis" (Appendix 3.7), "Peptide Mapping" (Appendix ==), "Isoelectric Focusing" (Appendix ==) or "Liquid Chromatography" (Appendix 3.5). For antibodies, identification is based on immunoglobulin class, isotype and/or specificity. In addition to the above-mentioned methods, "Immunochemical methods" (Appendix 14.5) and determination of activity are also considered suitable for identification.

TESTS

See under General Testing Methods.

Host-cell-derived proteins and residual host-cell or vector DNA Where relevant for the particular raw material, the content of residual host-cell or vector DNA and/or protein is determined using a suitable method unless the production process has been qualified to demonstrate suitable clearance. The content is within the limits defined for the particular raw material.

Related proteins Related proteins (e.g., polyclonal antibodies with undefined specificities, glycoforms, degradation and oxidation products, oligomers, and aggregates) are determined using liquid chromatography, electrophoretic, or immunological methods and are within the limits defined for the particular raw material.

ASSAY

Content The protein content is determined by an appropriate qualified method, for example by "Liquid Chromatography" (Appendix 3.5) or "Ultraviolet and Visible Spectrophotometry" (Appendix 2.2).

Biological activity The biological activity of a recombinant protein is determined using, for example, cell proliferation, cell differentiation, or an enzyme assay. Several acceptable bioassays may exist for a particular protein. For antibodies, cell-based immunoassays and assays based on ligand-binding and affinity may be used.

Where relevant, the biological activity is expressed per milligram of total protein (specific activity).

PROTEINS EXTRACTED FROM BIOLOGICAL MATERIAL

DEFINITION

Proteins extracted from biological material and used as raw materials include enzymes (e.g., porcine-derived trypsin and endonucleases), polyclonal antibodies, other proteins of biological origin (e.g., albumin and transferrin), and peptides of biological origin. They may be of human, animal, plant, or microbiological origin.

Proteins extracted from biological material are used in a wide range of applications such as growth promotion, differentiation or purification of cultured cells and extraction of active substances from tissues and/or fluids.

PRODUCTION

Proteins are extracted from the blood or tissue of animals or humans, or from plant or microbiological sources using mechanical and/or chemical techniques. They are then subjected to further purification processes using a variety of techniques such as centrifugation, filtration, chromatography, and concentration.

Polyclonal antibodies are produced by immunization with a specific antigen, followed by purification. Antibody purification involves selective enrichment or specific isolation of antibodies from serum based on physico-chemical fractionation, class-specific affinity, and/or antigen-specific affinity.

During production of these proteins, process-related impurities, such as blood components, tissue fragments, or contaminating proteins, must be reduced to acceptable levels. Particular attention is given to product -related impurities.

IDENTIFICATION

Identity is established by appropriate, qualified methods, such as "Electrophoresis" (Appendix 3.7), "Isoelectric Focusing" (Appendix ==), "Peptide Mapping" (Appendix ==), or "Liquid Chromatography" (Appendix 3.5) and "Immunochemical methods" (Appendix 14.5).

TESTS

See under General Testing Methods.

Process-related impurities Substances derived from the starting material (e.g., blood components, tissue fragments, or contaminating proteins) are determined using suitable methods and are within the limits defined for the particular raw material.

Related proteins Related proteins (e.g., antibodies with undefined specificity, degradation and oxidation products, oligomers, and aggregates) are determined using suitable methods and are within the limits defined for the particular raw material.

ASSAY

Content The protein content is determined using an appropriate qualified method, for example by Liquid Chromatography (Appendix 3.1), or UV spectrophotometry (Appendix 2.2).

Biological activity Where relevant, the biological activity of a protein is determined using, for example, enzyme assays, immunoassays, or assays based on cell proliferation/differentiation. For trypsin, the assay may be performed as described in the Trypsin. Where relevant, the biological activity is expressed per milligram of total protein (specific activity).

VECTORS

Vectors that may be used as raw materials in the production of cell-based and gene therapy medicinal products include DNA vectors (e.g., plasmids and transposon vectors) as well as viral vectors and bacteria (e.g., modified Lactococcus species). Vectors are usually considered as starting materials, thus not under the scope of this appendix. In cases where vectors are not considered as starting materials, such as vectors used as helper plasmids or helper viruses, the principles of this appendix and the principles of production and quality control as outlined in chapter "Gene Transfer Medicinal Products for Human Use" are to be followed.

GENE TRANSFER MEDICINAL PRODUCTS FOR HUMAN USE

GENERAL INFORMATION

This chapter provides information and suggests general requirements applicable to the production and control of gene therapy/transfer medicinal products for human use. For a specific medicinal product, application of these requirements and the need for any further information shall be decided by the competent authority. It is to be noted that the information is designed to be applicable to approved products; the need for application of part or all of the texts to products used during the different phases of clinical trials is decided by the competent authority.

DEFINITION

For the purposes of this appendix, gene transfer medicinal product (GTMP) shall mean a product obtained through a set of manufacturing processes aimed at the transfer, to be performed either *in vivo* or *ex vivo*, of a prophylactic, diagnostic or therapeutic gene (i.e., a piece of nucleic acid) to human/animal cells, and its subsequent expression *in vivo*. The gene transfer involves an expression system known as a vector, which can be of viral as well as non-viral origin. The vector can also be included in a human or animal cell.

Recombinant vectors, such as viral vectors and plasmids Recombinant vectors are either injected directly into the patient's body (*in vivo* gene transfer) or transferred into host cells before administration of these genetically modified cells to the patient (*ex vivo* gene transfer). Viral vectors are derived from various viruses (for example, adenoviruses, poxviruses, retroviruses, lentiviruses, adeno-associated-viruses, herpesviruses). These vectors can be replicative, non-replicative or conditionally replicative. Plasmid vectors include nucleic acids in a simple formulation (for example, naked DNA) or complexed to various molecules (synthetic vectors such as lipids or polymers). Genetic material transferred by GTMPs consists of nucleotide sequences, which may notably encode gene products, antisense transcripts, or ribozymes. Chemically synthesized oligonucleotides are not within the scope of this appendix. After transfer, the genetic material may remain either cytoplasmic or episomal, or may be integrated into the host cell genome, depending on the integrating or non-integrating status of the vector.

Genetically modified cells Genetically modified eukaryotic or bacterial cells are modified by vectors to express a product of interest.

PRODUCTION

Substances used in production. The raw materials used during the manufacturing process, including viral seed lot and cell bank establishment, where applicable, are qualified. Unless otherwise justified, all substances used are produced within a recognized quality management system using suitable production facilities. Suitable specifications are established to control notably their identity, potency (where applicable), purity, and safety in terms of microbiological quality and bacterial endotoxin contamination. The quality of water used complies with the relevant corresponding *Water for Injections* and *Purified Water*. Where bovine serum is used, it complies with the *bovine serum*. The use of antibiotics is avoided wherever possible during production.

Viral safety The requirements of "Viral Safety" (Appendix ==) apply.

Transmissible spongiform encephalopathies A risk assessment of the product with respect to transmissible spongiform encephalopathies is carried out and suitable measures are taken to "Minimize the Risk" (Appendix ==).

RECOMBINANT VECTORS

PRODUCTION

GENERAL PROVISIONS

For viral vectors, production is based on a cell bank system and a virus seed-lot system, wherever possible.

For plasmid vectors, production is based on a bacterial cell bank system.

The production method shall have been shown to yield a vector of consistent quality. Unless otherwise justified and authorized, the vector in the final product shall have undergone no more passages or subcultures from the master seed lot than were used to prepare the vector shown in clinical trials to be satisfactory with respect to safety and efficacy.

SUBSTRATE FOR VECTOR PROPAGATION

The substrates used comply with the "Chicken Flocks Free From Specified Pathogens for the Production and Quality Control of Vaccines" (Appendix ==), "Cell Substrates for the Production of Vaccines for Human Use" (Appendix ==), and the section Bacterial cells used for the manufacture of plasmid vectors for human use).

CHARACTERIATION OF THE VECTOR

Historical records of vector construction are documented, including the origin of the vector and its subsequent manipulation, notably deleted or modified regions.

The vector is characterized using suitable and validated methods.

The genetic stability of the vector at or beyond the maximum passage level or the maximum number of cell

doublings of the cell line used for production is assessed by suitable methods.

PROPAGATION AND HARVEST

All processing of the cell banks and subsequent cell cultures is done in an area where no other cells or vectors are handled at the same time. Any material of human or animal origin used in the preparation of cell suspensions and culture media is qualified. The purity of the harvest is verified by suitable tests as defined in the corresponding specific sections.

PURIFIED HARVEST

The bulk of active substance is defined as a lot of purified recombinant vectors (viral vectors, or naked or complexed plasmids).

FINAL LOT

Unless otherwise justified and authorized, formulation and distribution of the final bulk are carried out under aseptic conditions using sterile "Containers" (Appendix ==).

The stability of the final lot is assessed using stability protocols including the duration, storage conditions, number of lots to be tested, test schedule, and assays to be performed.

ASSAYS AND TESTS

The GTMPs comply with assays and tests described in the corresponding specific sections.

GENETICALLY MODIFIED CELLS

For cells to be modified with a recombinant vector, the data related to the recombinant vector are documented above, under Recombinant vectors.

PRODUCTION

CELL SUBSTRATE

For xenogeneic cell lines, including bacterial cells, a cell bank system comprising a master cell bank and working cell banks is established.

For autologous and allogeneic cells, a cell banking system comprising a master cell bank and working cell banks is established wherever possible.

TRANSFECTION / TRANSDUCTION

Cells are transfected or transduced using a recombinant vector (plasmid or viral vector) qualified as described under Recombinant vectors; the process is validated. They are handled under aseptic conditions in an area where no other cells or vectors are handled at the same time. All reagents used during cell manipulation steps are fully qualified. Antibiotics are avoided unless otherwise justified and authorized. Transfection or transduction is carried out under aseptic conditions.

FINAL LOT

In the case of frozen storage, the viability of genetically modified cells is assessed before freezing and after thawing.

If the cells are not used within a short period, stability is determined by verifying cell viability and expression of the genetic insert.

In the case of genetically modified cells encapsulated before implantation in man, any encapsulating component used is considered as part of the final product, and is therefore quality-controlled and fully characterized (for example, physical integrity, selective permeability and sterility).

ASSAYS AND TESTS

Controls of xenogeneic, allogeneic, or autologous cells include the following:

– identity, counting, and viability of cells;

– overall integrity, functionality, copies per cell, transfer, and expression efficiency of the genetic insert;

– microbiological controls, comply with the "Sterility Test" (Appendix 10.1) or "Microbiological examination of cell-based preparations" (Appendix ==), "Test for Bacterial Endotoxins", (Appendix 8.5), "Mycoplasmas" (Appendix ==), adventitious virus contamination and, where applicable, replicative vector generation.

The competent authority may approve a reduced testing programme where necessary because of limited availability of cells. Where necessary because of time constraints, the product may be released for use before the completion of certain tests.

ADENOVIRUS VECTORS FOR HUMAN USE

Category Vectors.

Adenovirus vectors for human use are freeze-dried or liquid preparations of recombinant adenoviruses, genetically modified to transfer genetic material to human somatic cells *in vivo* or *ex vivo*.

PRODUCTION

VECTOR CONSTRUCTION

There are different approaches for the design and construction of an adenovirus vector. The purpose of clinical use determines which approach is optimal. A method is chosen that minimizes the risk of generating replication-competent adenovirus vectors or that effectively eliminates helper viruses that might be used during production.

VECTOR PRODUCTION

The production method shall have been shown to yield a vector of consistent quality. Unless otherwise justified and authorized, the vector in the final product shall have undergone no more passages from the master

seed lot than were used to prepare the vector shown in clinical trials to be satisfactory with respect to safety and efficacy.

The genetic and phenotypic stability of the vector at or beyond the maximum passage level used for production is assessed by suitable methods.

SUBSTRATE FOR VECTOR PROPAGATION

The vector is propagated in continuous cell lines, comply with the "Cell Substrates for the Production of Vaccines for Human Use" (Appendix ==), based on a cell bank system. The occurrence of replication-competent adenoviruses may be significant when large regions of homology exist between the viral genome and the genome of the complementation cells. This occurrence may be minimized by minimizing the homology between both genomes. The use of cells with no sequence homology with the vector is recommended for production.

VECTOR SEED LOT

Production of the vector is based on a seed-lot system.

The strain of adenovirus used is identified by historical records that include information on its origin and its subsequent manipulation, notably deleted or modified regions. A detailed description of the genetic insert(s) and the flanking control regions is established, including the nucleotide sequence. The method by which the genetic insert is introduced into the vector is documented.

Only a seed lot that complies with the following requirements may be used for vector production.

Identification The vector is identified in the master seed lot and each working seed lot by "Immunochemical Methods" (Appendix 14.5), "Nucleic Acid Amplification Techniques" (Appendix 14.6), or restriction enzyme analysis.

Genetic and phenotypic characterization The following tests are carried out.

– The entire genome of the vector is sequenced at a passage level comparable to a production batch and the analytically determined sequence is compared to the theoretical sequence based on vector construction and available databases.

– Restriction enzyme analysis is performed on the vector DNA of the master seed lot, each working seed lot, and a production batch. The viral DNA is extracted, purified, and digested with sufficient resolution. The digested fragments are separated by gel electrophoresis or capillary electrophoresis and the observed restriction pattern is compared to the theoretical restriction pattern based on vector construction.

– A suitable number of isolated sub-clones are tested for expression of the genetic insert product(s) and biological activity at a passage level comparable to a production batch. Sub-clones giving lower levels of expression or biological activity need further characterization.

Vector concentration The titre of infectious vector or the concentration of vector particles in the master seed lot and each working seed lot are determined.

Extraneous agents The master seed lot and each working seed lot comply with the tests for extraneous agents.

Replication-competent adenoviruses Replication-competent adenoviruses are generated by homologous recombination between the recombinant viral DNA and the adenovirus sequences integrated into the genome of the complementation cells.

Detection of replication-competent adenoviruses is performed by a suitable method approved by the competent authority. It is generally performed by an infectivity assay on sensitive detector cell lines, which are not able to complement for the genes deleted from the vector. Other indicators of viral replication may be used as appropriate.

When replication-competent adenoviruses are not supposed to be present in the test sample, considering vector construction and cell lines used, at least 2, but preferably 3 or 4 successive passages are performed on the detector cell line, where applicable. Detection of a cytopathic effect at the end of the passages reveals the presence of replication-competent adenoviruses in the preparation. Positive controls are included in each assay to monitor its sensitivity.

When replication-competent adenoviruses are expected to be present in the test sample, plaque-assays or limit dilution assays on a detector cell line may be performed.

PROPAGATION AND HARVEST

All processing of the cell bank and subsequent cell cultures is done in an area with a suitable containment level where no other cells or vectors are handled at the same time. Any material of human or animal origin used in the preparation of cell suspensions and culture media is qualified. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration, but it is preferable to have a substrate free from antibiotics during production. Unless otherwise justified and authorized, at no stage during production is penicillin or streptomycin used. A portion of the production cell cultures is set aside as uninfected cell cultures (control cells).

Each single harvest that complies with the following requirements may be used in the preparation of the purified harvest.

Identification The vector is identified by

"Immunochemical Methods" (Appendix 14.5), "Nucleic Acid Amplification Techniques" (Appendix 14.6), or restriction enzyme analysis.

Vector concentration The titre of infectious vector and the concentration of vector particles in single harvests are determined.

Extraneous agents The single harvest complies with the tests for extraneous agents.

Control cells Control cells comply with a test for identification and a test for extraneous agents.

PURIFIED HARVEST

Several single harvests may be pooled before the purification process. The purification process is validated to demonstrate the satisfactory removal of impurities.

Purified harvests that comply with the following requirements may be used in the preparation of the final bulk.

Identification The vector is identified by "Immunochemical Methods" (Appendix 14.5), "Nucleic Acid Amplification Techniques" (Appendix 14.6), or restriction enzyme analysis.

Genomic integrity Genomic integrity of the vector is verified by suitable methods such as restriction enzyme analysis.

Vector concentration The titre of infectious vector and the concentration of vector particles in purified harvests are determined.

Residual host-cell protein The concentration of residual host-cell protein is determined by a suitable "Immunochemical Methods" (Appendix 14.5), unless the process has been validated to demonstrate suitable clearance.

Residual host-cell DNA The content of residual host-cell DNA is determined using a suitable method, unless the process has been validated to demonstrate suitable clearance. Quantitative polymerase chain reaction (PCR) is recommended for its sensitivity and specificity, but other suitable techniques may also be used.

Residual reagents Where reagents are used during the production process, tests for these substances are carried out on the purified harvest, unless the process has been validated to demonstrate suitable clearance.

Residual antibiotics Where antibiotics are used during the production process, their residual concentration is determined by a microbiological assay (adapted from general method) or by other suitable methods (for example, liquid chromatography), unless the process has been validated to demonstrate suitable clearance.

FINAL BULK

Several purified harvests may be pooled during preparation of the final bulk. A stabilizer and other excipients may be added. The formulated product is filtered through a bacteria-retentive filter.

Only a final bulk that complies with the following requirement may be used in the preparation of the final lot.

Sterility It complies with the "Sterility Test" (Appendix 10.1).

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use.

Provided that the tests for bovine serum albumin (when bovine serum is used to manufacture the vector) and replication-competent adenoviruses have been carried out with satisfactory results on the final bulk, they may be omitted on the final lot.

IDENTIFICATION

The vector is identified by "Immunochemical Methods" (Appendix 14.5), "Nucleic Acid Amplification Techniques" (Appendix 14.6), or restriction enzyme analysis.

TESTS

Osmolality Within the limits approved for the particular preparation (Appendix 4.35).

pH Within the limits approved for the particular preparation (Appendix 4.11).

Extractable volume It complies with the test for extractable volume.

Residual moisture Within the limits approved for the particular freeze-dried preparation.

Bovine serum albumin Not more than the limit approved for the particular preparation, determined by a suitable "Immunochemical Methods" (Appendix 14.5), where bovine serum has been used during production.

Replication-competent adenovirus concentration Within the limits approved for the particular preparation.

Vector aggregates Vector aggregates are determined by suitable methods (for example, light scattering).

Sterility It complies with the "Sterility Test" (Appendix 10.1).

Bacterial endotoxins Less than the limit approved for the particular preparation (Appendix 8.5).

Thermal stability Maintain samples of the vector final lot at a temperature and for a length of time that are adapted and authorized for the particular preparation.

Determine the total infectious vector concentration after heating, as described below under Assay. Determine in parallel the vector concentration of a non-heated sample. The estimation of the difference between the total vector concentration without heating and after heating is within the limits approved for the particular preparation.

ASSAY

Vector particle concentration Physical titration is performed by a suitable technique (for example, liquid chromatography, absorbance measurement or "Nucleic Acid Amplification Techniques" (Appendix 14.6), use an appropriate vector reference standard to validate each assay.

The vector particle concentration of the preparation to be examined is not less than the concentration stated on the label.

Infectious vector titre Titrate the preparation to be examined by inoculation into cell cultures. Titrate an appropriate vector reference standard to validate each assay.

The assay is invalid if:

– the confidence interval (P = 0.95) of the logarithm of the vector concentration is greater than a value authorized by the competent authority;

– the infectious vector titre of the reference standard is outside limit values defined by a control chart.

Ratio of vector particle concentration to infectious vector titre Within the limits approved for the particular preparation.

Expression of the genetic insert product The expression of the genetic insert product(s) is determined wherever possible, following inoculation of cell cultures with the particular preparation at a predetermined multiplicity of infection, by suitable "Immunochemical Methods" (Appendix 14.5), or biochemical assays or by flow cytometry.

Biological activity Unless otherwise justified and authorized, biological activity is determined by a suitable in vitro or in vivo test.

LABELLING The label on the container or the label on the package states at least (1) the content of active substance; (2) the recommended human dose, expressed in vector particle concentration; (3) for freeze-dried preparations, states the name or composition and the volume of the reconstituting liquid to be added and the time within which the product is to be used after reconstitution.

BACTERIAL CELLS USED FOR THE MANUFACTURE OF PLASMID VECTORS FOR HUMAN USE

Category Cells used for the manufacture of plasmid vectors.

Production of plasmid vectors for human use is based on the use of a bacterial cell-bank system with generation and characterization of a master cell bank (MCB), working cell banks (WCBs) and end-of-production cells (EOPCs). A bacterial cell bank for the manufacture of plasmid vectors is a collection of vials containing bacterial cells stored under defined conditions, with uniform composition, and obtained from pooled cells derived from a single clone of a transformed host strain. The MCB has a known, documented history; it is preferably derived from a qualified repository source. The WCB is produced by expanding one or more vials of the MCB. Methods and reagents used to produce the bank and storage conditions are documented.

MCBs and WCBs are qualified by testing an aliquot of the banked material or testing a subculture of the cell bank.

The following table indicates the tests required at each stage of production.

IDENTITY AND PURITY TESTING

Viability The number of viable cells is determined by plating a diluted aliquot of bacterial cells on an appropriate medium and counting individual colonies.

Biochemical and physiological bacterial strain characterization Depending on the bacterial strain used for production, relevant biochemical and physiological characterization is performed to confirm cell dentity at the species level.

Genotyping/phenotyping The genotype of bacterial cells is verified by determination of the suitable specific phenotypic markers or by appropriate genetic analysis.

Presence of the plasmid

SEQUENCING The whole nucleotide sequence of the plasmid is verified.

COPY NUMBER The plasmid DNA is isolated and purified from a known number of bacteria and the copy number determined by a suitable method such as "Nucleic Acid Amplification Techniques" (Appendix 14.6).

RESTRICTION MAP Restriction endonuclease digestion is performed with sufficient resolution to verify that the structure of the plasmid is unaltered in bacterial cells.

PERCENTAGE OF CELLS RETAINING THE PLASMID Bacterial elements present in the plasmid, such as selectable genetic markers, are used to define the percentage of bacteria retaining the plasmid.

Assay	Host strain	MCB	WCB	EOPCs[a]
Identity and purity				
Viability	+	+	+	+
Bacterial strain characterization	+	+	–	+
Genotyping / phenotyping	+	+	–	+
Presence of the plasmid				
–SequenceoftheDNAplasmid	–	+	–	+
–Copynumber	–	+	+	+
–Restrictionmap	–	+	+	+
– Percentage of cells retaining the plasmid	–	+	+	+
Adventitious agents				
Purity by plating	+	+	+	+
Presence of bacteriophage	+	+	–	+

[a]EOPCs are cells with a passage number at least equivalent to that used for production. The analysis has to be done once to validate each new WCB, except for purity, which has to be tested for each fermentation.

ADVENTITIOUS AGENTS AND ENDOGENOUS VIRUSES

Purity by plating Bacterial cells are streaked out onto suitable media and incubated in the required conditions in order to detect potential bacterial contaminants. In order to test for inhibition of the growth of contaminating organisms, additional tests in the presence of a definite amount of relevant positive control bacteria are carried out. A suitable number of colonies is examined; no contamination is detected.

Presence of bacteriophage Bacterial cells are plated and incubated in a medium allowing proliferation of bacteriophages, to test for bacteriophage presence. The test is validated by the use of a reference bacteriophage strain and permissive cells as positive controls. A suitable number of colonies are examined; no contamination is detected.

PLASMID VECTORS FOR HUMAN USE

Category Vectors.

Plasmid vectors for human use are double-stranded circular forms of bacterial DNA that carry a gene of interest or a nucleotide sequence encoding antisense sequences or ribozymes and its expression cassette; they are amplified in bacteria extrachromosomally. They are used to transfer genetic material into human somatic cells in vivo or to genetically modify autologous, allogeneic, xenogeneic, or bacterial cells before administration to humans. Plasmid vectors may be presented as naked DNA or may be formulated with synthetic delivery systems such as lipids (lipoplexes), polymers (polyplexes), and/or peptide ligands that facilitate transfer across the cell membrane and delivery to the cell, or that target delivery via specific receptors.

Plasmids formulated with synthetic delivery systems are not within the scope of this section.

PRODUCTION

PLASMID CONSTRUCTION

A typical plasmid vector is composed of:

– the plasmid vector backbone that contains multiple restriction endonuclease recognition sites for insertion of the genetic insert and the bacterial elements necessary for plasmid production, such as selectable genetic markers for the identification of cells that carry the recombinant vector;

– the required regulatory genetic elements to facilitate expression of the genetic insert;

– the genetic insert;

– a polyadenylation signal.

A complete description of the plasmid DNA, including its nucleotide sequence, is established with the identification, source, means of isolation and nucleotide sequence of the genetic insert. The source and function of component parts of the plasmid, such as the origin of replication, viral and eukaryotic promoters, and genes encoding selection markers, are documented.

GENERAL PROVISIONS

Cell banks Production of plasmid vectors is based on a bacterial cell-bank system with generation and characterization of a master cell bank (MCB), working cell banks (WCBs), and end-of-production cells (EOPCs), which comply with the section Bacterial cells used for the manufacture of plasmid vectors for human use. The raw materials used during the manufacturing process, including cell bank establishment, are qualified.

Selection techniques Unless otherwise justified and authorized, antibiotic-resistance genes used as selectable genetic markers, particularly for clinically useful antibiotics, are not included in the vector construct. Other selection techniques for the recombinant plasmid are preferred.

Reference substances A suitable batch of the formulated plasmid, preferably one that has been

clinically evaluated, is fully characterized and retained for use as a reference standard as necessary in routine control tests.

PROPAGATION AND HARVEST

Plasmid DNA is transferred to host strain bacterial cells and a single clone of transformed bacteria is expanded to create the MCB. The WCB is then derived from the MCB. The EOPCs are obtained from the WCB by fermentation in production conditions.

Plasmid DNA is isolated from harvested cells using an extraction step and is purified to obtain the bulk product.

Unless otherwise justified and authorized, caesium chloride-ethidium bromide density gradients are not used for production.

PURIFIED PLASMID

The production process is optimized to remove impurities consistently while retaining product activity. The requirement to test for a particular impurity depends on the following:

– the demonstrated capability of the manufacture and purification processes to remove or inactivate the impurity through process validation, using specific quantification methods;

– the potential toxicity associated with the impurity;

– the potential decrease of the efficacy of the genetic insert product associated with the impurity.

If selective resistance to specific antibiotics has been used for selection, data from validation studies of purification procedures are required to demonstrate the clearance capability for residual antibiotics.

Relevant in-process controls are performed to ensure that the process is continuously under control, for example, amount and form of plasmid after the extraction steps and amount of endotoxins after the extraction steps. Only a batch of purified plasmid that complies with the following requirements may be used.

Identity and integrity of the purified plasmid Identity and integrity of the purified plasmid are established by suitable methods such as sequencing or "Nucleic Acid Amplification Techniques" (Appendix 14.6); restriction enzyme analysis may be used where it is sufficient to detect potential critical modifications in the plasmid and confirm the plasmid identity.

Plasmid DNA The following indications are given as examples.

DNA concentrations greater than 500 ng per mL may be determined using absorbance measurement at 260 nm. A 50 µg per mL double-stranded DNA solution has an absorbance of 1 (specific absorbance 200).

DNA concentrations less than 500 ng per mL are determined following incubation with fluorescent dyes that bind specifically to double-stranded DNA, using a reference standard of DNA to establish a calibration curve.

Liquid chromatography may also be used to determine the concentration of plasmid DNA using a reference standard. In some cases, capillary electrophoresis is also acceptable.

DNA forms Plasmid DNA is characterized in terms of the proportions of supercoiled, multimeric, relaxed monomer and linear forms, using suitable analytical methods, examples of which are given below. For quantification of supercoiled forms, anion-exchange "High-Performance Liquid Chromatography" (Appendix 3.5) or "Capillary Electrophoresis" (Appendix 3.8) may be used. Capillary electrophoresis is also suitable for the quantification of other forms.

Residual host-cell DNA The content of residual host-cell DNA is determined using a suitable method, unless the process has been validated to demonstrate suitable clearance. Quantitative PCR is recommended for its sensitivity and specificity, but other suitable techniques may also be used.

Residual RNA The content of residual RNA is determined, unless the process has been validated to demonstrate suitable clearance. Reversed-phase HPLC (RP-HPLC) may be used, or quantitative reverse-transcriptase polymerase chain reaction (RT-PCR), comply with the "Nucleic Acid Amplification Techniques" (Appendix 14.6) when a lower limit of detection is required.

Residual host-cell protein The concentration of residual host-cell protein is determined using standard protein assays, comply with the "Total Protein" (Appendix ==), SDS-PAGE followed by silver staining, or specific immuno-assays such as western blot or ELISA, unless the process has been validated to demonstrate suitable clearance.

Microbiological control Depending on the preparation concerned, it complies with the "Sterility Test" (Appendix 10.1) or the bioburden is determined (2.6.12).

Bacterial endotoxins Less than the limit approved for the particular preparation, it complies with the "Test for Bacterial Endotoxins" (Appendix 8.5).

FINAL BULK

Several purified harvests may be pooled during preparation of the final bulk. A stabilizer and other excipients may be added. The formulated product is filtered through a bacteria-retentive filter.

Only a final bulk that complies with the following requirement may be used in the preparation of the final lot.

Sterility It complies with the "Sterility Test" (Appendix 10.1).

FINAL LOT

Only a final lot that complies with each of the requirements given below under Identification, Tests and Assay may be released for use.

IDENTIFICATION The plasmid vector is identified by restriction enzyme analysis or by sequencing. The test for biological activity also serves to identify the product.

TESTS

Appearance According to the manufacturer.

pH Within the limits approved for the particular preparation (Appendix 4.11).

Extractable volume It complies with the test for extractable volume.

Residual moisture Within the limits approved for the particular freeze-dried preparation.

DNA forms The percentage of the specific monomeric supercoiled form is determined as described for the purified plasmid.

Sterility It complies with the "Sterility Test" (Appendix 10.1).

Bacterial endotoxins Less than the limit approved for the particular preparation, it complies with the "Test for Bacterial endotoxins" (Appendix 8.5).

ASSAY

Plasmid DNA Not less than the quantity stated on the label, determined, for example, by one of the following methods.

DNA concentrations greater than 500 ng/mL may be determined using absorbance measurement at 260 nm. A 50 µg/mL double-stranded DNA solution has an absorbance of 1 (specific absorbance 200).

DNA concentrations less than 500 ng/mL are determined following incubation with fluorescent dyes that bind specifically to double-stranded DNA, using a reference standard of DNA to establish a calibration curve.

Liquid chromatography may also be used to determine the concentration of plasmid DNA using a reference standard. In some cases, capillary electrophoresis is also acceptable.

Biological activity Wherever possible, biological activity is assessed through *in vitro* or *in vivo* bioassays. A well-defined, representative reference standard is required as a positive control for the assay. Bioassays employed to assay plasmid vectors generally involve transfection of a relevant cell line *in vitro*, followed by some functional measure of the expressed genetic insert. Such functional assays provide information about the activity of the product encoded by the genetic insert instead of the expression level of the genetic insert itself.

It may be necessary to supplement the bioassay with western-blot and ELISA assays to assess the integrity and quantity of the expressed product.

LABELLING The label on the container or the label on the package states at least (1) the plasmid DNA concentration; (2) the recommended human dose; (3) for freeze-dried preparations; states the name and volume of the liquid to be added; the time within which the product is to be used after reconstitution.

APPENDICES

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APPENDIX 1 GENERAL INFORMATION

The specifications given below are strictly for the use of the materials as reagents. The inclusion of a material in this Appendix does not imply that it is suitable for use in medicines. Exceptionally, a trademark or supplier may be indicated for certain reagents whose availability is limited. It is however acceptable to use reagents from another source provided that they comply with the standards of the Pharmacopoeia.

1.1 REAGENTS

The name of a substance or solution indicates a reagent included in the following list. The specifications given for reagents do not necessarily guarantee their quality for use in medicines.

Some of the reagents included may be injurious to health. Important cautions have been stated for these reagents. They should be handled in accordance with good laboratory practice and any relevant regulations.

Reagents in aqueous solution are prepared using *water*. Where the name of the solvent is not stated, an aqueous solution is intended.

Unless otherwise specified, the reagents and reagent solutions are to be stored in well-closed containers. The labelling should comply with the relevant national legislation.

Acetoxyvalerenic Acid $C_{17}H_{24}O_4$ = 292.4

Use analytical reagent grade of commerce

DESCRIPTION Colourless or pale yellow viscous oil.

Anethole $C_{10}H_{12}O$ = 148.20

DESCRIPTION Colourless or faintly yellow liquid at or above 23°; odour aromatic. Affected by light.

SOLUBILITY Very slightly soluble in *water*; freely soluble in *ethanol*; readily miscible with *chloroform* and with *ether*.

FREEZING TEMPERATURE Not lower than 20° (Appendix 4.4).

BOILING RANGE 231° to 237° (Appendix 4.5).

REFRACTIVE INDEX 1.557 to 1.561 (Appendix 4.7).

OPTICAL ROTATION –0.15° to +0.15° (Appendix 4.8).

SPECIFIC GRAVITY 0.983 to 0.988 (Appendix 4.9).

Bergapten $C_{12}H_8O_4$ = 216.2

DESCRIPTION Colourless crystals.

SOLUBILITY practically insoluble in water, sparingly soluble in *ethanol* and slightly soluble in *glacial acetic acid*.

MELTING POINT about 188° (Appendix 4.3).

Bromocresol Purple $C_{21}H_{16}Br_2O_5S$ = 540.2

DESCRIPTION Pinkish powder

SOLUBILITY practically insoluble in *water*, soluble in *ethanol* and in dilute solutions of alkali hydroxides.

Borneol $C_{10}H_{18}O$ = 154.3

Bornyl Acetate $C_{12}H_{20}O_2$ = 196.3

DESCRIPTION Colourless crystals or a colourless liquid.

SOLUBILITY very slightly soluble in water, soluble in *ethanol*.

MELTING POINT about 28° (Appendix 4.3).

Cinchonine $C_{19}H_{22}N_2O$ = 294.40

DESCRIPTION White crystalline powder.

SOLUBILITY Very slightly soluble in water; soluble in ethanol.

MELTING TEMPERATURE About 263° (Appendix 4.3).

SPECIFIC ROTATION About 215° (Appendix 4.8), determined in a 1 per cent w/w solution in *ethanol*.

Concentrated SDS-PAGE Sample Buffer for Reducing Conditions

Dissolve 3.78 g of *tris(hydroxymethyl)aminomethane*, 10.0 g of *sodium dodecyl sulfate* and 100 mg of *bromophenol blue* in *water*. Add 50.0 mL of *glycerol* and dilute to 200 mL with *water*. Add 25.0 mL of *2-mercaptoethanol*. Adjust to pH 6.8 with *hydrochloric acid*, and dilute to 250.0 mL with *water*.

Alternatively, dithiothreitol may be used as reducing agent instead of *2-mercaptoethanol*. In this case prepare the sample buffer as follows: dissolve 3.78 g of *tris (hydroxymethyl)aminomethane*, 10.0 g of *sodium dodecyl sulfate* and 100 mg of *bromophenol blue* in *water*. Add 50.0 mL of *glycerol* and dilute to 200 mL with *water*. Adjust to pH 6.8 with *hydrochloric acid*, and dilute to 250.0 mL with *water*. Immediately before use, add *dithiothreitol* to a final concentration of 100 mM.

Chloramine T $C_7H_7ClNNaO_2S \cdot 3H_2O$ = 281.69

Use analytical reagent grade of commerce

***p*-Cymene** $C_{10}H_{14}$ = 134.2

Use analytical reagent grade of commerce containing not less than 96.0 per cent w/v of $C_{10}H_{14}$.

DESCRIPTION Colourless liquid.

SOLUBILITY practically insoluble in water, soluble in *ethanol*.

RELATIVE DENSITY about 0.858 (Appendix 4.9).

REFRACTIVE INDEX about 1.4895 (Appendix 4.7).

BOILING POINT 175° to 178° (Appendix 4.5).

Diisopropyl Ether (Isopropyl Ether) $C_6H_{14}O$ = 102.18

> ***Caution*** It is highly flammable. Do not use where it may be ignited. Do not evaporate to the point of near -dryness, since it tends to form explosive peroxides.

DESCRIPTION Colourless, mobile liquid.

SOLUBILITY Slightly soluble in *water*. Miscible with *ethanol* and with *ether*.

SPECIFIC GRAVITY 0.716 to 0.720 (Appendix 4.9).

BOILING RANGE Not less than 95 per cent distils between 65° to 70° (Appendix 4.5).

PEROXIDES To 10 mL, contained in a clean, glass-stoppered cylinder previously rinsed with a portion of the ether under examination, add 1 mL of *potassium iodide TS*. Shake, and allow to stand for 1 minute: no yellow colour is observed in ether layer.

NON-VOLATILE MATTER (**Note** If peroxide is present, do not carry out this procedure.) When evaporated and dried at 105° for 1 hours, leaves not more than 0.01 per cent w/w of residue.

ACIDITY Add 2 drops of *bromothymol blue TS* to 10 mL of *water* in a glass-stoppered 50-mL flask, and titrate with 0.010 M *sodium hydroxide* until a blue colour persists after vigorous shaking. Add 5.0 mL of the *ether* under examination, and titrate with 0.010 M *sodium hydroxide*: not more than 0.30 mL is required to restore the blue colour.

Note For spectrophotometric determinations, use *diisopropyl ether* that meets the following additional requirements:

ABSORBANCE Its absorbance at 255 nm, in a 1-cm quartz cell, dose not exceed 0.2, *water* being used as the blank.

Diphenylboric Acid Aminoethyl Ester (Diphenylboric Acid b-Aminoethyl Ester; Diphenylboric Acid-Ethanolamine Reagent) $C_{14}H_{16}BNO$ = 225.10

Use a suitable grade.

Dipotassium Hydrogenphosphate K_2HPO_4 = 174.18

Contains not less than 98.0 per cent and not more than 101.0 per cent of K_2HPO_4.

DESCRIPTION White, crystalline powder.

SOLUBILITY Soluble in *water*.

CHLORIDE Not more than 0.018 per cent w/w (Appendix 5.2). A 2.0-g sample shows no more chloride than that corresponds to 0.50 mL of 0.020 M *hydrochloric acid*.

SULFATE Not more than 0.06 per cent w/w (Appendix 5.2). An 800-mg sample shows no more sulfate than that corresponds to 0.50 mL of 0.010 M *sulfuric acid*.

ASSAY Dissolve about 5, accurately weighted, in 100 mL of *water* and titrate with 1 M *hydrochloric acid* VS, using *bromocresol green TS* as indicator to the green colour. Each mL of 1 M *hydrochloric acid VS* is equivalent to 174.2 mg of K_2HPO_4.

Dithiothreitol $C_4H_{10}O_2S_2$ = 154.2

DESCRIPTION Slightly hygroscopic needles.

SOLUBILITY freely soluble in *water*, in *acetone* and in *anhydrous ethanol*.

Store in an airtight container, protected from light

Geranyl acetate $C_{12}H_{20}O_2$ = 196.27

Use an analytical reagent grade of commerce containing not less than 97.0 per cent w/w of $C_{12}H_{20}O_2$.

DESCRIPTION Colourless or slightly yellow liquid, slight odour of rose and lavender.

Store in tightly closed containers, in a cool and dry place

Glutamyl Endopeptidase for Peptide Mapping

Endoproteinase Glu-C of high purity from *Staphylococcus aureus* strain V8.

Guaiazulene $C_{15}H_{18}$ = 198.3

DESCRIPTION Dark-blue crystals or blue liquid

SOLUBILITY very slightly soluble in *water*, miscible with *fatty* and *essential oils* and with *liquid paraffin*, sparingly soluble in *ethanol*, soluble in 50 per cent w/w solution of *sulfuric acid* and 80 per cent w/w solution of *phosphoric acid*, giving a colourless solution

MELTING POINT about 30°. (Appendix 4.3).

Store in an airtight container, protected from light

Glutaraldehyde $C_5H_8O_2$ = 100.1

DESCRIPTION Oily liquid

SOLUBILITY Soluble in *water*

REFRACTIVE INDEX About 1.434 (Appendix 4.7).

BOILING POINT About 188° (Appendix 4.5).

Hyoscine Hydrobromite (Scopolamine Hydrobromite) $C_{17}H_{22}BrNO_4 \cdot 3H_2O$ = 438.32

DESCRIPTION Colourless transparent crystals, or white crystalline powder; odourless.

SOLUBILITY Freely soluble in *water*; soluble in *ethanol*; slightly soluble in *chloroform*.

MELTING RANGE 195° to 199° (Appendix 4.3).

SPECIFIC ROTATION -24° to -26° at 20°, calculated on the anhydrous basis, determined in a 5.0 per cent w/v solution (Appendix 4.8).

Hyoscyamine sulfate $C_{34}H_{48}N_2O_{10}S \cdot 2H_2O$ = 713

DESCRIPTION White or almost white, crystalline powder or colourless needles.

SOLUBILITY Very soluble in water, sparingly soluble or soluble in *ethanol*.

PH 4.5 to 6.2. Dissolve 0.5 g in *carbon dioxide-free water* and dilute to 25 mL with the same solvent.

OPTICAL ROTATION –29° to –24° (anhydrous substance). (Appendix 4.8)

Store in an airtight container, protected from light

Hyperoside $C_{21}H_{20}O_{12}$ = 464.4

DESCRIPTION Faint yellow needles.

SOLUBILITY Soluble in *methanol*.

(+)-Isomenthone $C_{10}H_{18}O$ = 154.2

Use analytical grade of commerce containing not less than 80.0 per cent w/v of $C_{10}H_{18}O$.

DESCRIPTION A colourless liquid.

SOLUBILITY Very slightly soluble in *water*, soluble in *ethanol*

RELATIVE DENSITY About 0.904 (Appendix 4.9).

REFRACTIVE INDEX About 1.453 (Appendix 4.7).

SPECIFIC OPTICAL ROTATION about +93.2° (Appendix 4.8).

Isopulegol $C_{10}H_{18}O$ = 154.2

Use analytical grade of commerce containing not less than 99.0 per cent w/v of $C_{10}H_{18}O$.

RELATIVE DENSITY About 0.911 (Appendix 4.9).

REFRACTIVE INDEX About 1.472 (Appendix 4.7).

BOILING POINT About 210° (Appendix 4.5).

Limonene $C_{10}H_{16}$ = 136.23

Use an analytical reagent grade of commerce containing not less than 99.0 per cent w/w of $C_{10}H_{16}$.

DESCRIPTION Colourless liquid.

SOLUBILITY Practically insoluble in *water*, soluble in *ethanol*.

BOILING RANGE 175° to 177° (Appendix 4.5).

REFRACTIVE INDEX 1.471 to 1.474, at 20° (Appendix 4.7).

SPECIFIC ROTATION About +124 (Appendix 4.8).

SPECIFIC GRAVITY About 0.84 (Appendix 4.9).

Store in tightly closed containers, in a cool and dry place, protected from light.

Linalool $C_{10}H_{18}O$ = 154.2

Use analytical grade of commerce containing not less than 98.0 per cent w/v of $C_{10}H_{18}O$.

Mixture of two stereoisomers (licareol and coriandrol).

SOLUBILITY Liquid, practically insoluble in *water*.

RELATIVE DENSITY About 0.860 (Appendix 4.9).

REFRACTIVE INDEX About 1.462 (Appendix 4.7).

BOILING POINT About 200° (Appendix 4.5).

Linalyl Acetate $C_{12}H_{20}O_2$ = 196.3

Use analytical grade of commerce containing not less than 95.0 per cent w/v of $C_{12}H_{20}O_2$.

DESCRIPTION Colourless or slightly yellow liquid with a strong odour of bergamot and lavender.

RELATIVE DENSITY 0.895 to 0.912 (Appendix 4.9).

REFRACTIVE INDEX 1.448 to 1.451 (Appendix 4.7).

BOILING POINT about 215° (Appendix 4.5).

Luteolin-7-glucoside $C_{21}H_{20}O_{11}$ = 448.4

DESCRIPTION Yellow powder.

MELTING POINT About 247° (Appendix 4.3).

2-Mercaptoethanol C_2H_6OS = 78.1

SOLUBILITY Miscible with *water*.

RELATIVE DENSITY About 1.116 (Appendix 4.9).

BOILING POINT About 157° (Appendix 4.5).

Menthone $C_{10}H_{18}O$ = 154.2

Use analytical reagent grade of commerce containing not less than 90.0 per cent w/v of $C_{10}H_{18}O$.

DESCRIPTION Colourless liquid.

SOLUBILITY Very slightly soluble in water, very soluble in *ethanol*.

RELATIVE DENSITY About 0.897 (Appendix 4.9).

REFRACTIVE INDEX About 1.450 (Appendix 4.7).

Menthofuran $C_{10}H_{14}O$ = 150.2

Use analytical grade of commerce containing not less than 97.0 per cent w/v of $C_{10}H_{14}O$.

DESCRIPTION Slightly bluish liquid.

SOLUBILITY Very slightly soluble in *water*, soluble in *ethanol*.

RELATIVE DENSITY about 0.965 (Appendix 4.9).

REFRACTIVE INDEX about 1.480 (Appendix 4.7).

SPECIFIC OPTICAL ROTATION about +93° (Appendix 4.8).

BOILING POINT about 196° (Appendix 4.5).

Mentyl acetate $C_{12}H_{22}O_2$ = 198.3

Use analytical grade of commerce containing not less than 97.0 per cent w/v of $C_{12}H_{22}O_2$.

DESCRIPTION Slightly bluish liquid.

SOLUBILITY Very slightly soluble in *water*, soluble in *ethanol*.

RELATIVE DENSITY about 0.92 (Appendix 4.9).

REFRACTIVE INDEX about 1.447 (Appendix 4.7).

BOILING POINT about 228° (Appendix 4.5).

7-Methoxycoumarin $C_{10}H_8O_3$ = 176.17

Use a suitable grade with a content of not less than 98 per cent w/v of $C_{10}H_8O_3$.

Methyl 4-acetylbenzoate $C_{10}H_{10}O_3$ = 178.2

MELTING POINT about 94° (Appendix 4.3).

Naringin $C_{27}H_{32}O_{14}$ = 580.5

DESCRIPTION White or almost white crystalline powder.

SOLUBILITY Slightly soluble in *water*, soluble in *methanol* and in *dimethylformamide*.

MELTING POINT about 171° (Appendix 4.3).

***a*-Pinene** $C_{10}H_{16}$ = 136.2

Use analytical grade of commerce containing not less than 99.0 per cent w/v of $C_{10}H_{16}$.

SOLUBILITY Liquid not miscible with *water*.

RELATIVE DENSITY About 0.859 (Appendix 4.9).

REFRACTIVE INDEX About 1.466 (Appendix 4.7).

BOILING POINT 154° to 156° (Appendix 4.5).

Phenazone $C_{11}H_{12}N_2O$ = 188.2

DESCRIPTION White or almost white, crystalline powder or colourless crystals.

SOLUBILITY Very soluble in water, in *ethanol* and in *dichloromethane*.

Piperitone $C_{10}H_{16}O$ = 152.2

Use analytical grade of commerce

Pulegone $C_{10}H_{16}O$ = 152.2

Use analytical reagent grade of commerce containing not less than 98.0 per cent w/v of $C_{10}H_{16}O$.

DESCRIPTION Oily, colourless liquid.

SOLUBILITY Practically insoluble in water, miscible with *ethanol*.

RELATIVE DENSITY About 0.936 (Appendix 4.9).

REFRACTIVE INDEX 1.485 to 1.489 (Appendix 4.7).

BOILING POINT 222° to 224° (Appendix 4.5).

Puerarin $C_{21}H_{20}O_9$ = 416.4

Use analytical reagent grade of commerce.

Quinidine $C_{20}H_{24}N_2O_2$ = 342.42

DESCRIPTION White crystals.

SOLUBILITY Very slightly soluble in water; freely soluble in chloroform; sparingly soluble in ethanol; slightly soluble in methanol and in ether.

MELTING TEMPERATURE About 172° (Appendix 4.3).

Quinine $C_{20}H_{24}N_2O_2.3H_2O$ = 378.47

DESCRIPTION White, microcrystalline powder; odourless.

SOLUBIITY Very slightly soluble in *water*; very soluble in *ethanol*; soluble in *ether*.

SPECIFIC ROTATION About -165° (Appendix 4.8), calculated on the dried basis, determined in a 1 per cent w/v solution in *ethanol*.

Raney Nickel Catalyst A powdered alloy containing not less than 40.0 per cent and not more than 50.0 per cent of nickel; and not less than 50.0 per cent and nor more than 60.0 per cent of aluminium.

Rhaponticin $C_{21}H_{24}O_9$ = 420.4

DESCRIPTION Yellowish-grey, crystalline powder.

SOLUBILITY soluble in *ethanol* and in methanol.

Rutoside Trihydrate $C_{27}H_{30}O_{16} \cdot 3H_2O$ = 665

DESCRIPTION Yellow or greenish-yellow, crystalline powder

SOLUBILITY Practically insoluble in *water*, soluble in *methanol*, sparingly soluble in *ethanol*, practically insoluble in *methylene chloride*. It dissolves in solutions of alkali hydroxides.

Sodium 1-Hexanesulfonate Monohydrate $C_6H_{13}NaO_3S \cdot H_2O$ = 206.23

Use analytical reagent grade of commerce.

Sodium Metaperiodate $NaIO_4$ = 213.89

Contains not less than 98.0 per cent of $NaIO_4$.

DESCRIPTION White crystals or white, crystalline powder.

SOLUBILITY Soluble in *water*.

MANGANESE Boil 1.0 g with 40 mL of *water* and 10 mL of *sulfuric acid* for 1 minute, and allow to stand for 5 minutes: no pink colour is produced.

BROMITE, CHLORATE AND CHLORIDE Not more than 0.035 per cent w/w as Cl. Dissolve 1.0 g in a mixture of 50 mL of *water* and 2 mL of *nitric acid*, cool and add 100 mg of *sodium nitrite* and 1 mL of *silver nitrate TS*. Any opalescence produced is not greater than the standard opalescence produced by treating 0.50 mL of 0.020 M *hydrochloric acid* in a similar manner.

IODIDE Dissolve 1.0 g in 20 mL of *water* and 10 mL of *dilute sulfuric acid*, add 0.5 mL of *chloroform*, shake thoroughly, and allow to separate: the chloroform layer does not become pink or violet.

ASSAY Weigh accurately about 1 g, previously dried over *sulfuric acid* for 6 hours, dissolve in *water*, and dilute to 500.0 mL. To 50.0 mL of this solution in a glass-stoppered flask add 10 g of *potassium iodide* and 10 mL of a cooled solution of 3 M *sulfuric acid*. Allow to stand for 5 minutes, add 100 mL of cool *water*, and titrate the liberated iodine with 0.1 M *sodium thiosulfate VS*, using *starch TS* as indicator near the end of the titration. Each mL of 0.1 M *sodium thiosulfate VS* is equivalent to 2.674 mg of $NaIO_4$.

Sulfosalicylic Acid $C_7H_6O_6S.2H_2O$ = 254.2

DESCRIPTION White or almost white, crystalline powder or crystals

SOLUBILITY very soluble in water and in *ethanol*

MELTING POINT about 109° (Appendix 4.3).

α-Terpineol $C_{10}H_{18}O$ = 154.2

Use analytical grade of commerce containing not less than 97.0 per cent w/v of $C_{10}H_{18}O$.

DESCRIPTION Colourless crystals.

SOLUBILITY Practically insoluble in *water*, soluble in *ethanol*.

RELATIVE DENSITY About 0.935 (Appendix 4.9).

REFRACTIVE INDEX About 1.483 (Appendix 4.7).

SPECIFIC OPTICAL ROTATION About 92.5 (Appendix 4.8).

MELTING POINT About 35° (Appendix 4.3).

γ-Terpinene $C_{10}H_{16}$ = 136.2

Use analytical grade of commerce containing not less than 93.0 per cent w/v of $C_{10}H_{16}$.

DESCRIPTION Oily liquid

Valerenic Acid $C_{15}H_{22}O_2$ = 234.3

MELTING POINT 134° to 138° (Appendix 4.3).

1.4 pH INDICATORS

Bromocresol Purple $C_{21}H_{16}Br_2O_5S$ = 540.22

White to pink, crystalline powder. Insoluble in *water*;

soluble in *ethanol* and in solutions of alkali hydroxides. Transition interval: from pH 5.2 to 6.8. Colour change: from yellow to purple.

1.6 TEST SOLUTIONS

Alkaline Corallin Solution TS

Dissolve 5 g of *corallin* in 100 mL of *ethanol (90 per cent)*. Immediately before use add 1 mL of the solution to 20 mL of a 20 per cent w/v solution of *sodium carbonate*.

Mercuric Iodide TS

Slowly add *potassium iodide solution (1 in 10)* to red mercuric iodide until almost all of the latter is dissolved, and filter off the excess. A solution containing 10 g of *potassium iodide* in 100 mL dissolves approximately 14 g of HgI_2 at 20°.

Methyl 4-acetylbenzoate TS

Dissolve 0.25 g of *methyl 4-acetylbenzoate* in a mixture of 5 mL of *sulfuric acid* and 85 mL of cooled *methanol*.

Phloroglucinol TS Dissolve 500 mg of *phloroglucinol* in 25 mL of *ethanol*.

Store in tight containers, protected from light.

Sodium Hypobromite TS To a solution of 20 g of *sodium hydroxide* in 75 mL of *water* add 5 mL of *bromite*. After solution has taken place, dilute with *water* to 100 mL.

Prepare immediately before use.

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