Guide

for the elaboration of monographs on herbal drugs and herbal drug preparations

European Pharmacopoeia

European Directorate for the Quality of Medicines & HealthCare

edqm
European Directorate for the
Quality of Medicines & HealthCare

Edition 2007

CONTENTS

A monograph on a herbal drug or a herbal drug preparation is drafted with the same overall structure as a monograph on a chemical substance and both the latest versions of the *Technical guide for the elaboration of monographs* and of the *Style guide* apply to monographs on herbal drugs and herbal drug preparations. This Guide develops the specific points that are relevant to herbal drugs and herbal drug preparations and that are not presented in the above-mentioned general Guides.

It is recalled that all tests and assay methods described in a monograph must be validated according to the procedures stated in the *Technical guide*.

The general monograph *Herbal drugs (1433)* applies to all herbal drugs for medicinal use and its provisions must be taken into account when elaborating specific monographs. The general monograph *Herbal drug preparations (1434)* applies to all herbal drug preparations for medicinal use and its provisions must be taken into account when elaborating specific monographs.

HERBAL DRUGS

NOMENCLATURE

ENGLISH TITLE

Many herbal drugs have a well-established name in English and this is usually used as the title.

Example: Cascara

The plant part used may be included in the title, particularly where different herbal drugs are derived from the same plant.

Example: Hawthorn leaf and flower
Hawthorn berries

Where there is no common name, the title is derived from the scientific name.

Example: Psyllium seed

LATIN TITLE

The Latin title is derived from the scientific name of the source plant. It is formed by the genus (genitive) and/or species (genitive) names, followed by the name of the organ used (nominative and singular).

Example: Belladonnae folium

A more common name may also be used.

Example: Chamomillae romanae flos

Where appropriate the state of the drug is indicated in the English and Latin titles.

Example: Bilberry fruit, dried
Myrtilli fructus siccus

Example: Bilberry fruit, fresh
Myrtilli fructus recens

DEFINITION

Some or all of the following are usually included in the definition:

— the state of the drug: whole, fragmented, peeled, cut, fresh or dried;

— the complete scientific name of the plant (genus, species, subspecies, variety, author) as obtained from the *Kew Index* and its supplements (*International Plant Names Index IPNI*); commonly used synonyms may be mentioned;

— the part or parts of the plant used (written in the singular);

— where appropriate, the stage in the growth cycle when harvesting takes place, or other necessary information;

— wherever possible, the minimum content of **quantified constituents** (constituents with known therapeutic activity or markers); herbal drugs very often contain a mixture of related substances, in which case the total content of **quantified constituents** is determined and expressed as one of the constituents, usually the major constituent; separate limits may be given for different forms of the drug (whole/cut).

The statements '(dried drug)' or '(anhydrous drug)' imply that the monograph prescribes respectively a test for loss on drying (*2.2.32*) or a determination of water by distillation (*2.2.13*). In practice the 'dried drug' is dried again when the loss on drying test is performed.

The title is not repeated in the definition.

Example:

Dried flowering aerial parts of *Viola arvensis* Murr. or *Viola tricolor* L.

Content: minimum 1.5 per cent of total flavonoids, expressed as violanthin ($C_{27}H_{30}O_{14}$; M_r 578.5) (dried drug).

Example:

Dried cremocarp and mericarp of *Foeniculum vulgare* Mill. ssp. *vulgare* var. *vulgare*.

Content:

— *essential oil*: minimum 40 ml/kg (anhydrous drug);

— *anethole*: minimum 60.0 per cent in the essential oil;

— *fenchone*: minimum 15.0 per cent in the essential oil.

Example:

Whole or cut, dried rhizome and root of *Levisticum officinale* Koch.

Content:

— for the whole drug: minimum 4.0 ml/kg of essential oil (dried drug);

— for the cut drug: minimum 3.0 ml/kg of essential oil (dried drug).

CHARACTERS

This section contains a brief description of the physical characters of the drug. The information given is not to be regarded as representing mandatory requirements.

ORGANOLEPTIC CHARACTERS

The colour of the drug, where this is characteristic.

No reference is made to odour unless it is highly characteristic and can be described with reference to independent odours. Terms such as ‘aromatic’ and ‘characteristic’ are not used. No reference is made to taste unless there is a test for bitterness value (*2.8.15*) in the monograph or the taste is highly characteristic or the herbal drug is to be used as a flavour.

MACROSCOPIC AND MICROSCOPIC BOTANICAL CHARACTERS

The description of botanical characters is included under Identification. However, some botanical characters that are highly variable and considered not compulsory for the identification of the plant may be described under Characters.

Example:

The cremocarp is brown or light brown and is more or less spherical, about 1.5-5 mm in diameter, or oval and 2-6 mm long.

IDENTIFICATION

This section includes tests performed to identify the drug. All the identifications mentioned below are not necessarily included: some may be absent when they are not feasible or are not significant for the purpose of identification.

Example: no microscopy in *Mastix (1876)* and no TLC in *Oak bark (1887)*.

The monograph may have a First identification and a simpler Second identification that is suitable for use where the equipment required for the main identification tests is not available or the tests are not otherwise feasible but where the pharmacist, in some Member States, may have an obligation to identify a herbal drug, for example in a community pharmacy. Some tests may be specified in both the First and Second identifications. Application of the First and Second identifications is defined in the General Notices of the European Pharmacopoeia.

The identification section is introduced by a statement of the 2 identifications.

Example:

First identification: A, B, C, E.

Second identification: B, D.

MACROSCOPIC BOTANICAL CHARACTERS

The important macroscopic botanical characters of the drug are specified to permit a clear identification. When 2 species/subspecies of the same plant are included in the definition (example: *Thymus vulgaris* and *Thymus zygis*), the individual differences between them are indicated. Further information for rapid identification of the drug is provided if necessary.

Example:

A. The leaf is yellowish-green to brownish-green, with a prominent, whitish-green, almost parallel venation on the abaxial surface. It consists of a lanceolate lamina narrowing at the base into a channelled petiole. The margin is indistinctly dentate and often undulate. It has

3, 5 or 7 primary veins, nearly equal in length and running almost parallel. The indumentum is either rare or abundant, especially on the abaxial surface and the veins.

MICROSCOPIC BOTANICAL CHARACTERS

The microscopic examination of the drug reduced to a powder describes the dominant or the most specific characters, including, if necessary, examination of the stomata and stomatal index (*2.8.3*). The colour of the powder, the sieve number (use No. 355 (*2.1.4*) unless there is a valid reason for not doing so) and the reagents used for the microscopic examination are specified. It may be necessary to perform the microscopic examination using more than 1 mountant in order to identify the specific characters. A specific stain may be prescribed for particular characters. Negative statements should be avoided since they usually refer to adulteration rather than to identification.

Monographs may contain schematic drawings of the main microscopic features of powdered drugs.

Example:

B. Reduce to a powder (355) (*2.9.12*). The powder is brownish-grey (unpeeled root) or whitish (peeled root). Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters: fragments of colourless, mainly unlignified, thick-walled fibres with pointed or split ends; fragments of bordered, pitted or scalariformly thickened vessels; cluster crystals of calcium oxalate about 20-35 µm, mostly 25-30 µm, in size; parenchymatous cells containing mucilage; fragments of cork with thin-walled, tabular cells in the unpeeled root. Examine under a microscope using a 50 per cent *V/V* solution of *glycerol R*. The powder shows numerous starch granules about 3-25 µm in size occasionally with a longitudinal hilum. The starch grains are mostly simple, a few being 2-4 compounds.

THIN-LAYER CHROMATOGRAPHY

2 types of presentation are possible.

TLC PRESCRIBED ONLY FOR THE IDENTIFICATION OF THE HERBAL DRUG

TLC is used under Identification, even if other chromatographic methods, such as gas chromatography (GC) and liquid chromatography (LC), are subsequently used in the monograph. In this context the TLC is aimed at elucidating the chromatogram of the drug with respect to selected reference compounds that are described for inclusion as reagents (e.g. *rutin R*). Wherever possible, existing reagents described in chapter *4.1.1. Reagents* of the European Pharmacopoeia are used as reference compounds, otherwise a description of the reagent (name, molecular formula, relative molecular mass, CAS Registry Number, chemical nomenclature) is appended to the draft monograph for subsequent inclusion in chapter *4.1.1.* Availability of reference compounds as commercial reagents must be verified during monograph elaboration. Where they are not readily available, a chemical reference substance (CRS) will have to be established and availability of a suitable quantity must be verified during monograph elaboration.

The commercial name of the TLC plate used during monograph development is included as a footnote to the monograph and is transferred to the EDQM Knowledge database at the time of publication of the monograph in the European Pharmacopoeia.

A minimum of 2 reference compounds must be used to validate the separation and spacing between the zones, otherwise a resolution test is necessary.

All the information concerning the preparation of the reference solution and the test solution and the chromatographic conditions is clearly stated. The methodology used, where possible,

must be such that the application volume of the reference solution and the test solution is the same.

The general chapter on thin-layer chromatography covers both normal TLC and high performance TLC (HPTLC). Where these 2 methods give equivalent results with the development solvent and visualisation method prescribed, both may be included in the working conditions [HPTLC conditions in brackets after those for normal TLC], otherwise preference is given to normal TLC unless HPTLC is essential for proper identification.

The chromatograms are described in the form of a table showing the upper, middle and lower thirds of the plate, which may be represented as different sizes.

Only the principal zone(s) in the chromatogram obtained with the test solution are described in the table in relation to the position of the zones due to the reference compounds in the chromatogram obtained with the reference solution. The names of the constituents detected in the chromatogram obtained with the reference solution are always given. The names of the constituents detected in the chromatogram obtained with the test solution are given only if these constituents are present in the reference solution or if the nature of the substance is well established.

Chromatograms are never described in terms of R_F values (retardation factors).

It is usually necessary to indicate that zones other than those described (usually more faint) are also present in the chromatogram of the test solution.

A copy in colour of the suitable chromatogram has to be provided to the Secretariat.

Example: *Melilot (2120)*

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 0.3 g of the powdered drug (355) (*2.9.12*) add 3 ml of *methanol R*. Heat on a water-bath at 60 °C for 1 min. Filter.

Reference solution. Dissolve 50 mg of *coumarin CRS* and 20 mg of *o-coumaric acid R* in 50 ml of *methanol R*.

Plate: *TLC silica gel plate R* (5-40 µm) [or *TLC silica gel plate R* (2-10 µm)].

Mobile phase: upper phase of a mixture of 10 volumes of *dilute acetic acid R*, 50 volumes of *ether R* and 50 volumes of *toluene R*.

Application: 25 µl [or 5 µl] as bands of 10 mm [or 5 mm].

Development: over a path of 12 cm [or 6 cm].

Drying: in air.

Detection: spray with *2 M alcoholic potassium hydroxide R*; examine in ultraviolet light at 365 nm.

Detection: / heat at 100 °C for 5 min and / spray / the still-warm plate / with a 10 g/l solution of *diphenylboric acid aminoethyl ester R* in *methanol R*, and then with a 50 g/l solution of *macrogol 400 R* in *methanol R*; / heat at 100-105 °C for 5 min / allow to dry / in a current of warm air / in air / for 30 min / and examine in ultraviolet light at 365 nm.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other faint zones of various colours may be present in the chromatogram obtained with the test solution.

Top of the plate	
Coumarin: a greenish-yellow fluorescent zone _____ *o*-Coumaric acid: a greenish-yellow fluorescent zone _____	A greenish-yellow fluorescent zone (coumarin) _____ A fluorescent blue zone A greenish-yellow fluorescent zone (*o*-coumaric acid) may be present _____
Reference solution	**Test solution**

If a fluorescent coating is used, the zones are defined by their position only.

Plate: *TLC silica gel F_{254} plate R* (5-40 µm) [or *TLC silica gel F_{254} plate R* (2-10 µm)].

Top of the plate	
Eugenol: a quenching zone _____ Cinnamic aldehyde: a marked quenching zone _____	A quenching zone (eugenol) _____ A quenching zone (cinnamic aldehyde) _____
Reference solution	**Test solution**

Examination before and after visualisation

Example: *Fumitory (1869)*

C. Thin-layer chromatography (*2.2.27*).

Test solution. To 2 g of the powdered drug (355) (*2.9.12*) add 15 ml of *0.05 M sulphuric acid* and stir for 15 min. Filter. Dilute the filtrate to 20 ml with *0.05 M sulphuric acid*. Add 1 ml of *concentrated ammonia R* and 10 ml of *ethyl acetate R*. Stir and centrifuge. Collect the upper organic layer. Repeat the extraction in the same manner. Collect the organic layers and dry over *anhydrous sodium sulphate R*. Evaporate to dryness under reduced pressure. Take up the residue with 0.5 ml of *methanol R*.

Reference solution. Dissolve 5 mg of *protopine hydrochloride R* and 5 mg of *quinine R* in 10 ml of *methanol R*.

Plate: *TLC silica gel plate R*.

Mobile phase: *concentrated ammonia R*, *ethanol (96 per cent) R*, *acetone R*, *toluene R* (2:6:40:52 *V/V/V/V*).

Application: 30 µl as bands of 10 mm.

Development: over a path of 15 cm.

Drying: in air.

Detection A: examine in ultraviolet light at 365 nm.

Results A: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other blue fluorescent zones are present in the chromatogram obtained with the test solution.

Top of the plate	
Quinine: a blue fluorescent zone	4 blue fluorescent zones A greenish-blue fluorescent zone
Reference solution	**Test solution**

Detection B: spray with a mixture of 1 volume of *potassium iodobismuthate solution R2*, 2 volumes of *acetic acid R* and 10 volumes of *water R* until orange zones appear against a yellow background.

Results B: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution. Furthermore, other less intense orange zones are present in the chromatogram obtained with the test solution.

Top of the plate	
Protopine: an orange zone Quinine: an orange zone	An orange zone (protopine) 2 orange zones A faint orange zone (quinine)
Reference solution	**Test solution**

TLC PRESCRIBED UNDER TESTS AND IDENTIFICATION

If a TLC test is used both for the control of adulterations and for identification, the method is described entirely under Tests with a cross-reference under Identification.

Example: *Angelica root (1857)*

C. Examine the chromatograms obtained in the test for lovage root.

Results: see below the sequence of zones present in the chromatograms obtained with the reference solution and the test solution.

Top of the plate	
_____ Eugenol: (marked at 254 nm) Coumarin: (marked at 254 nm) _____	_____ _____ An intense blue fluorescent zone Yellow fluorescent zones A blue fluorescent zone A yellow fluorescent zone An intense blue fluorescent zone
Reference solution	**Test solution**

LIQUID OR GAS CHROMATOGRAPHY

Where LC or GC is used in a test or assay, it may also be referred to under Identification.

Example: *Narrow-leaved coneflower root (1821)* (LC)

D. Examine the chromatograms obtained in the assay.

Results: the chromatogram obtained with the test solution shows a major peak due to echinacoside and a minor peak due to cynarin; peaks due to caffeic acid, caftaric acid and chlorogenic acid are minor peaks or may be absent.

Example: *Juniper oil (1832)* (GC)

B. Examine the chromatograms obtained in the test for chromatographic profile.

Results: the characteristic peaks in the chromatogram obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

CHEMICAL REACTIONS FOR IDENTIFICATION

Chemical reactions are included only where TLC/HPTLC does not provide sufficient identification and if the reaction is particularly characteristic of a constituent or group of constituents. They must allow rapid identification without the use of complex equipment and not be so sensitive as to give a false positive result.

Example: *Yarrow (1382)*

D. Add 2.5 ml of *dimethylaminobenzaldehyde solution R8* to 0.1 ml of solution S (see Tests) and heat on a water-bath for 2 min. Allow to cool. Add 5 ml of *light petroleum R* and shake the mixture vigorously. The aqueous layer shows a blue or greenish-blue colour.

TESTS

TYPICAL TESTS

TOTAL ASH (*2.4.16*)

This test is always included unless otherwise justified. It is to be carried out on the powdered drug. It is not necessary to state the sieve number.

Example:

Total ash (*2.4.16*): maximum 14.0 per cent.

ASH INSOLUBLE IN HYDROCHLORIC ACID (*2.8.1*)

This test may be carried out depending on the nature of the particular herbal drug and is used to detect unacceptable quantities of certain minerals.

Example:

Ash insoluble in hydrochloric acid (*2.8.1*): maximum 2.0 per cent.

THIN-LAYER CHROMATOGRAPHY (*2.2.27*)

TLC can be used under Tests to detect plant species that are not part of the definition. The TLC method is described entirely under Tests and wherever feasible is also used to identify the herbal drug. The name of the unwanted plant species or their constituent(s) (e.g. **thujone** in three-lobed sage-leaf) is used as the title of the test. In the chromatogram obtained with the test solution only the position and colour of the zone(s) of the constituent(s) that must be absent are described by comparison with the chromatogram obtained with the reference solution. The zones present in the chromatogram obtained with the test solution are not described under Tests but under Identification.

Example: *Angelica root (1857)*

Levistici radix. Thin-layer chromatography (*2.2.27*).

… (description of the chromatographic procedure, which must meet all of the criteria outlined under 'TLC prescribed only for the identification of the drug').

Results: the chromatogram obtained with the test solution shows no pale blue or white fluorescent zone between the zones of coumarin and eugenol in the chromatogram obtained with the reference solution.

GAS CHROMATOGRAPHY (*2.2.28*) OR LIQUID CHROMATOGRAPHY (*2.2.29*)

The use of GC or LC is indicated under Tests to detect plant species that are not part of the definition (e.g. essential oils), to limit certain constituents (e.g. estragole in fennel) or to control the possible degradation or evaporation of any constituents that must be present in the drug at a certain level.

A system suitability criterion should be included and the commercial name of the column or columns found suitable during elaboration of the monograph are included in a footnote, and transferred to the EDQM Knowledge database at the time of publication of the monograph in the European Pharmacopoeia. A representative chromatogram is included in the draft monograph published in Pharmeuropa and is usually transferred to the EDQM Knowledge database at the time of publication of the monograph in the European Pharmacopoeia. Compounds prescribed as external standards for quantification of impurities are established as Chemical Reference Substances (CRS).

Example: *Ginseng (1523)*

***Panax quinquefolium*.** Liquid chromatography (*2.2.29*).

… (description of the chromatographic procedure).

The chromatogram obtained with the test solution shows a peak corresponding to ginsenoside Rf. In the case of a substitution by *Panax quinquefolium*, no peak corresponding to ginsenoside Rf is present.

When the same LC method is used both for the assay and for a test, the method is described entirely under Tests with a cross-reference under Assay.

FOREIGN MATTER (*2.8.2*)

Foreign matter consists of parts of the source plant that are not defined as the drug, and foreign elements of herbal origin that are not derived from the plant species given in the definition, or of mineral origin or any other matter not within the definition of the drug.

The general monograph *Herbal drugs (1433)* imposes a limit of 2 per cent of foreign matter, unless otherwise prescribed in a specific monograph. Where a limit for foreign matter greater than 2 per cent is to be prescribed, it is stated in the specific monograph with an indication of the type of foreign matter. Where necessary, the monograph indicates how the foreign matter is identified.

Example:

Foreign matter (*2.8.2*): maximum 8 per cent of lignified branches with a diameter greater than 2.5 mm and maximum 2 per cent of other foreign matter.

HEAVY METALS

A general method *Heavy metals in herbal drugs and fatty oils (2.4.27)* is included in the European Pharmacopoeia. The test is prescribed where there is the potential for contamination with heavy metals. A test for a specific heavy metal may be needed where a particular herbal drug is known to accumulate that metal.

Example: *Linseed (0095)*

Cadmium (*2.4.27*): maximum 0.5 ppm.

LOSS ON DRYING (*2.2.32*)

Herbal drugs are dried for preservation purposes: if they are insufficiently dried, growth of yeasts or moulds may occur. This test determines the maximum amount of water that may be present in the drug under the stated conditions. The limit is specified on the basis of the results obtained on a reasonable number of varied samples of acceptable quality. Monographs usually specify drying for a defined period (usually 2 h) rather than drying to constant mass. Unless otherwise justified, the loss on drying is not more than 10 per cent when drying for 2 h.

The monograph indicates the amount of herbal drug necessary for the determination and the fineness of the powder using the sieve number (*2.1.4*).

Example:

Loss on drying (*2.2.32*): maximum 10 per cent, determined on 1.000 g of the powdered drug (355) (*2.9.12*) by drying in an oven at 105 °C for 2 h.

WATER (*2.2.13*)

For herbal drugs containing more than 10 ml/kg (1 per cent) of essential oil, the determination of water by distillation (*2.2.13*) is carried out instead of the test for loss on drying. If required, the fineness of the powder is indicated using the sieve number (*2.1.4*).

Example:

Water (*2.2.13*): maximum 120 ml/kg, determined on 20.0 g of the powdered drug (710) (*2.9.12*) / crushed drug.

SWELLING INDEX (*2.8.4*)

Applicable to certain hydrocolloid-containing herbal drugs, for example: *Marshmallow root (1126)*, *Mallow flower (1541)*, *Fenugreek (1323)*, *Ispaghula seed (1333)*, *Ispaghula husk (1334)*, *Iceland moss (1439)*, *Kelp (1426)*, *Mullein flower (1853)*.

Example:

Swelling index (*2.8.4*): minimum 12, determined on the powdered drug (710) (*2.9.12*).

BITTERNESS VALUE (*2.8.15*)

Applicable to herbal drugs containing bitter principles, for example: *Gentian root (0392)*, *Wormwood (1380)*, *Yarrow (1382)*, *Centaury (1301)*, *Bogbean leaf (1605)*.

Example:

Bitterness value (*2.8.15*): minimum 4000.

EXTRACTABLE MATTER

It is considered useful to determine extractable matter only in herbal drugs where no constituent suitable for an assay is known or where the material is used to produce a preparation with a dry residue, for example: *Eleutherococcus (1419)*, *Restharrow root (1879)*, *Hop strobile (1222)*, *Gentian root (0392)*, *Couch grass rhizome (1306)*.

Example: *Restharrow root (1879)*

Extractable matter: minimum 15.0 per cent.

To 2.00 g of the powdered drug (250) (*2.9.12*) add a mixture of 8 g of *water R* and 12 g of *ethanol (96 per cent) R* and allow to macerate for 2 h, shaking frequently. Filter, evaporate 5 g of the filtrate to dryness on a water-bath and dry in an oven at 100-105 °C for 2 h. The residue weighs a minimum of 75 mg.

OTHER TESTS

In certain cases, additional microscopic examinations and/or additional chemical reactions are carried out. This is done particularly to detect adulteration by drugs that have a related morphological appearance, but which come from totally different species, to demonstrate for example that a given drug is free of toxic substances, such as alkaloids and cardiotonic steroids.

Specific tests may also be applied to a particular monograph when necessary, such as:

— **Starch** (*Devil's claw root (1095)*);

— **Deteriorated flower-heads** (*Roman chamomile flower (0380)*);

— **Diameter of flower-heads** (*Roman chamomile flower (0380)*);

— **Matter insoluble in ethanol** (*Myrrh (1349)*);

— **Broken drug** (*Matricaria flower (0404)*);

— ***Digitalis lanata* leaves** (*Ribwort plantain (1884)*).

ASSAY

Wherever possible, an assay is included. Substances used for quantification are established as chemical reference substances (CRSs); availability of a sufficient quantity of a batch of suitable quality must be verified during monograph elaboration.

Wherever possible, LC or GC are the methods of choice to determine the content of specific constituents rather than a global determination by spectrophotometry.

ULTRAVIOLET AND VISIBLE ABSORPTION SPECTROPHOTOMETRY

Spectrophotometry allows a global determination of constituents that are very often a group of related substances. It may be used for the quantification of constituents:

— that are quality markers, when the specific active constituents are not known;

— with known therapeutic activity that are a mixture of related substances.

It is used to determine, for example:

— flavonoids (*Birch leaf (1174)*, *Elder flower (1217)*, *Passion flower (1459)*, *Calendula flower (1297)*, *Hawthorn leaf and flower (1432)*);

— hydroxyanthracene derivatives (*Aloes (0257)*, *Cascara (0105)*, *Frangula bark (0025)*, *Senna leaf (0206)* and *Senna pods (0207)*);

— alkaloids (*Cinchona bark (0174)*).

Other methods, notably LC, are now often preferred.

Example of an assay for flavonoids

Stock solution. In a 100 ml round-bottomed flask introduce 0.200 g of the powdered drug (355) (*2.9.12*), 1 ml of a 5 g/l solution of *hexamethylenetetramine R*, 20 ml of *acetone R* and 2 ml of *hydrochloric acid R1*. Boil the mixture under a reflux condenser for 30 min. Filter the liquid through a plug of absorbent cotton into a 100 ml flask. Add the plug of absorbent cotton to the residue in the round-bottomed flask and extract with 2 quantities, each of 20 ml, of *acetone R*, each time boiling under a reflux condenser for 10 min. Allow to cool to room temperature, filter the liquid through a plug of absorbent cotton then through a filter paper into the volumetric flask, and dilute to 100.0 ml with *acetone R* by rinsing the flask and filter. Introduce 20.0 ml of the solution into a separating funnel, add 20 ml of *water R,* extract the mixture with 1 quantity of 15 ml and then with 3 quantities, each of 10 ml, of *ethyl acetate R*. Combine the ethyl acetate extracts in a separating funnel, rinse with 2 quantities, each of 50 ml, of *water R*, filter the extract over 10 g of *anhydrous sodium sulphate R* into a 50 ml volumetric flask and dilute to 50.0 ml with *ethyl acetate R*.

Test solution. To 10.0 ml of the stock solution add 1 ml of *aluminium chloride reagent R* and dilute to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Compensation liquid. Dilute 10.0 ml of the stock solution to 25.0 ml with a 5 per cent *V/V* solution of *glacial acetic acid R* in *methanol R*.

Measure the absorbance (*2.2.25*) of the test solution after 30 min, by comparison with the compensation liquid at 425 nm.

Calculate the percentage content of flavonoids, expressed as hyperoside, using the following expression:

$$\frac{A \times 1.25}{m}$$

i.e. taking the specific absorbance of hyperoside to be 500.

A = absorbance at 425 nm;

m = mass of the substance to be examined, in grams.

DETERMINATION OF TANNINS IN HERBAL DRUGS (*2.8.14)*

This assay is described as a general method. See examples in *Dried bilberry fruit (1588)*, *Hamamelis leaf (0909)*, *Rhatany root (0289)*, *Tormentil (1478)* and *Oak bark (1887)*.

VOLUMETRIC TITRATION

Examples are the assay of alkaloids in *Belladonna leaf (0221)*, *Hyoscyamus leaf (0225)*, *Stramonium leaf (0246)* and *Ipecacuanha root (0094),* and the assay of iodine in *Kelp (1426)*.

DETERMINATION OF ESSENTIAL OILS IN HERBAL DRUGS (*2.8.12*)

When a minimum content of essential oil is required in the Definition, the assay is carried out on the drug in the reduced form, if necessary, as prescribed in the monograph.

Example: *Sage leaf, three-lobed (1561)*

Carry out the determination of essential oils in herbal drugs (*2.8.12*). Use 20.0 g of drug, if necessary cut immediately before the assay, a 500 ml flask, 250 ml of *water R* as the distillation liquid, and 0.50 ml of *xylene R* in the graduated tube. Distil at a rate of 2-3 ml/min for 2 h.

LIQUID CHROMATOGRAPHY (*2.2.29*) AND GAS CHROMATOGRAPHY (*2.2.28*).

For the technical content and the style of these analytical methods, see both the *Technical guide* and the *Style guide*. The general methods *Chromatographic separation techniques (2.2.46)*, *Gas chromatography (2.2.28)* and *Liquid chromatography (2.2.29)* must also be consulted.

A system suitability criterion should be included and the commercial name of the column or columns found suitable during elaboration of the monograph are included in a footnote and transferred to the EDQM Knowledge database after publication of the monograph in the European Pharmacopoeia. A representative chromatogram is included in the draft monograph published in Pharmeuropa and transferred to the EDQM Knowledge database after publication of the monograph in the European Pharmacopoeia.

The expression used to calculate the result of the assay is given.

Example: *Agnus castus fruit (2147)*

Liquid chromatography (2.2.29).

…

Calculate the percentage content of casticin using the following expression:

$$\frac{S_1 \times m_2 \times p}{S_2 \times m_1}$$

S_1 = area of the peak due to casticin in the chromatogram obtained with the test solution;

S_2 = area of the peak due to casticin in the chromatogram obtained with the reference solution;

m_1 = mass of the drug to be examined used to prepare the test solution, in grams;

m_2 = mass of *casticin R* used to prepare the reference solution, in grams;

p = percentage content of casticin in *casticin R*.

Example: *Arnica flower (1391)*

Liquid chromatography (*2.2.29*).

…

Calculate the percentage content of total sesquiterpene lactones, expressed as dihydrohelenalin tiglate, using the following expression:

$$\frac{S_1 \times C \times V \times 1.187 \times 100}{S_2 \times m \times 1000}$$

S_1 = sum of the areas of all peaks due to sesquiterpene lactones appearing after the santonin peak in the chromatogram obtained with the test solution;

S_2 = area of the peak due to santonin in the chromatogram obtained with the test solution;

m = mass of the drug to be examined used to prepare the test solution, in grams;

C = concentration of santonin in the internal standard solution used to prepare the test solution, in milligrams per millilitre;

V = volume of the internal standard solution used to prepare the test solution, in millilitres;

1.187 = correction factor for dihydrohelenalin tiglate with respect to santonin.

STORAGE

Storage conditions described in the general monograph *Herbal drugs (1433)* are applicable unless otherwise specified: protected from light.

Where applicable, additional specific conditions are given in the individual monograph.

Example:

Do not store in powdered form.

REAGENTS

For the technical content and the style see both the *Technical guide* and the *Style guide*.

Commercial availability of constituents and markers that are described as reagents must be verified during elaboration of the monograph. Where a reagent may be difficult to obtain, the names and addresses of suppliers are included in a footnote to the monograph and transferred to the EDQM Knowledge database at the time of publication of the monograph in the European Pharmacopoeia.

The reagent specification includes: name, molecular formula, relative molecular mass, CAS Registry Number and chemical nomenclature. The EDQM adds a unique identifier (7-digit number in italics) when the reagent is included in chapter *4. Reagents*.

CHEMICAL REFERENCE SUBSTANCES (CRSs)

Substances used as external standards for quantification of impurities and as assay standards are established as chemical reference substances. Establishment of CRSs is co-ordinated by the Laboratory Department (Dlab) of the EDQM; the Group of Experts should advise on a supplier of a batch of suitable quality.

HERBAL DRUG PREPARATIONS

EXTRACTS

The provisions of the general monograph *Extracts (0765)* apply: account must be taken of the general monograph during elaboration of individual monographs, and the provisions of the general monograph are not repeated, but any specific information required for application of the general monograph is included in the individual monograph.

TITLE

The title is derived from that of the monograph on the herbal drug supplemented by an indication of the type and class of extract (types: liquid extract/tincture/dry extract/soft extract; classes: standardised/quantified). For the 3rd class of extract ('other extracts'), no indication is included in the title. The title may also include 'refined' (according to the definition in the general monograph *Extracts (0765)*).

Examples:

Belladonna leaf dry extract, standardised
Belladonnae folii extractum siccum normatum

Passion flower dry extract
Passiflorae herbae extractum siccum

Hawthorn leaf and flower liquid extract, quantified
Crataegi folii cum flore extractum fluidum quantificatum

DEFINITION

Reference is made to the monograph on the herbal drug from which the extract is prepared. Assay limits are included wherever possible. For standardised and quantified extracts, upper and lower assay limits should be given. For 'other extracts', a lower assay limit is given, wherever possible.

Example: *Hawthorn leaf and flower liquid extract, quantified (1864)*

Quantified liquid extract produced from *Hawthorn leaf with flower (1432).*

Content: 0.8 per cent to 3.0 per cent of total flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4).

Example: *Passion flower dry extract (1882)*

Dry extract produced from *Passion flower (1459).*

Content: minimum 2.0 per cent of total flavonoids, expressed as vitexin ($C_{21}H_{20}O_{10}$; M_r 432.4) (dried extract).

Example: *Hawthorn leaf and flower dry extract (1865)*

Dry extract produced from *Hawthorn leaf and flower (1432)*

Content:

— for aqueous extracts: minimum 2.5 per cent of total flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract);

— for hydroalcoholic extracts: minimum 6.0 per cent of total flavonoids, expressed as hyperoside ($C_{21}H_{20}O_{12}$; M_r 464.4) (dried extract).

PRODUCTION

Includes a statement of the extraction solvents used, based on approved medicinal products in Member States. This effectively defines the scope of the monograph, since the specifications must be established to take account of all such products. Where necessary, the monograph is drafted such that the specifications are related to the extraction solvent used. The drug/extract ratio (DER) is not stated; the general monograph *Extracts (0765)* requires that the DER be stated on the label.

Example: *Passion flower dry extract (1882)*

The extract is produced from the herbal drug by a suitable procedure using ethanol (40-90 per cent *V/V*), methanol (60 per cent *V/V*) or acetone (40 per cent *V/V*).

Example: *Myrrh tincture (1877)*

The tincture is produced from the herbal drug by a suitable procedure using 5 parts of ethanol (90 per cent *V/V*) for 1 part of drug.

Example: *Hawthorn leaf and flower dry extract (1865)*

The extract is produced from the herbal drug by a suitable procedure using either water or a hydroalcoholic solvent at least equivalent in strength to ethanol (45 per cent *V/V*).

CHARACTERS

Physical description of the extract. Taste is not mentioned unless the extract is used as a flavour and odour is only mentioned if it is very characteristic and can be described with reference to independent odours.

IDENTIFICATION

The preferred method is that used for the herbal drug, usually TLC [or HPTLC].

TESTS

Standard tests are included in the general monograph. Tests specific for the extract are described.

ETHANOL

A requirement of 95 per cent to 105 per cent of the content stated on the label is included for liquid extracts and tinctures.

Examples:

Ethanol (*2.9.10*): 95 per cent to 105 per cent of the quantity stated on the label.

RESIDUAL SOLVENTS IN DRY EXTRACTS

Unless otherwise prescribed in a specific monograph, the maximum acceptable levels of residual solvents are those shown in general chapter *5.4. Residual solvents*. Where the limit given in *5.4* is not suitable for a given extract, a specific limit and method of determination are included in the particular monograph.

LOSS ON DRYING

A limit for loss on drying, referring to general method *2.8.17,* is included for dry extracts.

Example:

Loss on drying (*2.8.17*): maximum 5.0 per cent.

ASSAY

Wherever possible, an assay is included. The same method as used for the herbal drug should be used, wherever possible.

STORAGE

Where necessary, an airtight container is prescribed.

LABELLING

The general monograph covers most labelling items.

PREPARED DRUGS

See the monographs *Belladonna, prepared (0222)*, *Ipecacuanha, prepared (0093)*, *Stramonium, prepared (0247)*.

The monograph is based on the monograph for the herbal drug, taking account of the powder form and possible presence of lactose or another added substance.

DEFINITION

Example:

Prepared belladonna is belladonna leaf powder (180) (*2.9.12*) adjusted if necessary by adding powdered lactose or belladonna leaf powder with a lower alkaloidal content to contain 0.28 per cent to 0.32 per cent of total alkaloids, calculated as hyoscyamine (M_r 289.4) with reference to the dried drug.

TESTS

Foreign matter. No test is included.

Loss on drying. A test and acceptance criteria are included.

ASSAY

The assay is usually the same as for the herbal drug.

HERBAL TEAS

Herbal teas are covered by the general monograph *Herbal teas (1435)*.