

Guide for the elaboration  
of monographs on  
**HERBAL DRUGS  
AND HERBAL DRUG  
PREPARATIONS**



European Pharmacopoeia

**EDQM**  
2<sup>nd</sup> Edition  
2023



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English version  
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# Guide for the elaboration of monographs on herbal drugs and herbal drug preparations

## I. Introduction

### I.1. Purpose of the guide

This document is a guide for the authors of monographs on herbal drugs and herbal drug preparations, and also a means of communicating the principles for the elaboration and revision of these monographs to the users of the European Pharmacopoeia (Ph. Eur.).

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 the latest versions of both the *Technical guide for the elaboration of monographs* and of the *Style guide* also apply to them. This guide describes in detail the specific points that are relevant to herbal drugs and herbal drug preparations and that are not presented in the above-mentioned general guides.

Analytical procedures included in monographs are validated as described in the section on analytical validation in the *Technical guide for the elaboration of monographs* (see section III) and other relevant specific parts of that guide. Validation reports are provided to the EDQM but are not published or otherwise made available to users.

### I.2. Reference standards

Substances used as external standards in assays or for quantification of impurities are established as Chemical Reference Standards (CRSs). They may also be used for qualitative purposes. The group of experts elaborating the monograph provides advice on potential suppliers of the candidate standard and its CAS number.

A Herbal Reference Standard (HRS) is either a herbal plant material or an extract. An HRS is usually established for qualitative purposes (for example, for system suitability or for peak identification) but may also be established for quantitative purposes. The group of experts elaborating the monograph provides advice on potential suppliers of the candidate standard.

In order to establish a CRS or HRS, a sufficient quantity of a batch of suitable quality must be available. The establishment process is co-ordinated by the EDQM Laboratory.

### 1.3. Provisions of general monographs

Monographs on herbal drugs and herbal drug preparations are covered by a number of general monographs in the Ph. Eur., i.e.:

- *Herbal drugs (1433)*;
- *Herbal drug preparations (1434)*;
- *Essential oils (2098)*;
- *Herbal drug extracts (0765)*;
- *Herbal teas (1435)*;
- *Herbal teas, instant (2620)*.

Unless otherwise stated, a general monograph describes requirements that must be fulfilled, not only for substances or preparations covered by an individual monograph but for all substances or preparations within the scope of the Definition section. For instance, standard tests and labelling requirements are included in the general monograph *Herbal drug extracts (0765)*, and these are applicable to all monographs on extracts unless otherwise indicated in the individual monograph.

## II. Monographs on herbal drugs

This section applies to dried herbal drugs. Monographs on fresh herbal drugs may have different requirements (e.g. *Fresh bilberry fruit (1602)*); the applicability of this section for the elaboration of monographs on fresh herbal drugs is decided on a case-by-case basis.

### II.1. Title

Many herbal drugs have a well-established common (vernacular) name in English and/or French and this is typically used as the title. The English and French names are given in capitals.

ANISEED; CASCARA

*Aniseed (07/2017:0262); Cascara (04/2011:0105)*

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

MALLOW FLOWER; MALLOW LEAF

*Mallow flower (01/2011:1541); Mallow leaf (07/2011:2391)*

The plant part is written in the singular (e.g. leaf, root, bark) except where it is the aerial parts (plural) that are described, since 'aerial parts' may cover different plant parts (e.g. stem, leaf, flower, fruit).

WILD PANSY (FLOWERING AERIAL PARTS)

*Wild pansy (flowering aerial parts) (01/2008:1855)*

Where no well-established common (vernacular) name exists, the title is derived from the scientific name (Latin name) and it is often limited to the genus name if no plant belonging to the same genus is the subject of another monograph.

DRYNARIA RHIZOME; ACANTHOPANAX BARK

*Drynaria rhizome (07/2017:2563); Acanthopanax bark (07/2017:2432)*

The title includes the species name if several plants belonging to the same genus are the subject of individual monographs.

ANGELICA ARCHANGELICA ROOT; ANGELICA DAHURICA ROOT; ANGELICA PUBESCENS ROOT; ANGELICA SINENSIS ROOT

*Angelica archangelica root (01/2013:1857); Angelica dahurica root (01/2018:2556); Angelica pubescens root (01/2018:2557); Angelica sinensis root (04/2017:2558)*

And exceptionally, the title may derive from the species scientific name, without the genus scientific name.

PSYLLIUM SEED

*Psyllium seed (01/2008:0858)*

#### II.1.1. Latin title

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

Belladonnae folium

*Belladonna leaf (01/2012:0221)*

A more common name may also be used.

Chamomillae romanae flos

*Chamomile flower, roman (01/2017:0380)*

### II.1.2. Other issues concerning titles both in English and Latin

Where appropriate, the state of the herbal drug (dried or fresh, cut, raw, degree of maturity, etc.) is indicated.

BILBERRY FRUIT, DRIED

*Bilberry fruit, dried (07/2021:1588)*

BILBERRY FRUIT, FRESH

*Bilberry fruit, fresh (01/2019:1602)*

VALERIAN ROOT, CUT

*Valerian root, cut (04/2017:2526)*

SOPHORA FLOWER-BUD

*Sophora flower-bud (01/2015:2427)*

OPIUM, RAW

*Opium, raw (01/2015:0777)*

Particular characters can be indicated in order to differentiate monographs of close taxa.

FENNEL, BITTER; FENNEL, SWEET

*Fennel, bitter (04/2013:0824); Fennel, sweet (04/2011:0825)*

### II.1.3. Chinese names used in monographs on herbal drugs used in traditional Chinese medicine

In monographs on herbal drugs used in traditional Chinese medicine (TCM), the Chinese names in pinyin and in sinograms (usually as taken from the Chinese Pharmacopoeia) are included in the draft monograph at *Pharmeuropa* stage and remain until the draft is presented to the Ph. Eur. Commission for adoption, but do not appear in the official published monograph. After adoption of the monograph, these names are given in the EDQM Knowledge database and published in general chapter 5.22. *Names of herbal drugs used in traditional Chinese medicine* for information.

BAICAL SKULLCAP ROOT; Huangqin; 黄芩

*Baical skullcap root (04/2021:2438)*

## II.2. Definition

### II.2.1. Description

Some or all of the following are usually included:

- The state of the herbal drug (see general monograph *Herbal drugs (1433)*): fresh or dried, whole, fragmented, broken, cut into slices, peeled, unpeeled, etc.
- The complete scientific name of the plant (genus, species, subspecies, variety if needed, author) as usually obtained from *Medicinal Plant Names Services* (MPNS, <https://mpns.science.kew.org/mpns-portal/>). If the plant is not included in this database, *Plants of the World Online*

(POWO, <https://powo.science.kew.org/>) or *The Plant List* (<http://www.theplantlist.org/>) and for fungi the *Index Fungorum* (<http://www.indexfungorum.org>) may be used.

- The author names written using the conventional abbreviations as indicated in the above databases.
- The part or parts of the plant used written in the singular (e.g. leaf, root, bark) and in the plural only in the case of aerial parts, since aerial parts may cover different plant parts (e.g. stem, leaf, flower, fruit).

Whole or cut, dried, flowering, aerial parts of *Alchemilla vulgaris* L. *s.l.*

*Alchemilla* (07/2019:1387)

For the scientific name, a commonly used synonym (exceptionally two) may be mentioned in parentheses:

Dried ripe fruit of *Persicaria orientalis* (L.) Spach (syn. *Polygonum orientale* L.).

*Polygonum orientale* fruit (01/2017:2726)

When different species belonging to the same genus are described in the definition, the name of the genus is written in full the first time and abbreviated afterwards.

Dried false fruits of *Crataegus monogyna* Jacq. or *C. laevigata* (Poir.) DC. (syn. *C. oxyacantha* L.) or their hybrids or a mixture of these false fruits.

*Hawthorn berries* (04/2020:1220)

When a monograph covers several plant species, the different scientific names are mentioned one after another. When separated by commas, by 'and', by 'and/or' or by 'as well as': all species are considered to be equivalent and may be used indifferently or as a mixture.

Rose hips made up by the receptacle and the remains of the dried sepals of *Rosa canina* L., *R. pendulina* L. and other *Rosa* species, with the achenes removed.

*Dog rose* (07/2019:1510)

Whole or fragmented dried leaf of *Betula pendula* Roth and/or *B. pubescens* Ehrh. as well as hybrids of both species.

*Birch leaf* (01/2017:1174)

When separated by 'or': only one of the species can be used, not their mixture.

Fragmented dried thallus of *Fucus vesiculosus* L. or *F. serratus* L. or *Ascophyllum nodosum* Le Jolis.

*Kelp* (01/2008:1426)

Where appropriate, the stage in the growth cycle when harvesting takes place, or other necessary information may be included.

Dried, whole or fragmented rhizome of *Atractylodes lancea* (Thunb.) DC. (syn. *Atractylodes chinensis* (Bunge) Koidz.) with the roots removed, collected in spring and autumn.

*Atractylodes lancea* rhizome (01/2022:2559)

### II.2.2. Content

A specification for content is always given when an assay is described in the monograph.

The limit(s) for content of quantified constituents is (are) determined by the analytical procedure(s) described under Assay and can be expressed either as a minimum (in the majority of cases) or as a range in case of toxic constituents (see under Assay). Herbal drugs very often

contain a mixture of related constituents, in which case the total content of quantified constituents is determined and expressed as one of the constituents, usually the major constituent or a surrogate (in this context a surrogate is a compound that is not a constituent of the herbal drug); separate limits may be given for different forms of the herbal drug (whole/cut).

The statement '(dried drug)' implies that the monograph prescribes a test for loss on drying (2.2.32). The statement '(anhydrous drug)' implies that the monograph prescribes a test for the determination of water (2.2.13, 2.5.12, 2.5.32).

The Assay, as well as the corresponding test for loss on drying or water, are performed on the herbal drug as is. Subsequently, when performing the calculation of content described in the Assay, the result is corrected by the corresponding value obtained in the test for loss on drying or in the test for water.

*Content:*

- *essential oil*: minimum 4 mL/kg (dried drug);
- *sesquiterpenic acids*: minimum 0.17 per cent *m/m* expressed as valerenic acid (C<sub>15</sub>H<sub>22</sub>O<sub>2</sub>; M<sub>r</sub> 234.3) (dried drug).

*Valerian root (04/2017:0453)*

*Essential oil content:*

- for the whole drug, minimum 20 mL/kg (anhydrous drug);
- for the cut drug, minimum 15 mL/kg (anhydrous drug)

*Eucalyptus leaf (07/2014:1320)*

### II.3. Characters

This section may contain a brief description of some physical, chemical, macroscopic and microscopic botanical characters of the herbal drug. The information given is not to be regarded as representing mandatory requirements.

#### II.3.1. Physical, chemical and organoleptic characters

The colour of the herbal drug is described, where this is characteristic.

*Appearance*: hard, friable, brownish to reddish-brown mass; thin fragments are brownish-yellow when examined against the light/powder or crumpled strips 2-5 mm wide or sometimes flakes translucent, somewhat tough and difficult to break, becoming more brittle on drying.

No reference is made to odour unless it is highly characteristic and can be described with reference to the odour of a single compound and is non-toxic/hazardous for the analyst. Terms such as 'aromatic' and 'characteristic' are to be avoided.

Reminiscent odour of vanillin/anethole.

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.

Very bitter and persistent/bitter/intense bitter somewhat astringent/strong and persistent bitter taste.

### II.3.2. 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 herbal drug may be described under Characters.

The fruit is a cremocarp and generally entire; a small fragment of the thin, rigid, slightly curved pedicel is frequently attached.

### II.4. Identification

This section includes tests performed to identify the herbal 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 microscopic examination in *Mastic* (1876) and no thin-layer chromatography (TLC)/high-performance thin-layer chromatography (HPTLC) in *Oak bark* (1887).

The monograph may have a first identification and a simpler second identification series. Application of the first and second identifications is defined in the *General Notices* of the Ph. Eur.: ‘The test(s) that constitute the second identification may be used in pharmacies only, provided it can be demonstrated that the article is fully traceable to a batch certified to comply with all the other requirements of the monograph. The implementation of the tests under the second identification is subject to national regulation’.

*First identification: A, B, D.*

*Second identification: A, B, C.*

A. The flower has a short peduncle...

B. Microscopic examination (2.8.23)...

C. Examine the chromatograms obtained in the test for lavender flower...

D. Examine the chromatograms obtained in the test for other species and varieties of lavender...

*Lavender flower (07/2018:1534)*

#### II.4.1. Macroscopic botanical characters

The main macroscopic botanical characters of the herbal drug are specified to permit a clear identification. When two or more plant species/subspecies are included in the definition, the individual differences between them are indicated. Further information for rapid identification of the herbal drug is provided if necessary.

The leaf of *Thymus vulgaris* is usually 4-12 mm long and up to 3 mm wide, sessile or with a very short petiole...

The leaf of *Thymus zygis* is usually 1.7-6.5 mm long and 0.4-1.2 mm wide; it is acicular or linear-lanceolate and the edges are markedly rolled towards the abaxial surface...

*Thyme (07/2014:0865)*

#### II.4.2. Microscopic botanical characters (2.8.23)

The microscopic examination of the herbal drug is carried out on the powdered herbal drug (355) (2.9.12) in accordance with general chapter 2.8.23 unless otherwise prescribed in the monograph. It 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 and the reagents used for the microscopic examination are specified. It may be necessary to use more than one 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.

When two or more plant species/subspecies are included in the definition, two separate descriptions may be given.

Monographs contain schematic drawings of the main microscopic features of powdered herbal drugs. These drawings complement the description given in the relevant identification test.

However, where a herbal drug does not present specific microscopic characters, a schematic drawing is not included in the monograph (e.g. *Mastic* (1876), *Poria* (2475))

B. Microscopic examination (2.8.23). The powder is greyish-brown (unpeeled root) or whitish (peeled root). Examine under a microscope using *chloral hydrate solution R*. The powder shows the following diagnostic characters (Figure 1126.-1): fragments of colourless, mainly unligified, thick-walled fibres [C, D, M] with split or pointed ends [D], sometimes accompanied by parenchymatous cells of the medullary rays [M], or grouped [C]; fragments of vessels, bordered-pitted or with reticulate or scalariform thickenings [G, H]; cluster crystals of calcium oxalate about 20-35 µm, mostly 25-30 µm in size, isolated [K] or included in parenchymatous cells [B]; fragments of parenchyma [E] with cells containing mucilage [Ea, F]; fragments of cork with thin-walled, tabular cells (surface view [A], transverse section [L]) (unpeeled root). Examine under a microscope using *ruthenium red solution R*. The powder shows groups of parenchyma containing mucilage, which stains orange-red. Examine under a microscope using *water R*. The powder shows numerous starch granules [J], about 3-25 µm in size, occasionally with a longitudinal hilum. The starch granules are mostly simple [Ja], a few being 2-4 compound [Jb].

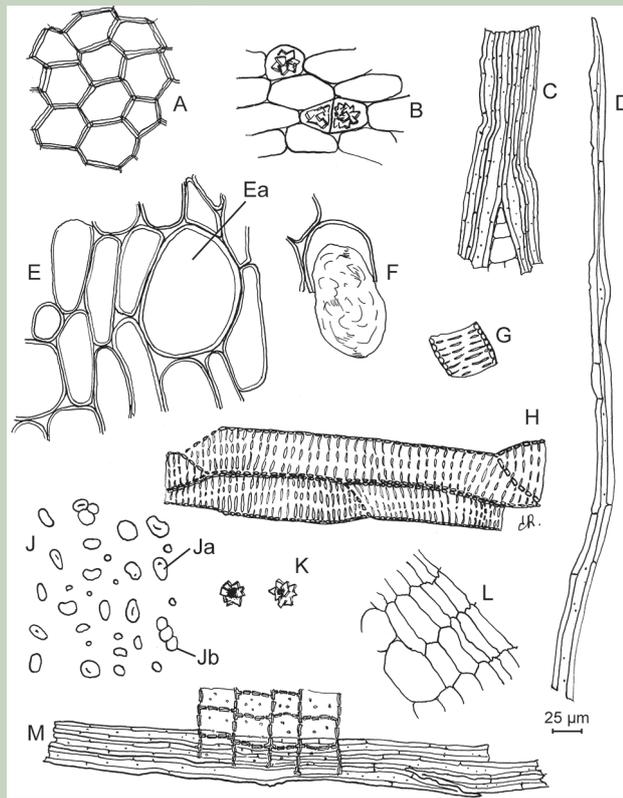


Figure 1126-1. – Illustration for identification test B of powdered herbal drug of...

*Marshmallow root (01/2012:1126)*

If the sieve size deviates from the size given in general chapter 2.8.23, it must be stated in the monograph:

Microscopic examination (2.8.23). Reduce to a powder (710) (2.9.12). The powder is greyish-green...

*Raspberry leaf (04/2020:2950)*

#### II.4.3. Thin-layer chromatography and high-performance thin-layer chromatography for identification of herbal drugs (2.2.27, 2.8.25)

Thin-layer chromatography (TLC/HPTLC) in accordance with general chapter 2.2.27 and high-performance thin-layer chromatography (HPTLC) in accordance with general chapter 2.8.25 are described here. Where appropriate HPTLC in accordance with general chapter 2.8.25 is used, TLC/HPTLC in accordance with 2.2.27 is replaced when possible.

TLC or HPTLC are the first choice chromatographic techniques for identification purposes, even if other chromatographic techniques, e.g. LC or GC, are used subsequently in other sections of the monograph.

The TLC or HPTLC is aimed at determining the characteristic chromatogram (fingerprint) with respect to the relative position, colour and intensity of characteristic zones. To this end, reference compounds, preferably reagents, are used.

The commercial name of the TLC or HPTLC plate used during monograph development is included as a footnote to the monograph and is transferred to the EDQM Knowledge database when the monograph is published in the Ph. Eur.

All the information concerning the preparation of the solutions and the chromatographic conditions, derivatisation if used and detection modes are 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 chromatograms are described in the form of a table. For editorial reasons the upper, middle and lower thirds of the plate may be represented at different scales in the Ph. Eur. Only the characteristic 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. Indication of very faint zones that may not be visible in all batches should be avoided.

Given the detection mode (daylight, UV 254 nm, UV 366 nm) and the possible treatment of the plate with derivatisation agents, the zones of the chromatogram are described by their position, intensity, colour or quenching behaviour.

The reference compounds used serve to indicate the position and separation between zones; they do not necessarily need to be constituents of the herbal drug.

The names of the constituents detected in the chromatogram obtained with the reference solution are usually 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, there is evidence that such a constituent is naturally occurring in the herbal drug and no other compounds are known to comigrate.

It is usually necessary to indicate that zones other than those described may also be present in the chromatogram obtained with the test solution.

Normally chromatograms are not described in terms of  $R_F$  values (retardation factors).

A colour copy of a suitable chromatogram is provided to the Secretariat. The chromatogram is published with the monograph in *Pharmeuropa*. The chromatogram will not ultimately be published in the Ph. Eur., but will be transferred to the EDQM Knowledge database.

In the description of the mobile phase, the solvents must be given in order of increasing volume. With equal volumes, the solvents are given in alphabetical order. The total sum of volumes should be equal to 100 whenever possible and practical.

#### **HPTLC in accordance with 2.8.25**

Preference is given to HPTLC in accordance with general chapter 2.8.25. According to this chapter, the intensities of zones are described in comparison to the intensities of zones due to one or more intensity marker(s) in the reference solutions at different concentrations and a system suitability requirement is always given.

The intensity of the characteristic zones due to the test solution is described as intense, equivalent, faint or very faint with respect to the intensity marker(s) at two different concentrations in the reference solution. The interpretation of these intensities is described in 2.8.25:

“Visual evaluation. The chromatograms obtained with the test and reference solutions are compared with the descriptions in the results section of the individual monograph, with respect to zone position and colour, as well as intensity for the test solution. Zones of the test solution described as ‘equivalent’ or without an indication of intensity have intensities similar to the zone of the intensity marker in the reference solution (R). Zones described as ‘intense’ are visually more intense than the zone of the intensity marker in the reference

solution; zones described as 'faint' are visually less intense than the zone of the intensity marker in the reference solution, but equal to or more intense than the zone of the intensity marker in the diluted reference solution ( $R_{1/4}$ ,  $R_{1/20}$ , etc.); zones described as 'very faint' are visually less intense than the zone of the intensity marker in the diluted reference solution."

In the result table, when the intensity of a zone is given as a range, the latter is described from the least intense to the most intense (e.g. very faint to equivalent).

The system suitability test is defined based on the separation of two reference compounds with close retardation factors, ideally between  $R_F = 0.3$  and  $R_F = 0.5$ . The monograph describes in which third of the HPTLC each of the zones due to these two reference compounds is located.

#### C. High-performance thin-layer chromatography (2.8.25).

*Test solution.* To 0.5 g of the powdered herbal drug (...) (2.9.12), add 5.0 mL of ...R. Sonicate for 15 min, centrifuge and use the supernatant/filter or centrifuge and use the filtrate or supernatant.

*Reference solution (a).* Dissolve 2.5 mg of ... R and 3.5 mg of ... R in ... R and dilute to 10.0 mL with the same solvent.

*Reference solution (b).* Dilute 2.5 mL of reference solution (a) to 10.0 mL with ... R.

*Reference solution (c).* Dissolve 2.5 mg of ... R and 3 mg of ... R in ... R and dilute to 10 mL with the same solvent.

*Intensity marker:* reference solutions (a) and (b):

- substance x.

Two intensity markers may be described for different coloured zones:

*Intensity markers:* reference solutions (a) and (b):

- substance x for the yellow fluorescent zones,
- substance y for the green or greenish-blue fluorescent zones.

*Plate:* TLC silica gel  $F_{254}$  plate R (2-10  $\mu\text{m}$ ).<sup>(1)</sup>

*Mobile phase:* ... R, ... R, ... R (10:10:80 V/V/V).

*Application:* 4  $\mu\text{L}$ , as bands of 8 mm.

*Development:* 70 mm from the lower edge of the plate.

*Drying:* in a current of air at room temperature/for 5 min.

*Detection:* treat with ... R and heat at 100 °C for 3 min/heat at 100 °C for 5 min/spray with ... R, then with ... R or, alternatively, dip the plate/the warm plate/in ... R, then in ... R; allow to dry in air for about 1 min and examine in daylight/in ultraviolet light at 366 nm.

*System suitability:* reference solution (c):

- the chromatogram shows in the lower/middle/upper third two distinct zones, which may be touching; the lower zone (...) shows a light blue fluorescence and the upper zone (...) shows a yellow or orange fluorescence/the lower zone (...) and the upper zone (...) show a violet-red/yellow or orange fluorescence.

1 Merck HPTLC Si 60  $F_{254}$  is suitable.

- the chromatogram shows at the border between the lower and middle/middle and upper thirds two distinct zones, which may be touching; the lower zone (...) is greenish/bluish and the upper zone (...) is greyish-green/reddish-orange.

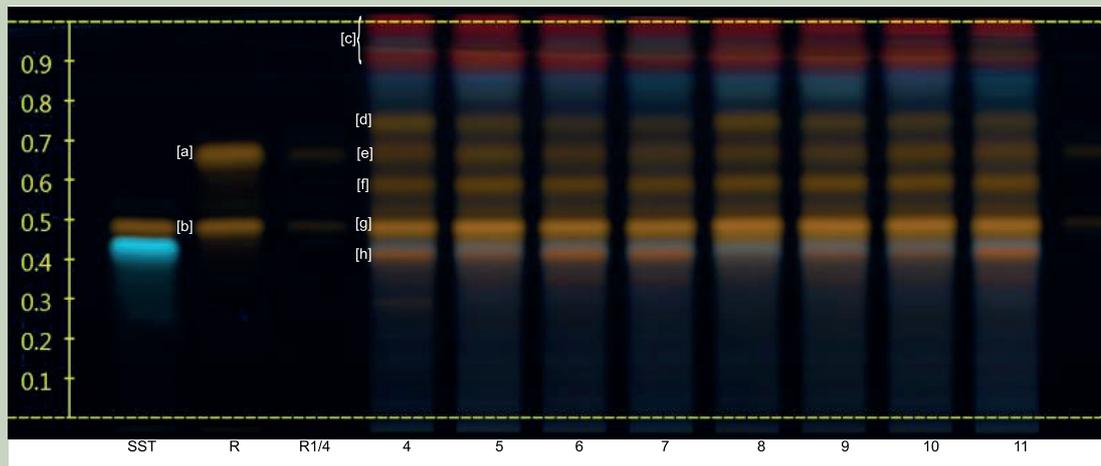
*Results:* see below the sequence of fluorescent zones/zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, other faint/blue/greenish/orange/or yellow fluorescent zones/zones may be present.

Top of the plate	
<p>[a] Substance x: a yellow or orange fluorescent zone</p> <p>—</p>	<p>[c] 2 red fluorescent zones, the lower one possibly overlapping with an orange fluorescent zone</p> <p>[d] A yellow or orange fluorescent zone or a yellow or orange fluorescent zone, faint</p> <p>[e] A yellow or orange fluorescent zone or a yellow or orange fluorescent zone, faint (substance x)</p> <p>—</p>
<p>[b] Substance y: a yellow or orange fluorescent zone</p> <p>—</p>	<p>[f] A yellow or orange fluorescent zone or a yellow or orange fluorescent zone, faint</p> <p>[g] A yellow or orange fluorescent zone, intense (substance y)</p> <p>[h] A yellow or orange fluorescent zone, very faint to intense</p> <p>—</p>
<b>Reference solution (a)</b>	<b>Test solution</b>

The following is only shown in *Pharmeuropa* drafts and in the Knowledge database after adoption of the text:

*The letters indicating the position of the zones refer to the chromatogram shown below for information. Like the chromatogram, they will not appear in the text published in the European Pharmacopoeia. However, the table with letters will be published with the chromatogram in the Knowledge database.*

*The following chromatogram is shown for information but will not be published in the European Pharmacopoeia. The zones in the chromatogram are identified by letters that correspond to the descriptions in the table above.*



SST: reference solution (c)    R1/4: reference solution (b)  
 R: reference solution (a)    4-11: test solutions from different batches  
 Figure XXXX.-X. – HPTLC chromatogram for identification test C of ...

Birch leaf (01/2017:1174)

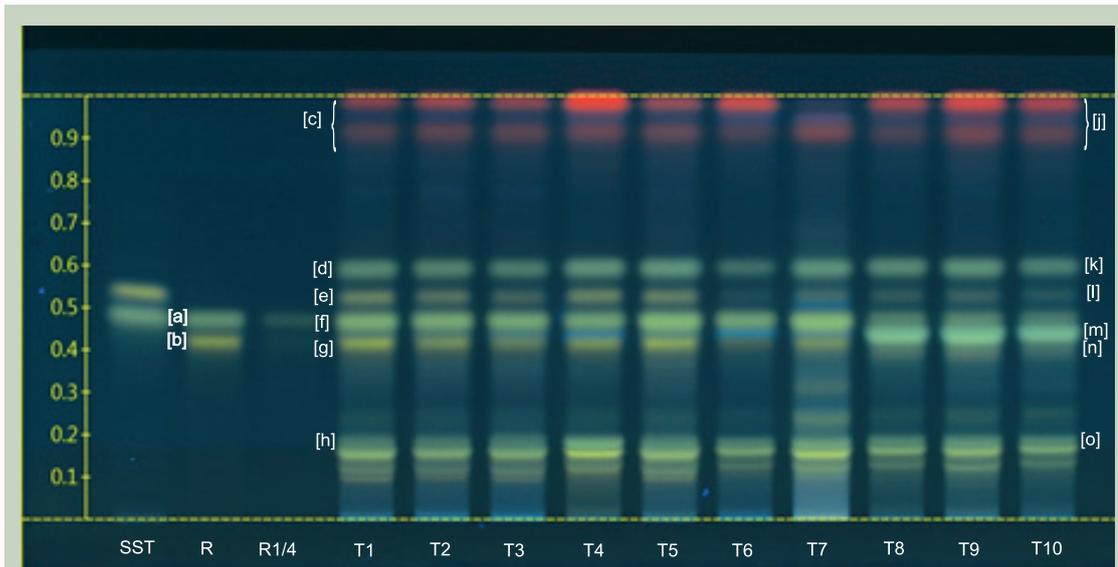
Different types of the same herbal drug may be described:

*Results:* see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a) and the test solution. Furthermore, in the chromatogram obtained with the test solution, the very faint to faint green or greenish-blue fluorescent zone in the lower third of the chromatogram may overlap with a faint yellow fluorescent zone just below it, and other very faint to faint yellow, greenish-yellow or brownish-yellow and green or greenish-blue fluorescent zones may be present, especially in the lower third of the chromatogram.

Top of the plate		
	[c] 2 red zones, intense	[j] 2 red zones, intense
—	—	—
[a] Substance x: a green or greenish-blue zone	[d] A green or greenish-blue zone, faint to equivalent [e] A yellow zone, faint to equivalent [f] A green or greenish-blue zone, faint to equivalent (substance x)	[k] A green or greenish-blue zone, faint to equivalent [l] A yellow zone, faint to equivalent
[b] Substance y: a yellow zone	[g] A yellow zone, faint to equivalent (substance y)	[m] A bluish-green zone (substance z) [n] A yellow zone, faint to equivalent (substance y)
—	—	—
	[h] A green or greenish-blue zone, very faint to faint	[o] A green or greenish-blue zone, very faint to faint
<b>Reference solution (a)</b>	<b>Test solution (substance x-type)</b>	<b>Test solution (substance z-type)</b>

The letters indicating ...

The following chromatogram ...



SST: reference solution (c)      T1-T7: test solutions prepared from different batches of passionflower herb, isovitexin-type

R: reference solution (a)

R1/4: reference solution (b)      T8-T10: test solutions prepared from different batches of passionflower herb, swertisin-type

Figure XXXX.-X. – HPTLC chromatogram for identification test C of ...

Passionflower herb (01/2021:1459)

More than one detection mode may be used:

*Detection A:* examine in ultraviolet light at 254 nm.

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

*Detection B:* treat with ... R. Heat at 100 °C for 3 min. Examine in ultraviolet light at 366 nm.

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

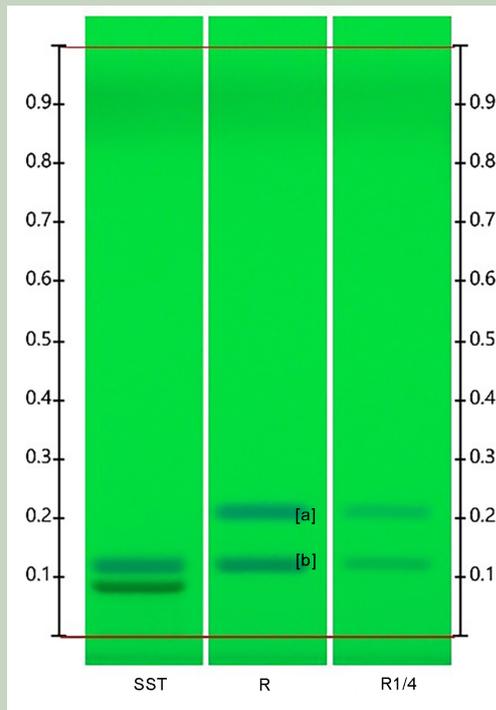
A detection mode may be used for assessing the system suitability only. When it is the case, only the chromatogram obtained with the reference solutions is included in the picture of the plate corresponding to the concerned detection mode:

*Detection A:* examine in ultraviolet light at 254 nm.

*System suitability:* reference solution (c):

– the chromatogram shows in the lower third 2 distinct zones, which may be touching; the upper zone (substance x) and the lower zone (substance y) are quenching zones.

*Detection B:* treat with a 10 per cent V/V solution of sulfuric acid R in methanol R; allow to dry in air for 5 min and examine in ultraviolet light at 366 nm. [...]



SST: reference solution (c)

R1/4: reference solution (b)

R: reference solution (a)

Figure XXXX.-X. – HPTLC chromatogram for identification test C of ... (reference solutions (a), (b) and (c), detection A at 254 nm)

[...]

*Saposhnikovia root (04/2023:2728)*

#### TLC [HPTLC] in accordance with 2.2.27

In some (especially older) monographs TLC and/or HPTLC may still be described in accordance with general chapter 2.2.27.

If the test has been developed both for TLC and HPTLC and equivalent results are obtained, HPTLC conditions (plate description, including particle size, application volume and development distance) are given in square brackets after those for classical TLC.

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

*Test solution:* To 2.0 g of the powdered herbal drug (...) (2.9.12) add 20 mL of ... R. Allow to stand for 2 h with occasional stirring. Filter.

*Reference solution:* Dissolve 5 mg of ... R and 5 mg of ... CRS in ... R and dilute to 10 mL with the same solvent.

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

*Mobile phase:* ... R, ... R, ... R, ... R (11:11:27:100 V/V/V/V).

*Application:* 10 µL [or 2 µL], as bands of 10 mm [or 8 mm].

*Development:* over a path of 13 cm [or 6 cm].

Drying, Detection and Results are described for TLC (2.2.27) as shown above in the section entitled HPTLC in accordance with 2.8.25.

If a fluorescent coating is used and the visualisation is done under UV at 254 nm, the zones are defined by their position, or their position and intensity.

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

Top of the plate	
—	A quenching zone, faint
Substance x: a quenching zone	— A quenching zone, faint/intense (substance x)
Substance y: a quenching zone	A quenching zone, intense
—	— Several quenching zones
Reference solution (a)	Test solution

When a TLC (2.2.27) or HPTLC (2.2.27 or 2.8.25) is used for the control of adulteration and for identification, or for identification and for the determination of minimum content ('semi-quantitative' approach), the analytical procedure is described fully under Tests with a cross-reference under Identification.

#### IDENTIFICATION [...]

C. High-performance thin-layer chromatography (2.8.25) as described in the test for *Solidago gigantea* Aiton and *Solidago canadensis* L./Examine the chromatograms obtained in the test for other officinal species of *Angelica*, *Levisticum* and *Ligusticum*.

[...]

*Results:* see below the sequence of fluorescent zones present in the chromatograms obtained with reference solution (a)/the reference solution and the test solution. Furthermore, other faint fluorescent zones may be present in the chromatogram obtained with the test solution.

[...]

#### TESTS

*Solidago gigantea* Aiton and *Solidago canadensis* L. High-performance thin-layer chromatography (2.8.25)/Other officinal species of *Angelica*, *Levisticum* and *Ligusticum*. Thin-layer chromatography (2.2.27).

[...]

*European goldenrod (07/2023:1893); Angelica dahurica root (01/2018:2556)*

#### II.4.4. Gas chromatography (2.2.28) and Liquid chromatography (2.2.29)

Where LC or GC is used in a test or assay, it may also be referred to under Identification. When the same LC procedure is used both for identification and as a test, it is described in full under Tests with a cross-reference under Identification. When the same LC procedure is used for both identification and the assay, it is described entirely under Assay with a cross-reference under Identification.

## IDENTIFICATION [...]

D. Examine the chromatograms obtained in the test for other species and varieties of lavender.

*Results:* the 5 principal peaks in the chromatogram obtained with the test solution are similar in retention time to the corresponding peaks in the chromatogram obtained with the reference solution; the 2 main peaks are due to linalol and linalyl acetate.

## TESTS [...]

**Other species and varieties of lavender.** Gas chromatography (2.2.28): use the normalisation procedure.

*Test solution* [...]

*Lavender flower (07/2018:1534)*

## II.4.5. Chemical reactions for identification

Chemical reactions are included if they are 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.

## IDENTIFICATION [...]

C. To 2.0 g of the powdered herbal drug (710) (2.9.12) add 25 mL of *ethyl acetate R...* The aqueous layer shows a blue or greenish-blue colour.

*Yarrow (07/2014:1382)*

## II.5. Tests

## Typical tests

Standard tests are included in the general monograph *Herbal drugs (1433)*. Tests specific for a given herbal drug are described in the corresponding individual monograph.

## II.5.1. Total ash (2.4.16)

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

As stated in the *General Notices*, the total ash is calculated with reference to the drug that has not been specially dried, unless otherwise prescribed in the corresponding individual monograph.

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

## II.5.2. 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 (e.g. sand).

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

### II.5.3. **Test for potential adulteration or unwanted constituents by thin-layer chromatography and high-performance thin-layer chromatography (2.2.27, 2.8.25)**

TLC and HPTLC can be used under Tests to detect plant species that are not part of the definition (adulteration) or are unwanted constituents. The TLC/HPTLC procedure is usually described entirely under Tests and wherever feasible it is also used to identify the herbal drug. The description of the chromatographic procedure meets all the criteria outlined under sections II.4.3.

The name of the unwanted plant species (the complete scientific name of the adulterant [genus, species, subspecies, variety, author]) or the name of the unwanted constituent(s) (e.g. thujone in *Three-lobed sage leaf (1561)*) is used as the title of the test. The name of the unwanted plant species is taken from the same scientific databases as those used to name the species described for the herbal drug (see II.2. Definition).

The title of the test is written in bold, the unwanted plant species are written in bold italics. In the chromatogram obtained with the test solution the position, colour and intensity of the zone(s) due to 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.

#### TESTS

***Bupleurum longiradiatum Turcz.*** High-performance thin-layer chromatography (2.8.25).  
[...]

*Results:* in the chromatogram obtained with the test solution, the presence of a greyish zone directly above the zone due to saikosaponin H (which co-migrates with saikosaponin C) indicates adulteration by *B. longiradiatum*.

*Bupleurum root (07/2021:2562)*

#### TESTS

**Thujone.** Thin-layer chromatography (2.2.27). [...]

*Results:* the chromatogram obtained with the reference solution shows in the middle part a blue zone (cineole) and in the upper part a pink-blue zone (thujone). The chromatogram obtained with the test solution shows no zone or a very faint pink-blue zone due to thujone.

*Sage leaf, three-lobed (07/2014:1561)*

### II.5.4. **Evaluation of the minimum content of a marker in a herbal drug by high-performance thin-layer chromatography (2.8.25)**

For herbal drugs that have analytical markers only and that are not used in products that have a marketing authorisation, the Assay may be replaced by an HPTLC test for minimum content for the marker, which is described under the Tests section ('semi-quantitative' approach). When this approach is chosen, a test for extractable matter may be described in addition. The monograph will not contain an Assay section.

The same HPTLC procedure used for the identification of the herbal drug may be employed to perform a semi-quantitative evaluation of the levels of the selected analytical marker(s). The intensity of the zone due to the selected marker in the test solution is compared with the intensity of the zone due to the same marker in the reference solution, the latter

representing an amount equal to the proposed limit for minimum content. The herbal drug passes the test when the zone due to the selected marker in the test solution is at least of the same intensity as the zone due to the same marker in the reference solution.

Intensity is evaluated by visual examination and/or using suitable software that evaluates the electronic images of the HPTLC plate. The luminance is calculated from the averaged signals of the red (R), green (G) and blue (B) channels of each pixel line of the track and it is plotted as a function of the retardation factor ( $R_F$ ) resulting in a peak profile for the track. Thus, zones are converted into peaks, which can be integrated to obtain quantitative information.

Note: to evaluate minimum levels, the chosen markers must be present on the HPTLC plate in an amount that is within the linear range of the signal obtained during the corresponding detection. This range and suitable concentrations of reference and test solutions are established during analytical procedure development and the corresponding validation data are provided.

**Peimine and peiminine.** High-performance thin-layer chromatography (2.8.25).

*Test solution.* Introduce 0.500 g of the powdered herbal drug (355) (2.9.12) into a centrifuge tube and add 2.5 mL of *concentrated ammonia R1*. Stopper the tube and allow to stand for 30 min. Add 12.5 mL of *methanol R* and shake for 20 min at 300 r/min. Centrifuge and use the supernatant.

*Stock solution A.* Dissolve 3.0 mg of *peimine R* in 15.0 mL of *methanol R*.

*Stock solution B.* Dissolve 3.0 mg of *peiminine R* in 15.0 mL of *methanol R*.

*Reference solution (a).* Dissolve 2 mg of *papaverine hydrochloride R* in 4 mL of *methanol R*.

*Reference solution (b).* Dissolve 1 mg of *yohimbine hydrochloride R* in 10 mL of *methanol R*.

*Reference solution (c).* To 1.0 mL of stock solution A, add 4.0 mL of *methanol R*.

*Reference solution (d).* To 1.0 mL of stock solution B, add 4.0 mL of *methanol R*.

*Reference solution (e).* To 1.0 mL of reference solution (c), add 3.0 mL of *methanol R*.

*Reference solution (f).* To 1.0 mL of reference solution (d), add 3.0 mL of *methanol R*.

*Reference solution (g).* Dilute 1.0 mL of stock solution A to 10.0 mL with *methanol R*.

*Reference solution (h).* Dilute 1.0 mL of stock solution B to 10.0 mL with *methanol R*. Dilute 6.7 mL of this solution to 20.0 mL with *methanol R*.

*Intensity markers:* peimine (reference solutions (c) and (e)) and peiminine (reference solutions (d) and (f)).

*Plate:* TLC silica gel plate R (2-10  $\mu\text{m}$ ).

*Mobile phase:* diethylamine R, acetone R, toluene R (6:45:45 V/V/V).

*Application:* 5  $\mu\text{L}$ , as bands of 8 mm, 8 mm from the lower edge and at least 15 mm from the left and right edges of the plate (see Table 2588.-1).

*Development:* 70 mm from the lower edge of the plate, in an unsaturated chamber.

*Drying:* in a current of cold air for 5 min.

*Detection A:* examine the chromatograms in ultraviolet light at 366 nm.

*System suitability:* the  $R_F$  values of the quenching zones due to yohimbine and papaverine are approximatively 0.61 and 0.66, respectively.

*Detection B:* treat with a 10 per cent V/V solution of sulfuric acid R in methanol R and dry at room temperature until the layer is uniformly white (approximately 1 min). Heat at 120 °C for 5 min and examine in ultraviolet light at 366 nm within 3 min after derivatisation.

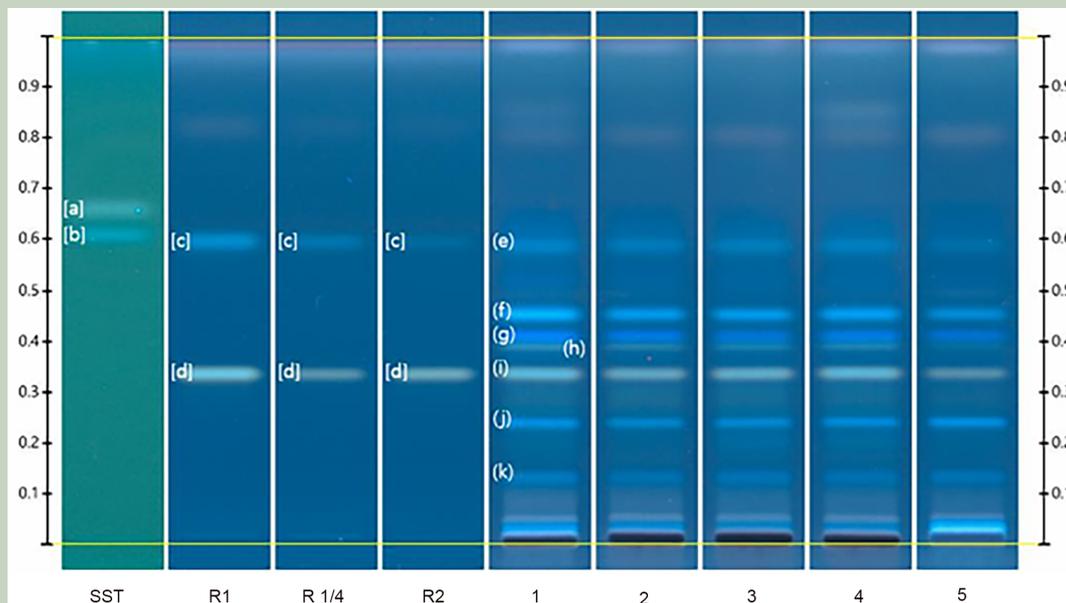
*Results C:*

- any zone due to peimine in the chromatogram obtained with the test solution is at least of the same intensity as the corresponding zone in the chromatogram obtained with reference solution (g) (minimum 0.06 per cent);
- any zone due to peiminine in the chromatogram obtained with the test solution is at least of the same intensity as the corresponding zone in the chromatogram obtained with reference solution (h) (minimum 0.02 per cent).

Table 2588.-1. – Application scheme

Track	1	2	3	4	5
Application volume (µL)	5	5	5	5	5
Solution	Reference solution (a) + reference solution (b)	Reference solution (c) + reference solution (d)	Reference solution (e) + reference solution (f)	Reference solution (g) + reference solution (h)	Test solution

Tracks 1-3 and 5 are used for Identification C; tracks 4 and 5 for the test for peimine and peiminine.



SST: reference solutions (a) and (b)

R1/4: reference solutions (e) and (f)

R1: reference solutions (c) and (d)

R2: reference solutions (g) and (h)

1-4: test solutions from different batches of Thunberg fritillary bulb

5: test solution from a batch of Thunberg fritillary bulb not compliant with the test

Figure 2588.-2. – HPTLC chromatogram for identification test C and the test for peimine and peiminine in Thunberg fritillary bulb

Thunberg fritillary bulb (01/2022:2588)

### 11.5.5. Gas chromatography (2.2.28) and Liquid chromatography (2.2.29)

The use of GC and LC is indicated under Tests to detect plant species that are not part of the definition, to limit certain constituents (e.g. estragole in *Bitter fennel* (0824)) and/or to control the possible presence of other compounds relevant to the purity of the herbal drug.

Substances used for quantification are established as CRSs. Where those compounds are not readily available, a surrogate CRS or a HRS may be established. The use of HRSs for quantitative purposes is only recommended when the other options are not feasible.

An HRS or the pure markers can be used as reagents for peak identification and the assessment of system suitability (for example, resolution). To establish CRSs or HRSs, a sufficient quantity of a batch of suitable quality must be available.

The commercial name of the column(s) found to be suitable during the elaboration of the monograph is included in a footnote, and transferred to the EDQM Knowledge database at the time of publication of the monograph in the Ph. Eur. Usually a representative chromatogram is included in the draft monograph published in *Pharmeuropa* and is transferred to the EDQM Knowledge database when the monograph is published in the Ph. Eur.

The mathematical formula used to calculate the corresponding content value may be given. The calculation is carried out with reference to the dried drug or anhydrous drug as described in the Definition. Hence, the value for the mass of the herbal drug is corrected for the loss on drying or the water content before its inclusion in the calculation formula.

When the same LC procedure is used for both the assay and a test, it is described in full under Tests with a cross-reference under Assay.

#### TESTS

**Nonivamide.** Liquid chromatography (2.2.29).

*Test solution.* To 2.5 g of the powdered herbal drug (500) (2.9.12) add 100 mL of ...

*Reference solution (a).* Dissolve 10.0 mg of *capsaicin* CRS and 2.0 mg of *nonivamide* CRS in ...

*Reference solution (b).* Dissolve 4.0 mg of *nonivamide* CRS in ...

*Column:*

- size:  $l = 0.25$  m,  $\varnothing = 4.6$  mm;
- stationary phase: base-deactivated end-capped phenylsilyl silica gel for chromatography R (5  $\mu$ m);
- temperature: 30 °C.

*Mobile phase:* acetonitrile R1, 1 g/L solution of *phosphoric acid* R (40:60 V/V).

*Flow rate:* 1.0 mL/min.

*Detection:* spectrophotometer at 225 nm.

*Injection:* 10  $\mu$ L.

*Run time:* 1.2 times the retention time of dihydrocapsaicin.

*Elution order:* nordihydrocapsaicin, nonivamide, capsaicin, dihydrocapsaicin.

*System suitability:* reference solution (a):

- resolution: minimum 1.5 between the peaks due to nonivamide and capsaicin.

Calculate the percentage content of nonivamide with reference to the total capsaicinoid content, using the following expression:

[...]

*Limit:*

– *nonivamide*: maximum 5.0 per cent of the total capsaicinoid content.

[...]

ASSAY

Liquid chromatography (2.2.29) as described in the test for nonivamide.

Calculate the percentage content of total capsaicinoids (C), expressed as capsaicin, using the following expression: [...]

*Capsicum* (01/2014:1859)

### II.5.6. Foreign matter (2.8.2)

Foreign matter consists of parts of the source plant that are not defined as the herbal drug, foreign elements of herbal origin that are not derived from the plant species given in the definition or are of mineral origin and other foreign elements not within the definition of the herbal drug and which are not covered by other tests for adulterants such as moulds and animal contamination or unwanted matter such as glass, metal or plastic.

The general monograph *Herbal drugs* (1433) specifies a maximum content of 2 per cent of foreign matter. Unless otherwise prescribed in an individual monograph, the general limit is applicable only to 'foreign organs' and 'foreign elements'; 'other foreign elements' as defined under 3 in general chapter 2.8.2 (i.e. matter such as moulds and animal contamination [e.g. insects, their eggs or larvae, spiders, rodents and excreta] and any other unwanted matter [e.g. glass, metal, plastics]), are not covered by the limit but should, as far as possible, be absent. Where a limit for foreign matter other than 2 per cent is to be prescribed, it is stated in the individual monograph with an indication of the type of foreign matter. Where necessary, the monograph indicates how the foreign matter is identified.

**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./maximum 8 per cent of fragments of coarse pedicels and other foreign matter and maximum 15 per cent of discoloured, brown flowers. Carry out the determination on 10 g/maximum 2 per cent, with no fruit of *Sambucus nigra* present.

Adulteration with *S. nigra* L. is indicated by the presence of glossy, violet-black, ovoid berries without the circular fold due to the calyx, and containing not more than 4 seeds.

*Hawthorn leaf and flower* (01/2021:1432); *Elder flower* (01/2013:1217); *Bilberry fruit, dried* (01/2013:1588)

### II.5.7. Elemental impurities

A general chapter *Heavy metals in herbal drugs and herbal drug preparations* (2.4.27) is included in the Ph. Eur. The general monograph *Herbal drugs* (1433) specifies maximum contents for cadmium, lead and mercury, which are applied unless otherwise stated in the individual monograph. A test for a specific elemental impurity may be included when needed, for example, where a particular herbal drug is known to accumulate that impurity.

**Arsenic (2.4.27):** maximum 90 ppm.

**Cadmium (2.4.27):** maximum 4 ppm.

**Lead (2.4.27):** maximum 5 ppm.

**Mercury (2.4.27):** maximum 0.1 ppm.

*Kelp (01/2008:1426)*

#### II.5.8. Loss on drying (2.2.32)

This test determines the amount of water present in the herbal 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 (e.g. *Achyranthes bidentate root (2999)*), the loss on drying is not more than 10.0 per cent when drying for 2 h in an oven at 105 °C.

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).

**Loss on drying (2.2.32):** maximum 10.0 per cent, determined on 1.000 g of the powdered herbal drug (355) (2.9.12) by drying in an oven at 105 °C for 2 h.

#### II.5.9. 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. Where appropriate, the fineness of the powder is indicated using the sieve number (2.1.4).

**Water (2.2.13):** maximum 100 mL/kg, determined on 20.0 g of the powdered herbal drug (355) (2.9.12)./maximum 120 mL/kg, determined on 20.0 g of the freshly powdered herbal drug (1400) (2.9.12) reduced using a knife mill.

*Eucalyptus leaf (07/2014:1320); Pepper (01/2013:2477)*

#### II.5.10. Swelling index (2.8.4)

Applicable to certain hydrocolloid-containing herbal drugs, for example: *Marshmallow root (1126)*, *Mallow leaf (2391)*, *Fenugreek (1323)*, *Ispaghula seed (1333)*, *Ispaghula husk (1334)*, *Iceland moss (1439)*, *Kelp (1426)*, *Mullein flower (1853)*.

**Swelling index (2.8.4):** minimum 7, determined on 1.0 g of the powdered herbal drug (710) (2.9.12)./minimum 9, determined on the powdered herbal drug (710) (2.9.12), moistened with 2 mL of *ethanol (96 per cent) R*.

*Mallow leaf (07/2011:2391); Mullein flower (07/2022:1853)*

#### II.5.11. Bitterness value (2.8.15)

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

**Bitterness value (2.8.15):** minimum 3 000.

*Bogbean leaf (01/2008:1605)*

#### II.5.12. Foam index (2.8.24)

Applicable to herbal drugs containing saponins.

**Foam index (2.8.24):** minimum 3,5, determined on 1.00 g of the powdered herbal drug (355) (2.9.12).

*Senega root (07/2019:0202)*

#### II.5.13. Extractable matter

The determination of extractable matter is usually prescribed for herbal drugs where no constituent suitable for an assay is known, where a test for minimum content by HPTLC is prescribed or where the material is used to produce a preparation with a dry residue.

This analytical procedure determines the amount of constituents extracted with solvents from a given amount of herbal material.

**Extractable matter:** minimum 15.0 per cent./minimum 30.0 per cent.

To ... g of the powdered herbal drug (250)/(355) (2.9.12) add a mixture of 8 g of *water R* and 12 g of *ethanol (96 per cent) R*/40 g of *water R* and allow to macerate for 2 h, shaking frequently. Filter, evaporate ...g of the filtrate to dryness on a water-bath and dry in an oven at 100-105°C for 2 h./Stir for 1h and filter. Evaporate ...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/0.15 g.

*Restharrow root (07/2014:1879); Dandelion herb with root (07/2012:1851)*

**Matter extractable by ethanol (70 per cent V/V):** minimum 25.0 per cent.

To 10.0 g of the powdered herbal drug (355) (2.9.12) add 300 mL of *ethanol (70 per cent V/V) R* ... The residue weighs a minimum of 0.250 g.

*Hop strobile (01/2011:1222)*

#### II.5.14. Aflatoxin B<sub>1</sub> (2.8.18)

Herbal drugs that are subject to contamination by aflatoxins are tested by a validated analytical procedure.

Unless otherwise indicated in the monograph, herbal drugs contain maximum 2 µg/kg of aflatoxin B<sub>1</sub>. The competent authority may also require compliance with a limit of 4 µg/kg for the sum of aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

The analytical procedure described in the general chapter *Determination of aflatoxin B<sub>1</sub> in herbal drugs (2.8.18)* is cited as an example of a procedure that has been shown to be suitable for devil's claw root, ginger and senna pods. Its suitability for other herbal drugs must be demonstrated or another validated analytical procedure used.

#### II.5.15. Ochratoxin A (2.8.22)

Herbal drugs that are subject to contamination by ochratoxin A are tested by a validated analytical procedure.

The analytical procedure described in the general chapter *Determination of ochratoxin A in herbal drugs (2.8.22)* has been shown to be suitable for liquorice extract and liquorice root. Its suitability for other herbal drugs must be demonstrated or another validated analytical procedure must be used.

**Ochratoxin A (2.8.22):** maximum 20 µg per kilogram of herbal drug.

*Liquorice root (01/2012:0277)*

#### II.5.16. Aristolochic acids (2.8.21)

Herbal drugs that, according to chemotaxonomic knowledge, are expected to be free from aristolochic acids, but that may be subject to adulteration or substitution with plant material containing aristolochic acids can be tested as described in the general chapter *Test for aristolochic acids in herbal drugs (2.8.21)*. The analytical procedures described in this general chapter are not designed for inclusion as assay procedures in monographs on those herbal drugs that produce aristolochic acids as secondary metabolites; for these, a more sensitive, validated analytical procedure is required.

*Aristolochia fangchi*. Test for aristolochic acids in herbal drugs (2.8.21). The drug to be examined complies with method A.

*Fourstamen stephania root (01/2013:2478)*

#### II.5.17. Contaminant pyrrolizidine alkaloids (2.8.26)

This general chapter covers trace analysis of target pyrrolizidine alkaloids (PAs) in herbal drugs and preparations thereof and in medicinal products. The target PAs are carcinogenic and naturally occur in common weeds that can contaminate raw plant materials used for the production of medicinal products. The general chapter does not cover the determination of PAs that occur naturally in the herbal drug as such.

It is not possible to describe a single procedure covering all target PAs in all possible matrices. Thus, instead the general chapter allows the use of any quantitative procedure consisting of chromatography coupled with MS/MS or high resolution MS, that meets the validation requirements given in the text. It also provides requirements for ongoing procedure verification during routine analysis. Finally, as an example, it describes a LC-MS/MS procedure shown to be suitable for some matrices.

#### II.5.18. 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. Current EMA guidance on the possible toxicity of constituents is taken into account.

*Aristolochia manshuriensis* Kom. and other species of *Aristolochia*. Examine the powdered herbal drug (355) (2.9.12) under a microscope using *chloral hydrate solution R*; no cluster crystals are visible.

*Clematis armandii stem (01/2018:2463)*

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

#### Starch

**Starch.** Examine the powdered herbal drug (355) (2.9.12) under a microscope using *water R*. Add *iodine solution R1*. No blue colour develops.

*Devil's claw root (01/2011:1095)*

### Deteriorated flower-heads

#### Diameter of the flower-heads

**Diameter of the flower-heads:** maximum 3 per cent of flower-heads have a diameter smaller than 8 mm.

**Deteriorated flower-heads:** brown or darkened flower-heads are absent.

*Chamomile flower, roman (01/2017:0380)*

#### Matter insoluble in ethanol

**Matter insoluble in ethanol:** maximum 75 per cent.

Place 1.00 g of the powdered herbal drug ...

*Myrrh (07/2017:1349)*

#### Broken drug

**Broken drug:** maximum 25 per cent, determined on 20.0 g, passes through a sieve (710) (2.9.12).

*Marticaria flower (07/2019:0404)*

## II.6. Assay

An assay is included to determine constituents with known therapeutic activity or constituents accepted to contribute to the therapeutic activity (active markers). If neither of such constituents are present, an assay for an analytical marker is usually included. Analytical markers should be phytochemically typical of the herbal drug and present in an adequate amount for quantitative determination. An assay is of little value for substances present in the herbal drug in very low amounts, for undesirable constituents or ubiquitous compounds not typical of the corresponding herbal drug. The selected markers (constituents with known therapeutic activity, active markers or analytical markers) are preferably determined using specific and accurate analytical procedures such as LC or GC.

The minimum content of the selected marker is given under Definition. If appropriate, in certain cases, a limit range may be given, for example when the assayed constituents are considered to be toxic at a certain concentration (level).

For the quantification of the selected marker, a CRS is established. If the selected marker cannot be procured, a readily available substance may be used as a 'surrogate' chemical reference standard, and where necessary, a correction factor shall be described in the monograph.

The selected marker(s) is (are) usually quantified versus a single CRS, established for the corresponding quantitative use. When the acceptance criterion is based on the sum of related constituents, the Definition describes a minimum content limit that is the sum of these constituents, usually expressed with respect to the most suitable constituent used for quantification or a surrogate.

Identification of peaks and system suitability (for example, resolution) can be performed using an HRS or the pure compounds as reagents.

Where possible, the same assay procedure is used for the herbal drug and the herbal drug preparation.

When appropriate, a global determination of constituents that are very often a group of related compounds may be used for the determination of, for example, flavonoids, tannins or essential oils. A spectrophotometric procedure may be used for the determination of flavonoids (e.g. *Safflower flower* (2386)).

Tannins are determined in accordance with general chapter 2.8.14. *Tannins in herbal drugs* (e.g. *Raspberry leaf* (2950)). The percentage content of tannins is expressed as pyrogallol.

The content of essential oils is determined by distillation in accordance with general chapter *Essential oils in herbal drugs* (2.8.12) (e.g. *Caraway fruit* (1080)).

Nevertheless, if an individual marker is known for a herbal drug, the assay is usually based on this individual marker instead.

When, for different reasons (e.g. in the absence of known markers), the introduction of an assay may not be justified/possible, a test for extractable matter (e.g. *Codonopsis root* (2714)) and/or a test for minimum content by HPTLC (e.g. *Thunberg fritillary bulb* (2588)) (see II.5.4. *Evaluation of the minimum content of a marker in a herbal drug by high-performance thin-layer chromatography* (2.8.25)) may be introduced into the Tests section of the monograph.

In monographs on herbal drugs used for their content in mucilage, an assay may be omitted and a test on swelling index may be introduced instead (2.8.4) (e.g. *Fenugreek* (1323)).

In monographs on herbal drugs used for their content in saponins, an assay may be omitted and a test on foam index may be introduced (2.8.24) (e.g. *Senega root* (0202)).

In monographs on herbal drugs used for their content in bitter principles, an assay may be omitted and a test on bitterness value (2.8.15) may be introduced (e.g. *Gentian root* (0392)).

#### II.6.1. Gas chromatography (2.2.28) and Liquid chromatography (2.2.29)

The description of the chromatographic procedure meets all the criteria outlined under II.5.5. The commercial name of the column(s) found to be suitable during elaboration of the monograph is included in a footnote, and transferred to the EDQM Knowledge database at the time of publication of the monograph in the Ph. Eur. The same applies to the representative chromatogram usually included in the draft monograph.

The mathematical formula used to calculate the corresponding content value is usually given. The calculation is carried out with reference to the dried drug or anhydrous drug as described in the Definition. Hence, the value for the mass of the herbal drug is corrected for the loss on drying or the water content before its inclusion in the calculation formula.

##### DEFINITION [...]

*Content:* minimum 0.15 per cent of forsythoside A (C<sub>29</sub>H<sub>36</sub>O<sub>15</sub>; M<sub>r</sub> 625) (dried drug).

[...]

##### ASSAY

Liquid chromatography (2.2.29). [...]

Calculate the percentage content of forsythoside A using the following expression:

$$\frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 2}$$

A<sub>1</sub> = area of the peak due to forsythoside A in the chromatogram obtained with the test solution;

$A_2$  = area of the peak due to forsythoside A in the chromatogram obtained with reference solution (a);

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

$m_2$  = mass of *forsythoside A CRS* used to prepare reference solution (a), in grams;

$p$  = percentage content of forsythoside A in *forsythoside A CRS*.

*Forsythia fruit (04/2021:2720)*

DEFINITION [...]

*Content:* minimum 0.2 per cent of total vitexin-2''-O-rhamnoside derivatives, expressed as vitexin-2''-O-rhamnoside ( $C_{27}H_{30}O_{14}$ ;  $M_r$  578.5) (dried drug).

[...]

ASSAY

Liquid chromatography (2.2.29). [...]

Calculate the percentage content of total vitexin-2''-O-rhamnoside derivatives, expressed as vitexin-2''-O-rhamnoside resulting from alkaline hydrolysis, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2.5}{A_2 \times m_1}$$

$A_1$  = area of the peak due to vitexin-2''-O-rhamnoside in the chromatogram obtained with the test solution;

$A_2$  = area of the peak due to vitexin-2''-O-rhamnoside in the chromatogram obtained with reference solution (a);

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

$m_2$  = mass of *vitexin-2''-O-rhamnoside CRS* used to prepare reference solution (a), in grams;

$p$  = percentage content of vitexin-2''-O-rhamnoside in *vitexin-2''-O-rhamnoside CRS*.

*Hawthorn leaf and flower (01/2021:1432)*

DEFINITION [...]

*Content:* minimum 1.5 per cent of triterpene glycosides, expressed as protoaescigenin ( $C_{30}H_{50}O_6$ ;  $M_r$  506.7) (dried drug).

[...]

ASSAY

Liquid chromatography (2.2.29). [...]

Calculate the percentage content of triterpene glycosides, expressed as protoaescigenin, using the following expression:

$$\frac{A_1 \times m_2 \times p \times 2}{A_2 \times m_1}$$

$A_1$  = total area (integrated as described above) of the peaks eluting between the peaks due to methyl salicylate and ibuprofen in the chromatogram obtained with the test solution;

$A_2$  = total area (integrated as described above) of the peaks eluting between the peaks due to methyl salicylate and ibuprofen in the chromatogram obtained with reference solution (a);

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

$m_2$  = mass of *aescin* for LC assay HRS used to prepare reference solution (a), in grams;

$p$  = percentage content of *aescin*, expressed as protoaescigenin, in *aescin* for LC assay HRS.

*Horse-chestnut* (01/2017:1830)

#### II.6.2. Ultraviolet and visible absorption spectrophotometry (2.2.25)

Spectrophotometry allows the determination of constituents that are very often a group or of classes of constituents that are characterised by a maximum absorption in the same range of wavelengths. It may be used for the quantification of constituents:

- that are analytical markers, when constituents with known therapeutic activity and active markers are not known;
- accepted to contribute to the therapeutic activity (active markers);
- with known therapeutic activity that are a mixture of similar substances.

It may be used to determine, for example:

- flavonoids (*Birch leaf* (1174), *Elder flower* (1217), *Calendula flower* (1297), *Sophora flower* (2639));
- alkaloids (*Cinchona bark* (0174)).

#### ASSAY

*Stock solution.* In a 100 mL round-bottomed flask introduce 0.200 g of the powdered herbal drug (355) (2.9.12), 1 mL of a 5 g/L solution of *hexamethylenetetramine* R, 20 mL of *acetone* R and ...

*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 herbal drug to be examined, in grams.

*Birch leaf* (01/2017:1174)

#### II.6.3. Determination of tannins in herbal drugs (2.8.14)

This assay is described in a general chapter and cross-referenced in some individual monographs such as *Dried bilberry fruit* (1588), *Hamamelis leaf* (0909), *Rhatany root* (0289), *Tormentil* (1478) and *Oak bark* (1887).

#### DEFINITION [...]

*Content:* minimum 3.0 per cent of tannins, expressed as pyrogallol (C<sub>6</sub>H<sub>6</sub>O<sub>3</sub>;  $M_r$  126.1) (dried drug).

[...]

ASSAY

**Tannins** (2.8.14). Use 0.700 g of the powdered herbal drug (710) (2.9.12).

Oak bark (01/2008:1887)

II.6.4. **Volumetric titration**

Examples are the assay of alkaloids in *Ipecacuanha root* (0094), the assay on acids in *Benzoin*, *Siam* (2158), *Roselle* (1623) and *Tolu balsam* (1596).

DEFINITION [...]

*Content*: minimum 2.0 per cent of total alkaloids, expressed as emetine (C<sub>29</sub>H<sub>40</sub>N<sub>2</sub>O<sub>4</sub>; M<sub>r</sub> 480.6) (dried drug).

[...]

ASSAY

Place 7.5 g of the powdered herbal drug (180) (2.9.12) in a dry flask, add 100 mL of *ether R* and shake for 5 min. Add 5 mL of *dilute ammonia R1*, shake for 1 h, add 5 mL of *water R* and shake vigorously. Decant the ether layer into a flask through a plug of cotton. Wash the residue in the flask with 2 quantities, each of 25 mL, of *ether R*, decanting each portion through the same plug of cotton. Combine the ether solutions and eliminate the ether by distillation. Dissolve the residue in 2 mL of *ethanol (90 per cent V/V) R*, evaporate to dryness and heat at 100 °C for 5 min. Dissolve the residue in 5 mL of previously neutralised *ethanol (90 per cent V/V) R*, warming on a water-bath. Add 15.0 mL of 0.1 M *hydrochloric acid* and titrate the excess acid with 0.1 M *sodium hydroxide* using 0.5 mL of *methyl red mixed solution R* as indicator.

1 mL of 0.1 M *hydrochloric acid* is equivalent to 24.03 mg of total alkaloids, expressed as emetine.

*Ipecacuanha root* (04/2016:0094)

II.6.5. **Determination of essential oils in herbal drugs (2.8.12)**

The determination of essential oils in herbal drugs is usually described for herbal drugs used for their essential oil content.

DEFINITION

[...]

*Essential oil content*:

- for the whole drug, minimum 18 mL/kg (anhydrous drug);
- for the cut drug, minimum 12 mL/kg (anhydrous drug).

[...]

ASSAY

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

*Sage leaf, three-lobed* (07/2014:1561)

## II.7. Storage

The 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.

The terminology given in the *General Notices* and in section 3.2. *Containers* of the Ph. Eur. should be used.

### STORAGE

In an airtight container/Protected from moisture.

## II.8. Reagents

Wherever possible, existing reagents described in general chapter 4.1.1. *Reagents* of the Ph. Eur. are used as reference compounds. In draft monographs containing a reagent not yet described in the Ph. Eur., its description is appended to the draft monograph for subsequent inclusion in general chapter 4.1.1.

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

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 usually included in a footnote to the monograph and transferred to the EDQM Knowledge database at the time of publication of the monograph in the Ph. Eur.

Where reagents are not readily available, the establishment of a CRS or HRS may be envisaged. In such cases, the availability of a suitable quantity must be verified during monograph elaboration.

### **III. Herbal drug preparations**

This part of the guide provides details on aspects that are specific to the different types of herbal drug preparations. Herbal drug preparations are extracts (dry extracts, liquid extracts, tinctures, soft extracts and oleoresins), essential oils, prepared herbal drugs, herbal teas and instant herbal teas. The overall structure of monographs on herbal drug preparations is similar to the structure used for herbal drugs.

### III.A. Extracts

The provisions of the general monograph *Herbal drug extracts (0765)* apply: the general monograph must be taken into account during the elaboration of individual monographs. 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.

In addition, the statements in the general monograph *Herbal drug extracts (0765)* are intended to be read in conjunction with general chapter 5.23. *Monographs on herbal drug extracts (information chapter)*.

#### III.A.1. Title

The title is derived from that of the monograph on the herbal drug and is supplemented by an indication of whether the extract is a liquid extract, tincture, soft extract, oleoresin or dry extract.

According to the general monograph *Herbal drug extracts (0765)*, there are three types of extracts: 'standardised extracts', 'quantified extracts' and 'other extracts'. The title indicates whether the extract is standardised or quantified. The term 'standardised' is used when the assay quantifies constituents with known therapeutic activity. The term 'quantified' is used when the assay quantifies constituents which are generally accepted to contribute to the therapeutic activity.

In the case of extracts for which the assay quantifies constituents chosen solely for analytical purposes, irrespective of any pharmacological or therapeutic activity they may be reported to possess ('other extracts'), no indication is included in the title.

For standardised and quantified extracts, the title may also include 'refined' depending on the purification procedures used (according to the general monograph *Herbal drug extracts (0765)*).

HAWTHORN LEAF AND FLOWER LIQUID EXTRACT

*Hawthorn leaf and flower liquid extract (01/2021:1864)*

ARNICA TINCTURE

*Arnica tincture (07/2022:1809)*

CAPSICUM SOFT EXTRACT, STANDARDISED

*Capsicum soft extract, standardised (01/2014:2529)*

CAPSICUM OLEORESIN, REFINED AND STANDARDISED

*Capsicum oleoresin, refined and standardised (01/2014:2336)*

PASSIONFLOWER HERB DRY EXTRACT

*Passionflower herb dry extract (01/2021:1882)*

ST JOHN'S WORT DRY EXTRACT, QUANTIFIED

*St John's wort dry extract, quantified (01/2017:1874)*

#### III.A.2. Definition

Reference is made to the monograph on the herbal drug from which the extract is prepared. Content limits are usually included. For standardised and quantified extracts, upper and lower assay limits should be given.

For standardised extracts, the content of the assayed constituents is stated as either a defined single content or a defined range of content.

As stated in the general monograph *Herbal drug extracts (0765)*:

*Defined single content.* For example, in the monograph *Ipecacuanha liquid extract, standardised (1875)*, the content of assayed constituents is stated as 1.80 per cent to 2.20 per cent. In this case, the declaration is based on a defined single content of 2.0 per cent with a tolerance of  $\pm 10$  per cent. The acceptable tolerance is usually within the range  $\pm 5$  per cent to  $\pm 10$  per cent taking into account the nature of the extract and the assay procedure.

*Defined range of content.* For example, in the monograph *Frangula bark dry extract, standardised (1214)*, the content of assayed constituents is stated as 15.0 per cent to 30.0 per cent. In this case, it is intended that an extract will consistently be produced to a defined single content selected from within the defined range taking into account an acceptable tolerance. Where there is an individual monograph in the Ph. Eur. for a standardised extract with a defined range of content, the acceptable tolerance will be stated in the individual monograph (for example, for *Frangula bark dry extract, standardised (1214)*, the acceptable tolerance is stated as  $\pm 10$  per cent relative to the declared content).

For 'other extracts', a limit for minimum content is usually given.

Refined and quantified oleoresin produced from *Capsicum (1859)*.

*Content:* 12.0 per cent to 18.0 per cent *m/m* of total capsaicinoids, expressed as capsaicin ( $C_{18}H_{27}NO_3$ ;  $M_r$  305.4).

*Capsicum oleoresin, refined and standardised (01/2014:2336)*

Refined and quantified dry extract produced from *Ginkgo leaf (1828)*.

*Content:*

- *flavonoids, expressed as flavone glycosides* ( $M_r$  756.7): 22.0 per cent to 27.0 per cent (dried extract);
- *bilobalide*: 2.6 per cent to 3.2 per cent (dried extract);
- *ginkgolides A, B and C*: 2.8 per cent to 3.4 per cent (dried extract);
- *ginkgolic acids*: maximum 5 ppm (dried extract).

*Ginkgo dry extract, refined and quantified (04/2008:1827)*

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

*Content:*

- *aqueous extracts*: minimum 1.0 per cent of total vitexin-2''-O-rhamnoside derivatives, expressed as vitexin-2''-O-rhamnoside ( $C_{27}H_{30}O_{14}$ ;  $M_r$  578.5) (dried extract);
- *hydroalcoholic extracts*: minimum 2.0 per cent of total vitexin-2''-O-rhamnoside derivatives, expressed as vitexin-2''-O-rhamnoside ( $C_{27}H_{30}O_{14}$ ;  $M_r$  578.5) (dried extract).

*Hawthorn leaf and flower dry extract (01/2021:1865)*

Standardised liquid extract produced from *Ipecacuanha root (0094)*.

*Content:* 1.80 per cent to 2.20 per cent of total alkaloids, expressed as emetine ( $C_{29}H_{40}N_2O_4$ ;  $M_r$  480.6).

*Ipecacuanha liquid extract, standardised (04/2016:1875)*

Standardised dry extract obtained from *Frangula bark (0025)*.

*Content:* 15.0 per cent to 30.0 per cent of glucofrangulins, expressed as glucofrangulin A ( $C_{27}H_{30}O_{14}$ ;  $M_r$  578.5) (dried extract). The measured content does not deviate from that stated on the label by more than  $\pm 10$  per cent.

*Frangula bark dry extract, standardised (07/2015:1214)*

### III.A.3. Production

The section includes a statement of the extraction solvents used, based on approved medicinal products in member states. This effectively describes 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/genuine extract ratio ( $DER_{genuine}$ ) is not stated; the general monograph *Herbal drug extracts (0765)* requires that the  $DER_{genuine}$  be stated on the label for quantified and 'other' extracts.

The extract is produced from the herbal drug by a suitable procedure using either hot water at not less than 65 °C or a hydroalcoholic solvent equivalent in strength to ethanol (45-75 per cent V/V)./The tincture is produced from 1 part of the herbal drug and 5 parts of ethanol (90 per cent V/V) by a suitable procedure./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).

*Boldo leaf dry extract (01/2011:1816); Myrrh tincture (07/2017:1877); Hawthorn leaf and flower dry extract (01/2021:1865)*

### III.A.4. Characters

This section may contain a brief description of some physical characters of the herbal drug extract. The information given is not to be regarded as representing mandatory requirements. No reference is made to taste unless there is a test for bitterness value (2.8.15) in the monograph or the extract is to be used for flavouring purposes.

The odour is only mentioned if it is very characteristic and can be described with reference to the odour of a single compound and it is non-toxic/hazardous for the analyst.

*Appearance:* yellowish-brown or brown powder./yellowish-brown or reddish-brown liquid.

*Liquorice dry extract for flavouring purposes (01/2012:2378); Gentian tincture (01/2008:1870)*

Very sweet taste./It has a strong bitter taste.

*Liquorice dry extract for flavouring purposes (01/2012:2378); Gentian tincture (01/2008:1870)*

It has a characteristic odour of vanillin.

*Benzoin tincture, Siam (01/2008:2157)*

### III.A.5. Identification

The preferred analytical procedure is that used for the herbal drug, usually HPTLC (2.8.25) or TLC/HPTLC (2.2.27), where appropriate.

### III.A.6. Tests

Standard tests are included in the general monograph *Herbal drug extracts (0765)*. Tests specific for a given extract are described in the corresponding individual monograph.

### III.A.6.1. *Ethanol content (2.9.10)*

#### *Test for methanol and 2-propanol (2.9.11)*

Liquid extraction preparations typically contain a test for ethanol. They may also contain a test for methanol and 2-propanol.

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

**Methanol and 2-propanol (2.9.11):** maximum 0.05 per cent V/V of methanol and maximum 0.05 per cent V/V of 2-propanol.

*Cinchona liquid extract, standardised (01/2008:1818)*

For extracts other than liquid extraction preparations, residual solvents are controlled as described in general chapter 5.4, unless otherwise prescribed or justified and authorised.

### III.A.6.2. *Loss on drying (2.8.17)*

#### *Semi-micro determination of water (2.5.12)*

According to general monograph *Herbal drug extracts (0765)*, dry extracts usually have a loss on drying of not greater than 5 per cent *m/m*. Where justified and authorised, a loss on drying (2.8.17) with a different limit or a test for water (2.5.12) may be prescribed.

**Loss on drying (2.8.17):** maximum 8.0 per cent.

*Olive leaf dry extract (04/2009:2313)*

**Water (2.5.12):** maximum 5.0 per cent, determined on 0.500 g.

*Passionflower herb dry extract (01/2021:1882)*

### III.A.6.3. *Dry residue (2.8.16)*

Monographs on liquid extraction preparations and soft extracts typically contain a test for dry residue. A minimum content is specified for the individual extracts in the monographs. A value that is too low indicates an unauthorised dilution of the preparation or an inferior source drug.

**Dry residue (2.8.16):** minimum 1.7 per cent.

*Arnica tincture (07/2022:1809)*

### III.A.7. **Assay**

In line with the definitions given in general chapter 5.23. *Monographs on herbal drug extracts (information chapter)*, monographs on standardised extracts describe assays determining constituents of known therapeutic activity. Monographs on quantified extracts prescribe assays determining constituents accepted to contribute to the therapeutic activity (active markers); monographs on 'Other extracts' describe assays determining analytical markers.

Analytical markers should be phytochemically typical for the extract and present in an adequate amount for quantitative determination. An assay is of little value for substances present in the herbal drug extract in very low amounts, for undesirable constituents or ubiquitous compounds not typical of the corresponding herbal drug. Where possible the same assay procedure is used for the herbal drug and the extract.

The selected markers (constituents with known therapeutic activity, active markers and analytical markers) are preferably determined using specific and accurate analytical procedures such as LC or GC.

The content requirement for a selected marker is given under Definition. For standardised and quantified extracts, the content of the assayed constituents is stated as either a defined single content or a defined range of content. In the case of 'other extracts' a minimum content is usually defined. If appropriate, in certain cases, a limit range for the content may be given, for example when the assayed constituents are considered to be toxic at a certain concentration (level) (e.g. ginkgolic acids in *Ginkgo dry extract, refined and quantified (1827)*).

For the quantification of the selected marker a CRS is established. If the selected marker cannot be procured, a readily available substance may be used as a 'surrogate' chemical reference standard, and where necessary, a correction factor shall be described in the monograph.

The selected marker(s) is (are) usually quantified versus a single CRS, established for the corresponding quantitative use. When the acceptance criterion is based on a mixture of related constituents, the Definition describes a limit for minimum content of the sum of these constituents, usually expressed with respect to the most suitable constituent used for quantification or a surrogate.

Identification of peaks and system suitability (for example resolution) can be performed using an HRS or the pure compounds as reagents.

The use of HRSs for quantitative purposes is only recommended when the other options are not feasible.

When appropriate, a global determination of constituents that are very often a group of related compounds may be used for the determination of, for example, flavonoids, tannins or essential oils. For the determination of flavonoids a spectrophotometric procedure may be used.

Tannins are determined in accordance with general chapter 2.8.14. *Tannins in herbal drugs* (e.g. *Rhatany tincture (1888)*).

The content of essential oils is determined by distillation (*Matricaria liquid extract (1544)*).

In monographs on herbal drug preparations used for their bitter principles, a test on the bitterness value (2.8.15) may be described (e.g. *Gentian tincture (1870)*).

Nevertheless, if an individual marker is known for a herbal drug extract, the assay is usually based on this individual marker instead.

#### III.A.8. Storage

The requirements of the general monograph *Herbal drug extracts (0765)* apply. Where applicable, additional specific conditions are given in the individual monograph.

#### III.A.9. Labelling

The requirements of the general monograph *Herbal drug extracts (0765)* apply. Where applicable, additional specific conditions are given in the individual monograph.

### III.B. Essential oils

The overall structure of monographs for essential oils is similar to the structure for herbal drugs; this part of the guide provides details on aspects that are specific to essential oils.

The provisions of the general monograph *Essential oils (2098)* apply: the statements in that monograph are intended to be read in conjunction with individual monographs on essential oils in the Ph. Eur.

In addition, the statements in the general monograph *Essential oils (2098)* are intended to be read in conjunction with general chapter 5.30. *Monographs on essential oils (information chapter)*.

Different types of certain essential oils (including chemotypes) may exist. The reason for this could be, for example, the origin of the plant material, the chemical composition or the primary processing. The Ph. Eur. monograph on an essential oil may cover one or more of these types. For example, several chemotypes exist for the essential oil of thyme, but only the thymol type is included in the Ph. Eur. (*Thyme oil, thymol type (1374)*).

The monograph may describe different sets of specifications for the different types of essential oil covered (e.g. *Rosemary oil (1846)*) or a single specification can cover different types of essential oil (e.g. *Lemon oil (0620)*). The relevance of distinguishing between different types in a monograph is analysed on a case-by-case basis and the decision is taken based mainly on the differences in the composition and the suitability for the intended use.

Monographs on essential oils do not apply to essential oils obtained from the primary production steps unless they are used as is in medicinal products.

An essential oil whose composition has been significantly modified may be known as:

- *Rectified essential oil*: an essential oil from which part of the constituents has been partially or totally removed by rectification; rectification can also be used to enrich an essential oil in a particular component (e.g. 1,8-cineole in *Eucalyptus oil (0390)*);
- *Deterpenated essential oil*: an essential oil from which monoterpene hydrocarbons have been partially or totally removed by rectification or any other suitable process;
- *Deterpenated and desesquiterpenated essential oil*: an essential oil from which monoterpene and sesquiterpene hydrocarbons have been partially or totally removed by rectification or any other suitable process.

#### III.B.1. Title

The title is derived from the name of the herbal drug and if necessary is supplemented by an indication of the type of the oil.

LEMON OIL

*Lemon oil (04/2022:0620)*

THYME OIL, THYMOL TYPE

*Thyme oil, thymol type (01/2012:1374)*

Any modification is usually indicated in the name of the essential oil.

MINT OIL, PARTLY DEMENTHOLISED

*Mint oil, partly dementholised (01/2008:1838)*

Certain rectified essential oils sometimes may keep a 'traditional' name that does not include the word 'rectified'.

#### EUCALYPTUS OIL

*Eucalyptus oil (07/2021:0390)*

#### III.B.2. Definition

Reference to a plant material from which the essential oil is obtained by a suitable method (steam distillation, dry distillation, or a suitable mechanical process without heating) is included. The complete scientific name of the plant (genus, species, subspecies, variety, author) as usually obtained from the scientific databases as described under herbal drugs (see section II.2. Definition) is provided. The reference to the state of the plant material (fresh, lightly wilted, wilted, partially dried, dried, whole, fragmented, broken or cut) may be included. If the herbal drug used must comply with the corresponding Ph. Eur. monograph, reference is made to that monograph by giving its title and number in the definition of the corresponding essential oil monograph. In all cases, the considerations laid down in the general monograph *Herbal drugs (1433)* apply. Usually all statements concerning the production of an essential oil are included in the Definition, i.e. whether it is produced by distillation (steam or dry) or by a mechanical process (cold-pressed oils). If appropriate, the method of subsequent treatment and the use of a suitable antioxidant are indicated.

The composition of the oil is defined in the chromatographic profile but no content requirements are given in the Definition.

Essential oil obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branchlets of various species of *Eucalyptus* rich in 1,8-cineole. The species mainly used are *Eucalyptus globulus* Labill., *Eucalyptus polybractea* R.T.Baker and *Eucalyptus smithii* R.T.Baker.

*Eucalyptus oil (07/2021:0390)*

The type of modification must be indicated in the Definition section of the individual monograph.

Essential oil obtained by steam distillation from the fresh, flowering aerial parts, recently gathered from *Mentha canadensis* L. (syn. *M. arvensis* L. var. *glabrata* (Benth) Fern., *M. arvensis* var. *piperascens* Malinv. ex Holmes), followed by partial separation of menthol by crystallisation.

*Mint oil, partially dementholised (01/2008:1838)*

#### III.B.3. Characters

This section may contain a brief description of some physical characters of the essential oil. The information given is not to be regarded as representing mandatory requirements. No reference is made to taste. No reference is made to odour unless it is highly characteristic and can be described with reference to the odour of a single compound and is non-toxic/hazardous for the analyst. Terms such as 'aromatic' and 'characteristic' are to be avoided.

*Appearance:* colourless or pale yellow liquid/clear, yellow liquid, which becomes brown when exposed to air.

*Odour:* reminiscent of 1,8-cineole.

*Eucalyptus oil (07/2021:0390)*

**Solubility:** miscible with methylene chloride, with toluene and with fatty oils.

*Clove oil (01/2008:1091)*

#### III.B.4. Identification

Usually two sets of identification tests (first and second identification) are included. The preferred analytical procedure for the first identification is GC and for the second identification is HPTLC in accordance with general chapter 2.8.25. Where applicable, the HPTLC analytical procedure is harmonised with that used for the herbal drug.

[...]

##### IDENTIFICATION

*First identification: B.*

*Second identification: A.*

A. Thin-layer chromatography (2.2.27).

[...]

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.

*Anise oil (01/2008:0804)*

#### III.B.5. Tests

The following are examples of tests that may be found in individual monographs on essential oils:

**Relative density (2.2.5):** 0.878 to 0.892.

**Refractive index (2.2.6):** 1.455 to 1.466.

**Optical rotation (2.2.7):**  $-12.5^{\circ}$  to  $-6.0^{\circ}$ .

**Acid value (2.5.1):** maximum 1.0, determined on 5.00 g of the essential oil to be examined dissolved in 50 mL of the prescribed mixture of solvents.

*Lavender oil (07/2018:1338)*

**Freezing point (2.2.18):** 15 °C to 19 °C.

*Anise oil (01/2008:0804)*

**Peroxide value (2.5.5):** maximum 20.

*Dwarf pine oil (07/2019:2377)*

**Water in essential oils (2.8.5).**

**Foreign esters (2.8.6).**

**Residue on evaporation (2.8.9):** 1.8 per cent to 3.6 per cent after heating on a water-bath for 4 h.

*Lemon oil (04/2022:0620)*

**Solubility in alcohol (2.8.10):** 1 volume is soluble in 2 volumes and more of *ethanol (80 per cent V/V) R*.

*Spanish sage oil (07/2008:1849)*

**Adulteration.**

**Chromatographic profile.** Gas chromatography (2.2.28): use the normalisation procedure.

[...]

*Elution order:* substance x, substance y, substance z/order indicated in the composition of reference solution (a); record the retention time of these substances.

*Identification of peaks/components:* use the chromatogram supplied with ... for peak identification HRS and the chromatogram obtained with reference solution (a) to identify the peaks due to .../using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution.

*System suitability:* reference solution (a):

- *resolution:* minimum 1.4 between the peaks due to ... and ...

Determine the percentage content of each of these components. The percentages are within the following ranges:

- *limonene:* maximum 1.0 per cent;
- *1,8-cineole:* maximum 2.5 per cent;

[...]

- *reporting threshold:* the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

The ratio of ... content to ... content is greater than 1.0.

**Chromatographic profile.** Gas chromatography (2.2.28): use the normalisation procedure.

*Test solution.* Dissolve 200 µL of the substance to be examined in 1.0 mL of *hexane R*.

*Reference solution.* To 1.0 mL of *hexane R*, add 20 µL of *linalol R*, 20 µL of *estragole R*, 20 µL of *α-terpineol R*, 60 µL of *anethole R* and 30 µL of *anisaldehyde R*.

[...]

*Elution order:* order indicated in the composition of the reference solution; record the retention times of these substances.

*System suitability:* reference solution:

- *resolution:* minimum 1.5 between the peaks due to estragole and α-terpineol.

Using the retention times determined from the chromatogram obtained with the reference solution, locate the components of the reference solution in the chromatogram obtained with the test solution and locate *cis*-anethole and foeniculin using the chromatogram shown in Figure 2108.-1 (disregard any peak due to hexane).

Determine the percentage content of these components. The percentages are within the following ranges:

- *linalol:* 0.2 per cent to 2.5 per cent,
- *estragole:* 0.5 per cent to 6.0 per cent,
- *α-terpineol:* maximum 0.3 per cent,
- *cis-anethole:* 0.1 per cent to 0.5 per cent,
- *trans-anethole:* 86 per cent to 93 per cent,

- *anisaldehyde*: 0.1 per cent to 0.5 per cent,
- *foeniculin*: 0.1 per cent to 3.0 per cent.

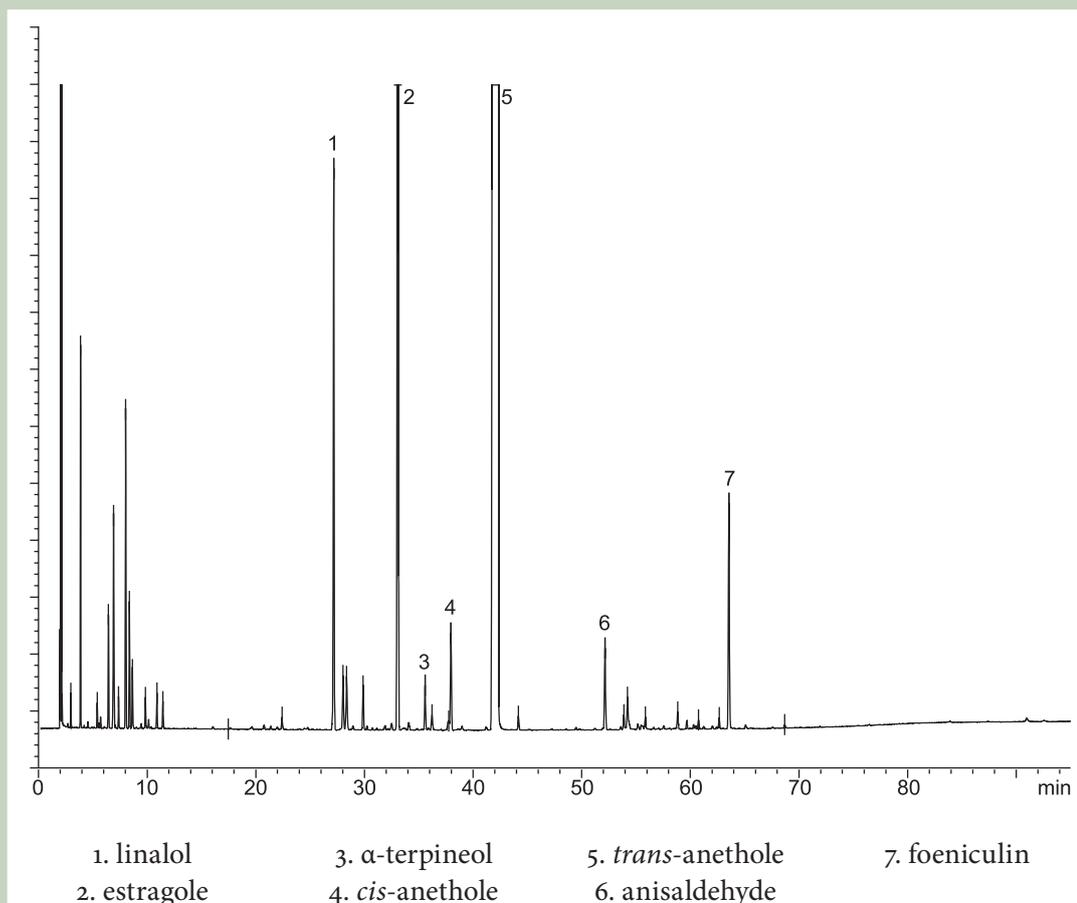


Figure 2108.-1. – Chromatogram for the test for chromatographic profile of star anise oil

*Star anise oil (01/2008:2108)*

**Chiral purity.** Gas chromatography (2.2.28).

[...]

*Elution order:* (*R*)-linalol, (*S*)-linalol, (-)-borneol, (*R*)-linalyl acetate, (*S*)-linalyl acetate; depending on the operating conditions and the state of the column, (-)-borneol may be eluted before or after (*S*)-linalol.

*System suitability:* reference solution:

- *resolution:* minimum 5.5 between the peaks due to (*R*)-linalol and (*S*)-linalol; minimum 2.9 between the peaks due to (*S*)-linalol and (-)-borneol; minimum 2.0 between the peaks due to (*R*)-linalyl acetate and (*S*)-linalyl acetate.

Calculate the percentage content of the specified (*S*)-enantiomers using the following expression:

$$\frac{A_S}{A_S + A_R} \times 100$$

$A_S$  = area of the peak due to the corresponding (*S*)-enantiomer;

$A_R$  = area of the peak due to the corresponding (*R*)-enantiomer.

*Limits:*

- (S)-linalol: maximum 12 per cent;
- (S)-linalyl acetate: maximum 1 per cent.

*Lavender oil (07/2018:1338)***III.B.6. Assay**

Usually no assay procedure is included.

**III.B.7. Storage**

The requirements of the general monograph on *Essential oils (2098)* apply. Where applicable, additional specific conditions are given in the individual monograph.

**STORAGE**

At a temperature not exceeding 25 °C./In an inert container and at a temperature not exceeding 25 °C./Protected from heat.

**III.B.8. Labelling**

The requirements of the general monograph *Essential oils (2098)* apply. Specific additional labelling requirements may be given in the individual monograph, for example, when different types are covered by one monograph, this is indicated in the Labelling section.

**LABELLING**

The label states that the content is Spanish type or Moroccan and Tunisian type.

*Rosemary oil (01/2008:1846)*

### III.C. Prepared herbal drugs

See the monographs *Belladonna, prepared* (0222), *Ipecacuanha, prepared* (0093), *Opium, prepared* (1840), *Stramonium, prepared* (0247).

Prepared herbal drugs are adjusted to a defined content of one or more constituents with known therapeutic activity. This is achieved by adjustment of the prepared herbal drug with inert excipients or by blending different batches of the herbal drug.

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

#### III.C.1. Definition

Reference is made to the monograph on the herbal drug from which the prepared drug is produced.

*Ipecacuanha root* (0094) powdered (180) (2.9.12) and adjusted, if necessary, by the addition of powdered lactose or ipecacuanha root powder with a lower alkaloidal content.

*Content:* 1.9 per cent to 2.1 per cent of total alkaloids, expressed as emetine ( $C_{29}H_{40}N_2O_4$ ;  $M_r$  480.7) (dried drug).

*Ipecacuanha, prepared* (04/2016:0093)

#### III.C.2. Tests

**Foreign matter.** No test is included as the test is already performed on the herbal drug as such.

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

#### III.C.3. Assay

The analytical procedure in the monograph of the prepared herbal drug is usually the same as the one in the monograph for the corresponding herbal drug.

### III.D. Herbal teas

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

### III.E. **Instant herbal teas**

Instant herbal teas are covered by the general monograph *Instant herbal teas* (2620).



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