3

Pharmacognostic Methods for Analysis of Herbal Drugs, According to European Pharmacopoeia

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1. Introduction

Plants had been used for medical purpose long before recorded history.

At the present time, according with the WHO reports, about 80 % of the world's population use herbal medicines for some aspects of their primary health care. This phenomena is in relationship with a rapidly expanding of the phytopharmaceutical industry and market, especialy for dietary supplements. Unfortunately, these supplements are insufficiently studied and have a low quality. For this reason, today, the tendency is to militate for to decrease the number of supplements and to increase the number of herbal medicinal products, which are more rigorously analyzed before marketing authorization and after that, according to European Medicines Agency guidelines.

European Pharmacopoeia is the official book about the quality of medicines, recognized in Europe. It was inaugurated in 1964 through the Convention on the elaboration of a European pharmacopoeia, under the auspices of Council of Europe. The current seventh edition became effective on the 1st January 2011.

This chapter presents the quality specification and specific methods (pharmacognostic methods) for analysis of herbal drugs, according to European Pharmacopoeia.

2. Herbal drug: Definition, nomenclature, types (classification)

2.1 Definition

According to European Pharmacopoeia (EP), a herbal drug is mainly a whole, fragmented, or a cut plant, part of a plant, algae, fungi or lichen, in an unprocessed state, usually in dried form but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal drugs.

The herbal drug may be used in therapy, due to its content of active principle. Active principle means an organic compound or a mixture of organic compounds, which are present in a herbal drug and has a specific pharmacological activity.

2.2 Nomenclature

Herbal drugs are defined by the botanical scientific name, according to the binominal system. The first word defines genus and/or species and / or variety, but sometimes organoleptic characteristics, processing status s.a. The second word defines the type of vegetal drug (botanical organ). Some examples are included in table 1.

Herbal drug	**Botanical origin**
Althaeae folium	*Althaea officinalis* L.
Belladonnae folium	*Atropa belladonna* L.
Echinaceae angustifoliae radix Echinaceae pallidae radix	*Echinacea angustifolia* D.C. *Echinacea pallida* Nutt.
Foeniculi dulcis fructus	*Foeniculum vulgare* Miller sp. *vulgare* var. *dulce*
Myrtilli fructus recens Myrtilli fructus siccum	*Vaccinium myrtillus* L.

Table 1. Nomenclature for herbal drugs.

2.3 Types of herbal drugs

European Pharmacopoeia contains more than 120 specific monographs about herbal drugs.

A vegetal drug which have a plant origin may consist of subteran organs (radix, rhizoma, tubera, bulbus), bark (cortex) or aerial organs (folium, flos, fructus, pseudofructus, pericarpium, semen, seminis tegumentum, herba). List of monographs is included in table 2.

This chapter discusses only about these ,,classic" vegetal drugs. Other herbal drugs (like lichens, algae, resins, volatile oils s.a) are not discussed in this chapter.

The grade of fragmentation point of view in pharmacognostic analysis are used the following types of herbal drugs: in toto, concissum, pulveratum. The grade of pulverisation is defined in EP chapter 2.1.4.

3. Pharmacognostic analysis

An adequate methodology must be used for to analyse a vegetal raw material. We will call this methodology as ,,pharmacognostic analysis".

Herbal drug	**Herbal drug**	**Herbal drug**
Agni casti fructus	Frangulae cortex	Polygalae radix
Agrimoniae herba	Fraxini folium	Polygoni avicularis herba
Alchemillae herba	Fumariae herba	Pruni africanae cortex
Althaeae folium	Gentianae radix	Psylli semen
Altaeae radix	Ginkgonis folium	Quercus cortex
Angelicae radix	Ginseng radix	Ratanhiae radix
Anisi fructus	Graminis rhizoma	Rhamni purshianae cortex
Anisi stelati fructus	Hamamelidis folium	Rhei rhizoma
Arnicae flos	Harpagophyti radix	Rosae pseudo-fructus

Herbal drug	**Herbal drug**	**Herbal drug**
Astragali mongholici radix	Hederae folium	Rosmarini folium
Auranti amari epicarpium et mesocarpium	Hibisci sabdarifae flos	Rusci rhizoma
Aurantii amari flos	Hydrastidis rhizoma	Sabalis serrulatae fructus
Ballotae nigrae herba	Hyperici herba	Salicis cortex
Belladonnae folium	Ipecacuanhae radix	Salviae officinalis folium
Betulae folium	Iuniperi pseudo-fructus	Salviae trilobae folium
Bistortae rhizoma	Lavandulae flos	Sambuci flos
Boldi folium	Leonuri cardiacae herba	Sanguisorbae radix
Calendulae flos	Levistici radix	Schizandrae chinensis fructus
Capsici fructus	Lini semen	Scutellariae baicalensis radix
Carthami flos	Liquiritiae radix	Sennae folium
Centauri herba	Lupuli flos	Sennae acutifoliae fructus
Centellae asiaticae herba	Lythri herba	Sennae angustifoliae fructus
Chamomillae romanicae flos	Malvae silvestris flos	Serpylli herba
Chelidonii herba	Malvae folium	Silybi mariani fructus
Cinchonae cortex	Marrubii herba	Stephaniae tetrandrae radix
Cinnamomi cortex	Matricariae flos	Stramonii folium
Colae semen	Meliloti herba	Tanaceti parthenii herba
Crataegi fructus	Menthae piperitae folium	Thymi herba
Crataegi folium cum flos	Menyanthidis trifoliatae folium	Tiliae flos
Curcumae xanthorrhizae rhizoma	Millefolii herba	Tormentillae rhizoma
Cynarae folium	Myrtilli fructus recens	Trigonellae foenugreci semen
Echinaceae angustifoliae radix	Myrtilli fructus siccum	Urticae folium
Echinaceae pallidae radix	Notoginseng radix	Uvae ursi folium
Echinaceae purpureae herba	Oleae folium	Valerianae radix
Echinaceae purpureae radix	Ononidis radix	Valerianae radix minutata
Eleuterococci radix	Orthosiphonis folium	Verbasci flos
Equiseti herba	Passiflorae herba	Verbenae citriodoratae folium
Ephedrae herba	Pelargonii radix	Verbenae herba
Eucapypti folium	Plantaginis lanceolatae folium	Violae herba cum flore
Fagopyri herba	Plantaginis ovatae semen	Zingiberis rhozoma
Filipendulae ulmariae herba	Plantaginis ovatae seminis tegumentum	Polygalae radix

Table 2. List of EP monographs (for „classic" vegetal drugs)

It comprises qualitative and quantitative tests in order to verify or to establish the identity, purity and quality of a herbal drug.

Identity parameters: macroscopic examination; microscopic examination; qualitative chemical analysis; chromatographic analysis.

Purity parameters: foreign matter.

Quality parameters: loss on drying; soluble-substances; total ash and ash insoluble in hydrochloric acid; heavy metals; swelling index; bitter value; assay of active principles; microbiological examination (bacteria, yeasts and moulds, specified microorganisms); pesticide residues; aflatoxines; ochratoxines.

3.1 Macroscopic examination

This test have in view to determ the morphological characteristics. It gives details concerning the drug aspect, size, colour, odour and taste.

3.1.1 Methodology

Morphological characters and the colour may be examination with the naked eye or by using a magnifyed glass.

The size can be determ by using a ruler or a caliper.

The odour can be determ by shattering the drug between two fingers and smell, or using an extractive solution.

The taste can be determ by putting a piece of drug or an extractive solution in the mouth.

3.1.2 Evaluation of results

There are not general recomandations in EP in order to the macroscopical examination.

The main characteristics which are frequently analyse are included in table 3.

3.1.3 Limits

The morphologic characteristics vary in large limits, due to the vegetal drug.

Organoleptic characteristics may inform about chemical composition: coloured in yellow conduct to flavones, xanthones, carotenoids; coloured in red for anthocyanins; bitter taste for antracenic-derivatives, alkaloids, cardiotonic glycosides.

3.2 Microscopic examination

This test have in view to determ the anatomic characteristics.

3.2.1 Methodology

According to EP chapter 2.8.23. , the microscopic examination of herbal drugs is carried out on the powdered drug (355). Chloral hydrate is the most commonly prescribed reagent.

Herbal drug	**Aspect**	**Size**	**Colour**	**Odour**	**Taste**
Radix, rhizoma, tubera, bulbus	- striated / smooth - shape - decorticated / nondecorticated - harsh / soft - fracture - consistency - tissues' ratio	- length - diameter	external, internal	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Cauli	- branched / unbranched - striated / smooth - hollow or not / pubescent / glabrous	- length - diameter	external surface	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Folium	- pubescent / glabrous - sessile / petiolate - thin / thick / coriaceous - the shape of lamina - the margin, the base, and the top of lamina - venation	- length of petiole and lamina - width of lamina	upper and lower surfaces	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Flos	- isolated flower / inflorescence (type) - bud / mature flower - complete / incomplete - flower formula (description)	- length, width of each component	especially for corola	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Fructus	- type - freshy / dry - shape - epicarp characteristics	- length - width - diameter	- internal and external surfaces	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Semen	- shape - hylum, rafee - ratio between tegument, endosperme and embryo	- length - width - diameter	external surface	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific
Herba	- the position of leaves and flowers on the stem - aspect of stem, leaves and flowers (*see cauli, folium, flos*)	*see cauli, folium, flos*	*see cauli, folium, flos*	*see cauli, folium, flos*	*see cauli, folium, flos*
Cortex	- shape - thin / thick - outer surface (i.e. lenticels, lichens) - striated / smooth - fracture	- length - width	internal and external surfaces	- present / absent - non-specific/ specific	- present / absent - non-specific/ specific

Table 3. Macroscopic characteristics

Other reagents are: lactic reagent, alcoholic solution of phloroglucinol and hydrochloric acid, ruthenium red solution, glycerol.

3.2.2 Evaluation of results

Phloroglucinol is used to identify the presence of lignin, ruthenium red solution is used to show the presence of mucilage, glycerol is used to show the presence of starch and inulin.

In the case of Plantaginis ovatae semen and Plantaginis ovatae semen tegmentum, lactic reagent makes it possible to visualize lignified cells, cutinized membranes and starch.

Common anatomic elements (which are used for to confirm the organ) and specific elements (which are used for to identify the herbal drug) are analyzed (see table 4).

Herbal drug	**Common elements**	**Specific elements**
Radix, rhizoma, tubera, bulbus	large xylem vessels, parenchyma, coak	fibres (celulozic, lignified), starch, calcium oxalate (prisms, cluster-crystals, raphides), sclereids, medullary rays
Cauli	xylem vessels, parenchyma, coak	fibres (celulozic, lignified), starch, calcium oxalate (prisms, cluster-crystals, raphides), sclereids, covering trichomes
Folium	chlorophylian tissue, spiral and annular vessels, stomata	type of stomata, covering- and glandular trichomes, calcium oxalate (prisms, cluster-crystals, raphides), fibres, secretory cells
Flos	papillose cells, pollen grains, endothecium, spiral and annular vessels,	description of pollen grain (size, shape, number of pores, exine aspect), covering- and glandular trichomes, calcium oxalate (prisms, cluster-crystals, raphides), fibres
Fructus	fragments of epicarp, spiral and annular vessels	sclereids, secretory cells, fragment of mesocarp with volatile oil, fatty oil or pigments, fibres, calcium oxalate, covering trichomes
Semen	fragments of endosperma and cotiledone	pigmentary tissue, fibres, starch, mucilaginous cells, aleurone grains, globules of fixed oil
Herba	*see cauli, folium, flos*	*see cauli, folium, flos*
Cortex	parenchyma, coak; xylem vessels are absent	phloem fibres (celulozic, lignified), calcium oxalate (prisms, cluster-crystals, raphides), sclereids, medullary rays

Table 4. Microscopic examination

In EP, a special chapter is named ,,Stomata and Stomatal index" (chapter 2.8.3.).

The Stomatal index is the ratio (expressed as „%") of the number of stomata in a given area of leaf and the number of total epidermal cells (including stomata, trichomes) in the same area of leaf.

3.2.3 Limits

Using stomatal index, it may distinguish *Cassia acutifolia* (stomatal index 10-12.5-15) from *Cassia angustifolia* (stomatal index 14-17.5-20).

3.3 Qualitative chemical analysis

For unknown vegetal product, this test may establish the chemical composition, and for known herbal drugs this test may confirm the presence of a chemical compound (which may be not the main active principle).

3.3.1 Methodology

Because EP is a reference used for the control of herbal drugs, only reactions in an extractive solution apply.

3.3.2 Evaluation of results

EP examples are included in table 5.

Compound	Herbal drug	Reagent	Result - colour
Antracenic derivatives	Frangulae cortex, Rhei rhizoma, Rhamni purshianae cortex, Sennae fructus	Dilute ammonia	a red colour
Tannins	Quercus cortex	vanillin in hydrochloric acid	a red colour
Tropan alkaloids	Belladonnae folium, Stramonii folium	fuming nitric acid + 30 g/L solution of potassium hydroxide in ethanol 96%	a violet colour
Iridoids	Verbasci flos	hydrochloric acid	a greenish-blue color, and after a few minutes, cloudiness and then a blackish precipitate
Sesquiterpens	Millefolii herba	dimethylaminobenzaldehyde	blue or greenish-blue
Cardenolic glycosides	Digitalis purpureae folium	dinitrobenzoic acid + 1M sodium hydroxide	reddish-violet colour

Table 5. Reactions in an extractive solution.

3.4 Qualitative chromatography

Chromatography is a method of separation based on adsorbtion, repartition, ion exchange. It brings supplementary informations about chemical composition.

Chromatographic techniques: TLC, HPLC, GC

3.4.1 Thin Layer Chromatography (TLC)

3.4.1.1 Methodology

Experimental conditions differ depending of chemical compound have to identify. Examples of mobile phases, references substances and reagents used for some active principles and some herbal drugs are included in tabel 6.

Herbal drug	Active principle	Mobile phase	Reference solution	Reagent, examination
Salicis cortex	salicin	water: methanol: ethyl acetate (7.5:10:75, v/v/v)	salicin, chlorogenic acid	R1, daylight
Agrimoniae herba	Flavones (quercitroside, isoquercitroside,hyperoside, rutin)	anhydrous formic acid: water: ethyl acetate (10:10:80, v/v/v)	isoquercitroside, rutin	R2, UV (365 nm)
Carthami flores	yellow and red pigments (including flavones)	acetic acid: anhydrous formic acid: water: ethyl acetate (11:11:27:100, v/v/v/v).	rutin, quercetin	daylight; R2, UV (365 nm)
Malvae silvestris flos	anthocyanins (6″-malonyl malvin, malvin)	acetic acid: water: butanol (15:30:60, v/v/v).	quinaldine red	daylight
Malvae folium	fluorescent compounds (including flavones)	anhydrous formic acid: anhydrous acetic acid: water: ethyl formate: 3-pentanone (4:11:14:20: 50, v/v/v/v/v).	rutin, hyperoside	R2, UV (365 nm)
Althaeae folium	fluorescent compounds (including flavones and polyphenol-carboxylic acids)	acetic acid: anhydrous formic acid: water: ethyl acetate (11:11:27:100, v/v/v/v).	chlorogenic acid, quercitrin	R2, UV (365 nm)
Ephedrae herba	alkaloids (ephedrine)	concentrated ammonia: methanol: methylene chloride (0.5:5:20, v/v/v).	ephedrine, 2-indanamine	R3, daylight
Sennae folium, Sennae fructus (acutifoliae, angustifoliae)	antracenic derivatives (including sennosides)	glacial acetic acid: water: ethyl acetate: propanol (1:30:40:40, v/v/v/v)	senna extract	R4, daylight

Legend: R1 - sulphuric acid; R2- diphenylboric acid aminoethyl ester + macrogol 400; R3 – ninhydrin; R4 - nitric acid + potassium hydroxide.

Table 6. TLC experimental conditions

3.4.1.2 Evaluation of results

Each compound has a characteristic spot, with a definit Rf-value, colour and / or fluorescence.

3.4.1.3 Limits

Most of pharmacopoeia's monographs include TLC as an identification test. Exceptions: Althaeae radix, Graminis rhizoma, Lini semen, Psylli semen.

In some cases, using this technique it may distinguish vegetal sources / herbal drugs, like *Panax sp.*; *Panax ginseng* C. A. Meyer is the vegetal source for Ginseng radix, and *Panax pseudoginseng* Wall. var. *notoginseng* (Burk.) Hoo et Tseng is the source for Notoginseng radix.

TLC may be used as a purity test, too (see section 2.5. Foreign matter).

3.4.2 High Pressure Liquid Chromatography (HPLC)

This technique is used both for identification and for assay.

For example, HPLC technique is used as an identification test in the case of the following herbal drugs: Echinaceae angustifoliae radix, E. pallidae radix, E. purpureae folium, E. purpureae herba.

3.4.3 Gas-chromatography (GC)

This technique is used both for identification and for assay.

Identification by using GC technique is mentioned for the following herbal drugs: Thymi herba, Lavandulae flos, and Sabalis serrulatae fructus.

3.5 Foreign matter

According to EP chapter 2.8.2., foreign matter is material consisting of foreign organs (matter coming from the source plant but not defined as the drug) and / or foreign elements (matter not coming from the source plant and either of vegetable or mineral origin).

3.5.1 Methodology

A macroscopic examination, microscopic examination, reactions or chromatography are used for to identify foreign matters.

A quantitative evaluation may be applied, too.

3.5.2 Evaluation of results

Organoleptic, morphologic, anatomic and chemical characteristics for the sample are compared with the ones are known for ,,pure" herbal drug.

The content of foreign matter is expressed as ,,%, m/m".

3.5.3 Limits

The EP recommendation (monograph ,,Herbal drugs") is that the content of foreign matter is not more than 2%, unless otherwise prescribed or justified and authorized.

Some impurities are limited, and others are excluded. Some examples are included in table 7.

Herbal drug (active principle)	**Foreign matter (active principle)**	**Test**
Papaveris rhoeados flos	maximum 2% of capsules and maximum 1% of other foreign matter	general quantitative evaluation
Sambuci flos	maximum 8%of fragments of coarse pedicels and other foreign matter and maximum 15% of discolored, brown flowers	general quantitative evaluation
Malvae folium	maximum 5% of foreign organs (flowers, fruits and parts of the stem), maximum 5% of leaves with blisters of spores of Puccinia malvacearum and maximum 2% of foreign elements	general quantitative evaluation; microscopic examination of spores
Tiliae flos, from *Tilia cordata* Miller, *Tilia platyphyllos* Scop., *Tilia*×*vulgaris* Heyne (essential oil – sedative)	*Tilia tomentosa* Moench. (essential oil in a lower content)	macroscopic examination
Thymi herba (essential oil)	*Thymus serpyllum* (essential oil; lower content, and different fingerprint)	macroscopic examination
Digitalis purpureae folium (cardenolic glycosides – purpurea glycosides)	Digitalis lanatae folium (cardenolic glycosides – lanatosides; the pharmacokinetic profile is different)	microscopic examination
Bistortae rhizoma (tannins)	*Paris polyphylla* or *Paris quadrifolia* (steroidal saponins; toxic)	microscopic examination
Graminis rhizoma (inulin, triticin)	*Cynodon dactilon*, *Imperata cylindrica* (starch)	microscopic examination, using iodine solution
Plantaginis lanceolatae folium (mucilages)	*Digitalis lanata* L. (cardenolic glycosides)	TLC
Primulae radix (triterpenic saponins – expectorant)	*Vincetoxicum hirundinaria medicus* (vincetoxine – toxic)	TLC
Stephaniae tetrandrae radix (bisbenzylisoquinoline alkaloids)	*Aristolochia fangchi (*aristolochic acids – toxic)	Test for aristolochic acids in herbal drugs – method A (TLC)

Table 7. Foreign matter

3.6 Loss on drying

This parameter is stricken by the humidity of the environment. On the other hand, it may affect the quality of the herbal drugs among the storage. A high content of water may favor the growth of microorganisms (fungi which produce mycotoxins), or may activate enzymatic systems which will generate compounds with a less activity (specific hydrolases may degrade primary cardenolic glycosides to secondary cardenolic glycosides, which have less activity).

3.6.1 Methodology

Usually, the powdered drug is dried in an oven at 105 ^{0}C for 2 h.

When the content of essential oil is high (Carvi fructus, Eucalypti folium, Foeniculi fructus, Iuniperi pseudo-fructus, Menthae piperitae folium, Zingiberis rhizoma, Thymi herba), EP recommendation is to determ the content of water (according to cap 2.2.13) and the content of essential oils (chapter 2.8.12).

3.6.2 Evaluation of results

The result is expressed as ,,%, m/m" or ,,mL/kg".

3.6.3 Limits

Usually, the limits are about 10 – 12% (100 – 120 mL/kg).

Unusual limits (for example maximum 6% for Digitalis purpureae folium, maximum 8% for Lini semen and Syllibi mariani fructus, max. 80 mL/kg for Foeniculi amari fructus and Foeniculi dulcis fructus, and maximum 70 mL/kg for Anisi fructus) are exceptions. These lower limits are in relationship with the stability of the active compounds - cardenolic glycosides (Digitalis purpureae folium), lipids and mucilages (Lini semen), only lipids (the other upper-mentioned vegetal drugs).

In the case of Lini semen, mucilages may favor the growth of microorganisms (fungi which produce mycotoxins), if the content of water is higher.

In Digitalis purpureae folium, water may activate enzymatic systems (specific hydrolases) and so, primary cardenolic glycosides degrade to secondary cardenolic glycosides, which have less activity.

If the vegetal drug with a high content of lipids is stored in a light, hot and wet place, unsaturated fatty acids degrade (peroxidation, polymerization); the lipids are ranciding.

3.7 Soluble substances

This parameter refers to all vegetal compounds which can be extracted with a certain solvent, in certain experimental conditions. When the solvent is water, the parameter calls ,,water-soluble extractive". When another solvent is used, it calls ,,extractable matter".

3.7.1 Methodology

Any general EP method exists. Generally, the powdered drug is extracted with the solvent (definite quantity) under the conditions specified in monograph, the solvent is evaporated, the residue is dried up to fixed mass and finally weighed.

Experimental protocols are included in table 8.

3.7.2 Evaluation of results

The result is expressed as ,,%, m/m".

3.7.3 Limits

The limits vary, due to the vegetal drug (table 8).

Herbal drug	Sieve (µm)	Solvent	Method	Vegetal drug:solvent ratio	Limits
Aurantii amari epicarpium et mesocarpium	250	a mixture of water and ethanol (3:7)	shake, 2 h	1:5	min. 6%
Gentianae radix	710	boiling water	shake, 10 min.	1:40	min. 33%
Graminis rhizoma	355	boiling water	shake, 10 min.	1:40	min. 25%
Lupuli flos	355	ethanol 70 %	heat on a water-bath under a reflux condenser, 10 min.	1:30	min. 25%
Pruni africanae cortex	250	methylene chloride	continuous extraction apparatus (Soxhlet type), 4h	-	min. 0.5%

Table 8. Soluble substances

For a medicinal product development, the results obtained by using a vegetal raw material and different solvents and different experimental parameters help to evaluate the efficiency of the extraction, to establish the proper solvent and the optimum working technology.

3.8 Total ash and ash insoluble in hydrochloric acid

These parameters express the content of metallic ions (mineral compounds) of a vegetal drug, and they are in relationship with the pedoclimatic conditions.

3.8.1 Methodology

Essentially, according to EP general monograph (chapter 2.4.16), for to determ the total ash, the vegetal drug is ignited to constant mass in a muffle furnace at about 600 ^{o}C.

According to EP general monograph (chapter 2.8.1.), for to determ the ash insoluble in hydrochloric acid, the following method apply: the residue from the determination of total ash is boiled with dilute hydrochloric acid, and the solution is filtered through a ashless filter; the filter is dried, is ignited, allow to cool in a desiccator and finally is weighed.

3.8.2 Evaluation of results

The result is expressed as ,,%, m/m".

3.8.3 Limits

Parameter ,,Total ash" is included in all monographs.

Parameter ,,Ash insoluble in hydrochloric acid" is included in most of monographs; exceptions are the following: Agni casti fructus, Agrimoniae herba, Alchemillae herba,

Althaeae radix, Anisi stellati fructus, Arnicae flos, Aurantii amari epicarpium et mesocarpium, Aurantii amari flos, Ballotae nigrae herba, Betulae folium, Boldi folium, Calendulae flos, Capsici fructus,Caryophylli flos, Carvi fructus, Centaurii herba, Centellae asiaticae herba, Chamomillae romanae flos, Chelidonii herba, Cinchonae cortex, Cinnamomi cortex, Coriandri fructus, Crataegi fructus, Crataegi folium cum flore, Cynarae folium, Colae semen, Curcumae xanthorrhizae rhizoma, Echinaceae purpureae herba, Eleutherococci radix, Ephedrae herba, Eucalypti folium, Fagopyri herba, Filipendulae ulmariae herba, Foeniculi amari fructus, Foeniculi dulcis fructus, Frangulae cortex, Fraxini folium, Fumariae herba, Gentianae radix, Ginkgonis folium, Harpagophyti radix, Hederae folium, Hibisci sabdariffae flos, Hyperici herba, Iuniperi pseudo-fructus, Lavandulae flos, Leonuri cardiacae herba, Lini semen, Lythri herba, Lupuli flos, Matricariae flos, Meliloti herba, Melissae folium, Menyanthidis trifoliatae folium, Myrtilli fructus siccus, Myrtilli fructus recens, Oleae folium, Ononidis radix, Orthosiphonis folium, Papaveris rhoeados flos, Passiflorae herba, Plantaginis lanceolatae folium, Plantaginis ovatae semen, Plantaginis ovatae seminis tegumentum, Polygoni avicularis herba, Pruni africanae cortex, Psyllii semen, Quercus cortex, Ratanhiae radix, Rhamni purshianae cortex, Rosae pseudo-fructus, Rosmarini folium, Sabalis serrulatae fructus, Salicis cortex, Salviae officinalis folium, Salviae trilobae folium, Sambuci flos, Schisandrae chinensis fructus, Silybi mariani fructus, Solidaginis virgaureae herba, Tanaceti parthenii herba, Tiliae folium, Tormentillae rhizoma, Trigonellae foenugraeci semen, Uvae ursi folium, Violae herba cum flore, Zingiberis rhizoma.

The limits vary, due to the vegetal drug (for examples see table 9).

Herbal drug	**Total ash**	**Ash insoluble in hydrochloric acid**
Anisi fructus	max. 12%	max. 2.5%
Belladonnae folium	max. 16%	max. 4%
Bistortae rhizoma	max. 9%	max.1%
Carthami flos	max. 10%	max. 3%
Echinaceae angustifoliae radix	max. 9%	max. 3%
Echinaceae pallidae radix	max. 7%	max. 2%
Echinaceae purpureae herba	max. 12%	-
Echinaceae purpureae radix	max. 9%	max. 2%
Ephedrae herba	max. 9%	max. 3%
Equiseti herba	12 - 27%	3 - 15%
Malvae folium	max. 17%	max. 3%
Rhei radix	max. 12%	max. 2%
Urticae folium	max. 20%	max. 4%
Verbasci flos	max. 6%	max. 2%

Table 9. Total ash and ash insoluble in hydrochloric acid (examples)

3.9 Heavy metals

This parameter espress the pollution.

3.9.1 Methodology

Atomic absorption spectrometry is used. This is described in EP general chapter 2.4.27.

3.9.2 Evaluation of results

The limits of suitability are given as a maximum value expressed as units ppm.

3.9.3 Limits

In monograph „Herbal drugs", the following limits are mentioned: cadmium – max. 1.0 ppm; lead – max. 5.0 ppm; mercury – max. 0.1 ppm.

Other limits for cadmium are mentioned in monographs for Fumariae herba (max. 1.5 ppm), Lini semen (max. 0.5 ppm), Salicis cortex (max. 2.0 ppm) and Tormentillae rhizoma (max. 2.0 ppm).

3.10 Swelling index

The swelling index is the volume (expressed in milliliters) occupied by 1 gram of an herbal drug and the adhering mucilage, after it has swollen in an aqueous liquid.

It expressed a high content of mucilage in an herbal drug.

3.10.1 Methodology

A general EP method is described in chapter 2.8.4. Generally, 1 gram of herbal drug (the degree of comminution prescribed in the monograph) is placed in a 25 mL ground-glass stoppered cylinder graduated and then is moistened with alcohol. Add 25 mL water, close the cylinder and shake it for 1 h, with a standard frequency. Allow to stand 3 h. Finally, note the volume occupied by the drug and the adhering mucilage.

3.10.2 Evaluation of results

The result is given by the mean of the 3 tests.

3.10.3 Limits

The limits vary, due to the vegetal drug (table 10).

Herbal drug	**Swelling index**
Althaeae radix	Min. 10
Althaeae folium	Min. 12
Malvae folium	Min. 7
Malvae sylvestris flos	Min. 15
Verbasci flos	Min. 9
Violae herba cum flores	Min. 9
Lini semen	Min. 4
Plantaginis ovatae semen	Min. 9
Plantaginis ovatae seminis tegumentum	Min. 40
Psyllii semen	Min. 10
Trigonellae foenugraeci semen	Min. 6

Table 10. Swelling index

3.11 Bitterness value

The bitterness value is the reciprocal of the maximum dilution of a vegetal drug that still has a bitter taste. It is determined by comparison with quinine hydrochloride, the bitterness value of which is set at 200000.

It expressed a high content of bitter-compounds in an herbal drug, active principles which are used to stimulate the appetite. But, attention: not all vegetal substances with bitter taste are used to stimulate the appetite (e.g. cardenolic glycosides, antracenic derivatives and alkaloids).

3.11.1 Evaluation of results

Depending to the dilutions of the reference substance (quinine hydrochloride) and of the test solution, expressions for correction factor and for bitterness value are described in the monograph.

The result is given by the mean of all 6 tests.

3.11.2 Limits

The acceptance limits vary, due to the vegetal drug (table 11).

Herbal drug	Bitterness value
Centauri herba	min. 2000
Gentianae radix	min. 10000
Menyanthidis trifoliatae folium	min. 3000

Table 11. Bitterness value

3.12 Colouring intensity

This parameter expresses the content of pigments (yellow and /or red pigments).

3.12.1 Methodology

Spectral methods apply. These methods consist of the measuring the absorbance at a specific wavelength for a solution having a definit concentration.

3.12.2 Evaluation of results

The absorbance is recorded using a suitable spectrophotometer.

3.12.3 Limits

Specific limits of admisibility are mentioned in monographs. The details are mentioned in tabel 12.

3.13 Assay

In the case of herbal drugs with constituents of known therapeutic activity or with active markers, assays of their content are applied. When the constituents responsible for the therapeutic activity are unknown assays of analytical markers are required.

Herbal drug	Protocol	Limits (absorbance)
Carthami flos	Yellow pigment: macerate 0.1 g of the powdered drug (355) in 150 mL of water, stir for 1 h, filter through a sintered-glass filter (40) and dilute to 500.0 mL; record the absorbance at 401 nm.	min. 0.40
	Red pigment: to 0.25 g of the powdered drug (355) add 50 mL of a mixture of 20 volumes of water and 80 volumes of acetone; heat on a water-bath at 50 °C for 90 min.; allow to cool, filter through a sintered-glass filter (40) and dilute to 100.0 mL; record the absorbance at 518 nm.	min. 0.40
Hibisci sabdariffae flos	To 1.0 g of the powdered drug (355) add 25 mL of boiling water and heat for 15 min on a water-bath with frequent shaking. Filter the hot mixture into a 50 mL graduated flask; after cooling, dilute to 50 mL with water. Dilute 5 mL of this solution to 50 mL with water. Record the absorbance at 520 nm using water as the compensation liquid.	min. 0.350 for the whole drug; min. 0.250 for the cut drug.
Papaveris rhoeados flos	To 1.0 g of the powdered drug (355) add 100 mL of ethanol 30% (V/V) and macerate for 4 h with frequent stirring; filter and discard the first 10 mL; to 10 mL of the filtrate add 2 mL of hydrochloric acid and dilute to 100 mL with ethanol 30%; allow to stand for 10 min. record the absorbance at 523 nm using ethanol 30% as the compensation liquid	min. 0.6

Table 12. Colouring intensity

Titrimetric, spectrofotometric or chromatographic methods are described in EP monographs.

3.13.1 Titrimetric methods

Titrimetry consists in determining the number of moles of reagent (titrant), required to react quantitatively with the substance being determined.

3.13.1.1 Methodology

The standard technique involves the addition of a controlled volume of a reagent-solution (titrant) to a known volume of a sample solution. In some cases, an exces of reagent is added and the excess is measured by back titration.

Various methods are available for end-point determination: spectrophotometry, potentiometry, amperometry, conductometry etc. The potentiometric end-point determination is the most widely used. In this method, the end-point of the titration is

determined by following the variation of the potential difference between two electrodes immersed in a sample solution as function of the quantity of the titrant solution added.

Other times, visual indicators may be used, too.

3.13.1.2 Evaluation of results

The content of active or analitycal marker is calculated by having in view the stoechiometry of the titration reaction.

3.13.1.3 Limits

Specific limits of admisibility are mentioned in monographs. The details are mentioned in tabel 13.

Herbal drug	Protocol	Limits
Belladonnae folium	Back titration: 0.01M sulfuric acid + 0.02M sodium hydroxide; end-point-methyl red	min. 0.30% of total alkaloids, expressed as hyoscyamine
Stramonii folium		min. 0.25% of total alkaloids, expressed as hyoscyamine
Fumariae herba	standard titration: 0.02M perchloric acid; potentiometric end-point determination	min. 0.40% of total alkaloids, expressed as protopine
Hibisci sabdariffae flos	standard titration: 0.1M sodium hydroxide; potentiometric end-point determination	min.13.5% of acids, expressed as citric acid

Table 13. Assay - titrimetric methods

3.13.2 Spectrophotometric methods

Spectrophotometric analysis is based on the measurement of radiation intensity as a function of wavelength.

These methods may be used because the active / analytical marker or its derivatives has a definite UV or VIS spectrum.

3.13.2.1 Methodology

Specific spectrophotometric methods are described in monographs. Usually, the following parameters vary: the degree of comminution of the herbal drug, solvent for extraction, methodology for obtaining the sample and the reference solutions, coloring reagent, wavelength for detection. Some details are mentioned in tabel 14.

A general method for determination of tannins in herbal drugs is described in EP chapter 2.8.14. This method consists of the following steps: the herbal drug is extracted with water on a water-bath; total polyphenols are determined in this solution, using a spectrophotometric method; shake the solution with hide powder, filter and assay the polyphenols not adsorbed by the hide powder in the filtrate, using the same spectrophotometric method.

Herbal drug	Reagent	Detection (wavelength)	Evaluation of results
Cinchonae cortex	-	316 nm and 348 nm	alkaloids, expressed as quinine-type alkaloids; comparing with reference solutions (quinine, cinchonine)
Millefolii herba	-	608 nm	proazulenes, expressed as chamazulene; A= 23.8
Hyperici herba	-	590 nm	total hypericins, expressed as hypericin; A = 870
Myrtilli fructus recens	-	528 nm	anthocyanins, expressed as cyanidin 3-O-glucoside chloride (chrysanthemin); A = 718
Rosmarini folium	hydrochloric acid + (sodium nitrite + sodium molybdate) + sodium hydroxide	505 nm	total hydroxycinnamic derivatives, expressed as rosmarinic acid; A = 400
Ballotae nigrae herba, Plantaginis lanceolatae folium		525 nm	total ortho-dihydroxycinnamic acid derivatives, expressed as acteoside; A = 185
Fraxini folium		525 nm	total hydroxycinnamic acid derivatives, expressed as chlorogenic acid; A = 188
Passiflorae herba	boric acid + oxalic acid	401 nm	total flavonoids, expressed as vitexin; A = 628
Violae herba cum flore		405 nm	flavonoids, expressed as violanthin; A = 400
Crataegi folium cum flore		410 nm	total flavonoids, expressed as hyperoside; A = 405
Curcumae xanthorrhizae rhizoma		530 nm	dicinnamoyl methane derivatives, expressed as curcumin; A = 2350
Carthami flos	aluminium chloride	420 nm	total flavonoids, expressed as hyperoside; A = 400
Betulae folium, Calendulae flos, Leonuri cardiacae herba, Polygoni avicularis herba, Solidaginis herba, Solidaginis virgaureae herba	aluminium chloride + sodium acetate	425 nm	total flavonoids, expressed as hyperoside; A = 500
Equiseti herba, Sambuci flos		425 nm	flavonoids, expressed as isoquercitroside; A = 500
Auranti amari flos	magnesium + hydrochloric acid	530 nm	total flavonoids, expressed as naringin; A = 52
Crataegi fructus	hydrochloric acid	545 nm	procyanidins, expressed as cyanidin chloride; A = 75
Frangulae cortex	magnesium acetate	515 nm	glucofrangulins, expressed as glucofrangulin; A = 204
Rhamni purshianae cortex			hydroxyanthracene glycosides, expressed as cascaroside A; A = 180
Rhei rhizoma			hydroxyanthracene derivatives,

Herbal drug	Reagent	Detection (wavelength)	Evaluation of results
			expressed as rhein; A = 468
Sennae folium, Sennae fructus acutifoliae, Sennae fructus angustifoliae			hydroxyanthracene glycosides, expressed as sennoside B; A = 240
Digitalis purpureae folium	dinitrobenzoic acid + sodium hydroxide	540 nm	cardenolic glycosides, expressed as digitoxin; comparing with reference solution (digitoxin)
Chelidoni herba	chromotropic acid, sodium salt	570 nm	total alkaloids, expressed as chelidonine; A = 933
Rosae pseudo-fructus	dinitrophenylhydrazine + sulfuric acid	520 nm	ascorbic acid; comparing with reference solution (ascorbic acid)
Agrimoniae herba, Bistortae rhizoma, Hammamelidis folium, Lythri herba, Quercus cortex, Ratanhiae radix, Sanguisorbae radix, Tormentillae rhizoma	phosphomolybdotungstic reagent + sodium carbonate (general method, chapter 2.8.14)	760 nm	tannins, expressed as pyrogallol; comparing with reference solution (pyrogallol)

Table 14. Assay - spectrophotometric methods

3.13.2.2 Evaluation of results

The content of active or analitycal marker is calculated by using the specific absorbance or by comparing with the reference solution.

In the case of the method described in chapter 2.8.14., the formula for the content of tannins have in view the diference between the value for total polyphenols and the value for polyphenols not adsorbed by the hide powder.

3.13.2.3 Limits

Specific acceptance limits are mentioned in monographs.

3.13.3 High-Performance Liquid Chromatography (HPLC)

Is a chromatographic technique that is used to separate, identify, quantify and purify the individual components of the mixture, due to a different migration of the compounds through the column (solid stationary phase).

In the case of herbal drugs, the separations are based upon partition mechanisms using chemically modified silica as the stationary phase and polar solvents as the mobile phase.

3.13.3.1 Methodology

The apparatus consists of a pumping system (which must deliver the mobile phase at a constant flow rate), an injector (which can operate at high pressure and is capable to release an exact volume of solutions), a proper chromatographic column (eventually having a

temperature controller), a detector (commonly a ultraviolet / visible spectrophotometer) and a data aquisition system.

Usually, the following parameters vary: column characteristics (type, dimensions, particle size), qualitative and quantitative composition of the mobile phase, method of separation (isocratic flow / gradient elution), the gradient, characteristics of the sample and reference solutions, using an external or an internal standard, flow rate, injection volumes, run time, wavelength for detection. Some details are mentioned in tabel 15.

3.13.3.2 Evaluation of results

When an extern standard is used, the content of active or analitycal marker is calculated by comparing the response of the sample with the response of the reference.

Herbal drug	**Marker**	**Method of separation**	**Type of standard**
Uvae ursi folium	arbutin	isocratic flow	external standard (arbutin)
Salicis cortex	total salicylic derivatives, expressed as salicin	isocratic flow	external standard (salicin + picein)
Cynarae folium	Chlorogenic acid	gradient elution	external standard (chlorogenic acid)
Urticae folium	caffeoylmalic acid + chlorogenic acid, expressed as chlorogenic acid	gradient elution	external standard (chlorogenic acid)
Echinaceae purpureae herba, Echinaceae purpureae radix	caftaric acid + cichoric acid	gradient elution	external standard (chlorogenic acid + caffeic acid)
Echinaceae palidae radix, Echinaceae angustifoliae radix	echinacoside	gradient elution	external standard (chlorogenic acid + caffeic acid)
Meliloti herba	coumarin	isocratic flow	external standard (coumarin)
Fagopyri herba	rutin	gradient elution	external standard (rutin)
Ginko folium	flavonoids, expressed as flavone glycosides	gradient elution	external standard (quercetol)
Matricariae flos	apigenin-7-glucozide	isocratic flow	external standard (apigenin-7-glucoside)
Scutellariae baicalensis radix	baicalin	gradient elution	external standard (baicalin + methyl parahydroxybenzoat)
Silybi mariani fructus	silymarin, expressed as silibinin	gradient elution	external standard (milk thistle standardised dry extract)
Harpagophyti radix	harpagoside	isocratic flow	external standard (harpagoside)
Marrubii herba	marubin	gradient elution	external standard (marubin)
Oleae folium	oleuropein	gradient elution	external standard (oleuropein)
Agnus casti fructus	casticin	gradient elution	external standard (casticin)
Valerianae radix	sesquiterpenic acids, expressed as valerenic	gradient elution	external standard (valerian dry extract)

Herbal drug	**Marker**	**Method of separation**	**Type of standard**
	acid		
Arnicae flores	total sesquiterpene lactones, expressed as dihydrohelenalin tiglate	gradient elution	internal standard (santonin)
Tanaceti parthenii herba	parthenolide	isocratic flow	external standard (parthenolide)
Rusci rhizoma	sapogenins, expressed as ruscogenins	gradient elution	external standard (ruscogenins)
Centelae asiaticae herba	total triterpenoid derivatives, expressed as asiaticoside	gradient elution	external standard asiaticoside
Hederae folium	hederacoside	gradient elution	external standard (ivy leaf standardized tincture)
Liquiritiae radix	glycyrrhizic acid	isocratic flow	external standard (monoammonium glycyrrhizate)
Ginseng radix	ginsenoside Rg1 + ginsenoside Rb1	gradient elution	external standard (ginsenoside Rg1 + ginsenoside Rb1 + ginsenoside Re+ ginsenoside Rf)
Notoginseng radix	ginsenoside Rg1 + ginsenoside Rb1	gradient elution	external standard (ginsenoside Rg1 + ginsenoside Rb1 + ginsenoside Rf)
Eleutheroccoci radix	eleutheroside B + eleutheroside E	isocratic flow	external standard (ferulic acid)
Astragali mongholici radix	astragaloside IV	gradient elution	external standard (astragaloside IV)
Boldi folium	total alkaloids, expressed as boldine	isocratic flow	external standard (boldine)
Hydrastidis rhizoma	hydrastine, berberine	isocratic flow	external standard (hydrastine hydrochloride + berberine chloride)
Stephaniae tetrandrae radix	tetrandrine + fangchinoline, expressed as tetrandrine	isocratic flow	external standard (tetrandrine)
Colae semen	caffeine	isocratic flow	external standard (caffeine + theobromine)
Ephedrae herba	ephedrine	isocratic flow	external standard (ephedrine hydrochloride + terbutaline sulfate)
Capsici fructus	total capsaicinoids, expressed as capsaicin	isocratic flow	external standard (capsaicin + nonivamide)
Orthosiphonis folium	sinensetin	isocratic flow	external standard (sinensetin)
Schisandrae chinensis fructus	schisandrin	gradient elution	external standard (schisandrin)

Table 15. Assay - HPLC methods

When an internal standard is used, the content of this standard must have in view.

3.13.3.3 Limits

Specific limits of admisibility are mentioned in monographs.

3.13.4 Gas Chromatography (GC)

Is a chromatographic technique that is used to separate, identify, quantify and purify the volatile components (or volatile derivatives of the components) of the mixture, due to a different migration of the species through a solid or a liquid stationary phase.

3.13.4.1 Methodology

The apparatus consists of an injector, a chromatographic column (which is included in an oven), a detector (commonly a flame-ionisation detector) and a data acquisition system.

Usually, the following parameters vary: the type and the characteristics of the stationary phase, the carrier gas, method of separation (normalisation / derivatisation), temperature (for column, injection port, detector), characteristics of the sample and reference solutions, flow rate, injection volumes, split ratio, run time. Some details are mentioned in tabel 16.

3.13.4.2 Evaluation of results

The content of active or analitycal marker is calculated by comparing the response of the sample with the response of the reference. The results is expressed as a minim value (%) in the essential oil.

3.13.4.3 Limits

Specific limits of admisibility are mentioned in monographs.

Herbal drug	Marker	Method of separation	Type of standard
Anisi stelati fructus	Trans-anethole	normalisation procedure	external standard (estragole + α-terpineol + anethole)
Foeniculi amari fructus	Anethole, fenchone	normalisation procedure	external standard (anethole + fenchone)
Foeniculi dulcis fructus	Anethole	normalisation procedure	external standard (anethole)
Thymi herba, Origani herba	Thymol + carvacrol	normalisation procedure	external standard (thymol + carvacrol)
Sabalis serrulatae fructus	Total fatty acids	derivatisation procedure, using trimethylsulfonium hydroxide	internal standard (methyl margarate + methyl pelargonate)

Table 16. Assay - GC methods

3.13.5 Determination of essential oils in herbal drugs

A general method for extraction and assay of essential oils in herbal drugs is described in EP chapter 2.8.12.

3.13.5.1 Methodology

Essentially, the determination is carried out by steam distilation in a special apparatus in the conditions described in chapter 2.8.12. The distilate is collected in the graduated tube, using (usually) xylene to take up the essential oil, and the aquueous phase is automatically returned to the distillation flask. The mass of herbal drug used for extraction, the type and the volume of solvent, distilation rate and distilation time vary, and so that these parameters are mentioned in specific monographs.

3.13.5.2 Evaluation of results

The volume of liquid collected in the graduated tube is readed, the volume of xylene is substracted and so the volume of essential oil is obtained. The result is expressed as „mL/ kg".

3.13.5.3 Limits

Specific limits of admisibility are mentioned in monographs.

The content of essential oil is mentioned in the following monographs: Angelicae radix, Anisi fructus, Anisi stelati fructus, Auranti amari epicarpium et mesocarpium, Aurantii amari flos, Carvi fructus, Caryophylli flos, Chamomillae romanicae flos, Cinnamomi cortex, Coriandri fructus, Curcumae xanthorrhizae rhizoma, Eucapypti folium, Filipendulae ulmariae herba, Foeniculi amari fructus, Foeniculi dulcis fructus, Iuniperi pseudo-fructus, Lavandulae flos, Levistici radix, Matricariae flos, Menthae piperitae folium, Millefolii herba, Origani herba, Rosmarini folium, Salviae officinalis folium, Salviae trilobae folium, Serpylli herba, Thymi herba, Valerianae radix, Valerianae radix minutata, Verbenae citriodoratae folium, Verbenae herba, Zingiberis rhizoma.

3.14 Pesticide residues

According to EP, chapter 2.8.13, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of herbal drugs. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops, either before or after harvest, to protect the commodity from deterioration during storage and transport.

EP chapter refers especially to the organochlorine, organophosphorus and pyrethroid insecticides.

3.14.1 Methodology

A general procedure is described in EP, but this is only for information.

It consists of the following three steps: extraction the pesticides; purification (using size-exclusion chromatography); quantitative analysis (examine by gas-chromatography, using carbophenothion as the internal standard).

3.14.2 Evaluation of results

The content of an insecticide is calculated from the peak area and the concentrations of the solutions. Lists of relative retention times for main organophosphorus insecticides, and

the organochlorine and pyrethroid insecticides respectively, are attached in the monograph.

3.14.3 Limits

The limits are expressed as ,,mg/ kg". A list containing 69 pesticides is presented in the EP chapter. For other substances, the limits are calculated using an expression which have in view acceptable daily intake, body mass and daily dose of the herbal drug.

3.15 Microbial contamination

The presence of micro-organisms may reduce or inactivate the therapeutic activity of the herbal drug, and implicitly of the pharmaceutical product.

This parameter refers to the total aerobic microbial count (TAMC), total combined yeasts / moulds count (TYMC) and specific micro-organisms (e.g. Escherichia coli).

According to monograph ,,Herbal drugs", it is a compulsory test.

3.15.1 Methodology

Microbial analysis is performed according to specific microbiologic methods. The following methods are discussed in EP (chapters 2.6.12.) for TAMC and TYMC: membrane filtration method and plate-count methods (including pour-plate method, surface-spread method and most-probable-number method).

In chapter 2.6.13. are described tests which allow determination of the absence or limited occurrence of specified micro-organisms (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella sp., Candida albicans, Clostridia, Bile-tolerant gram-negative bacteria).

3.15.2 Evaluation of results

The limits of suitability are given as a maximum value of units CFU.

3.15.3 Limits

The general chapters 5.1.4 ,,Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use" and 5.1.8 ,,Microbiological quality of herbal medicinal products for oral use" not include limits for herbal substances. So, acceptance limits should be set in relation to the specific herbal substance and subsequent processing. Reduction of the microbial count at level of herbal substance (e.g. geographical origin, appropriate harvest/collection and drying procedures, treatment with water vapour) should be taken into account when setting the limits.

For herbal medicinal products for oral use, containing herbal drugs, intended for the preparation of infusions and decoctions using boiling water (e.g. herbal teas) the limits are: TAMC - max. 10^7CFU/g; TYMC – max. 10^5 CFU/g; Escherichia coli – max. 10^5 CFU/g; Salmonella sp.- absence/25g.

3.16 Determination of aflatoxins

Aflatoxins are naturally occurring mycotoxins that are produced by many species of Aspergillus (a fungus). Aflatoxins are toxic and among the most carcinogenic substances known. Aflatoxin-producing members of Aspergillus are common and widespread in nature. They can colonize and contaminate grain before harvest or during storage. Host crops are particularly susceptible to infection by Aspergillus following prolonged exposure to a high humidity environment, or damage from stressful conditions such as drought, a condition which lowers the barrier to entry. The native habitat of Aspergillus is in soil, decaying vegetation, hay and grains undergoing microbiological deterioration and it invades all types of organic substrates whenever conditions are favorable for its growth. Favorable conditions include high moisture content (at least 7%) and high temperature. The toxin can also be found in the milk of animals which are fed contaminated feed.

3.16.1 Methodology

A specific liquid chromatographic method, using an isocratic flow, fluorescence detection and post-column derivatisations apply. An immunoaffinity column containing antibodies against aflatoxin B_1 is used for to obtain the test solution. The method is described in EP general chapter 2.8.18. It is cited as an example of a method that has been shown to be suitable for devil's claw root, ginger and senna pods.

3.16.2 Evaluation of results

The limits of suitability are given as a maximum value expressed as ,,ng/g", or ,, µg/kg" .

3.16.3 Limits

The EP requires a limit of not more than 2 µg/kg of aflatoxin B_1 and a limit of 4 µg/kg for the sum of aflatoxins B_1, B_2, G_1 and G_2.

3.17 Determination of ochratoxin A

Ochratoxins are a group of mycotoxins produced by some Aspergillus species and Penicillium species including Aspergillus ochraceus and Penicillium viridicatum. Ochratoxin A is the most prevalent and relevant fungal toxin of this group, while ochratoxins B and C are of lesser importance. Ochratoxin A is known to occur in commodities such as cereals, coffee, dried fruit and red wine. It is nephrotoxic and nephrocarcinogenic.

3.17.1 Methodology

A specific liquid chromatographic method, using a gradient elution and a fluorescence detection apply. An immunoaffinity column containing antibodies against aflatoxin B_1 is used for to obtain the test solution. The method is described in EP general chapter 2.8.22. It is suitable for Liquiritiae radix. The EP recommendation is that the suitability of this method for other herbal drugs must be demonstrated or another validated method used.

3.17.2 Evaluation of results

The limits of suitability are given as a maximum value expressed as ng/g.

3.17.3 Limits

In the case of Liquiritiae radix, the acceptance limit of maximum 20 μg/kg is required.

4. Conclusion

A herbal drug is a particular and a complex raw material. Its analysis involves specific pharmacognostic methods, which can be undertaken from European Pharmacopoeia or must be developed by the scientist.

Owing to the complexity of all above-mentioned aspects in studying the medicinal plants (herbal drugs), pharmacists, biologists, chemists and biochemists must co-operate.

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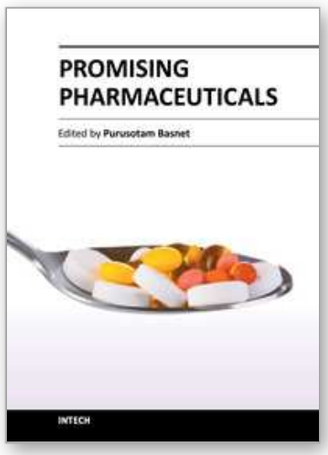

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From the dawn of civilization, humans have been dreaming of happy, healthy and long-life. Our life expectancy is twice longer than 100 years ago. We know more about the diseases. Therefore we have developed new drugs to fight against them. The demand for drugs was so high that we developed Pharma industries. Although Pharma industries took responsibility of producing the needed drugs and gave us a quality of life, misuse of drugs brought further complication. Therefore, discovery, production, distribution, and the phase of administration of patients' quality assurance has to be controlled with a technological procedure and tight regulations to make the system as effective as possible for the benefit of human health. Our book provides selected but vital information on the sources, tools, technologies and regulations regarding the current status of medicine development.

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