Quality of Herbal Drugs and Their Preparations: Critical Criteria and Management

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INTRODUCTION

PhytoLab (PL) is a service laboratory and is part of the nature network of the MB-Group in Vestenbergsgreuth. PL has comprehensive experience of herbal products and is specialised in product development, analysis of herbal starting materials and their preparations as well as regulatory affairs concerning herbal medicinal products. PL compiles marketing authorisation procedure dossiers, with functions which include project co-ordination, method development and validation, stability testing, biopharmaceutical investigations, organisation of pre-clinical and clinical studies and writing of expert reports on efficacy, safety and quality of herbal products. PL has over 150 skilled employees, including 30 scientists with degrees in Pharmacy, Chemistry, Food Chemistry, Biology or Medicine.

Herbal Drugs and Herbal Drug Preparations, Definitions

Herbal drugs are defined in the European Pharmacopoeia (Ph.Eur.) as whole, fragmented, cut or powdered plants or plant parts usually in dried form or sometimes fresh. They are defined by their botanical scientific name, their genus, species, variety and author and have a complex composition which varies depending on habitat, climate zone, annual variations and other endogen and exogen factors.

Herbal drug preparations are extracts, tinctures, essential oils, distillates, resins, etc. Their composition not only depends on the quality of the drugs used in their preparation, but also on the method of manufacture, solvent kind and concentration, extraction time and temperature among other factors.

The Active Principle of Herbal Drugs and Herbal Drug Preparations

The active principle of herbal medicinal products is the herbal drug itself or its preparation as a whole whether or not constituents with therapeutic activity or contributing to it are known. The Ph.Eur. distinguishes between the following types of extracts:

- Standardised extracts are adjusted within an acceptable tolerance to a given content of constituents with known therapeutic activity. Standardisation is achieved by adjustment of the genuine extract with inert material or by blending batches of extracts with different composition.
- Quantified extracts are adjusted to a defined range of constituents; adjustments are made by blending different batches of extracts.
- Other extracts are essentially defined by their production process (e.g. state of the herbal drug to be extracted, solvent, extraction conditions) and their specifications.

Variability of Constituents of Herbal Drugs and Their Preparations

A few years ago, there was a serious shortage of St. John's Wort. At that time the drug was purchased world wide. But very soon considerable variability of characteristic markers was apparent in the drugs offered for sale. Given the variation of the constituents, it was suggested that the drugs could not be considered as pharmaceutically and therapeutically equivalent. A TLC (Fig. 1) shows that in the samples 4 to 7 the characteristic zone of Rutosid was lacking. In the samples 2 Chinese St. John's Wort and 4 *H. maculatum* Hypericin (RF=0.50) and/or Pseudohypericin (RF=0.45) were missing.

Herbal Drugs from Collection and Cultivation

A point to be considered regarding cultivation and collection is the fact that wild plants may have different pattern of constituents compared to their cultivated counterparts. The seeds used in agricultural production frequently come from breeding aiming at an increase of therapeutically active constituents in the drug. A comparative TLC of the *Camomile* breed “Mabamille” with *Camomile* from Egypt and Bulgaria shows that the tetraploid “Mabamille” is a chemical race of *Camomile* with an increased quantity of the anti-inflammatory Bisabolol (Fig. 2).

The Role of Marker Substances in the Evaluation of Herbal Drugs

The prerequisite for an herbal medicinal product with reliable efficacy is a starting material with a precisely defined identity. It is not to be acceptable, for example, to use alternatively the 5 different species of *Crataegus* described in the Monograph “Hawthorn leaf with flowers” of the Ph.Eur. The TLC prepared by M. Schüssler (1992) shows that the species *C. monogyna*, *C. laevigata*, and the species *C. pentagyna*, *C. nigra* and *C. azarolus* have a very different pattern of constituents. Given the variation of marker substances shown in the chromatogram (Fig. 3) one would not expect that all 5 species would have the same therapeutic effect.

Adulterations of Plantain from Collection

In 1997 the Food and Drug Administration (FDA) in the US discovered an adulteration of plantain (*Plantago lanceolata*) with foxglove (*Digitalis lanata*). The adulterated plantain had its origin in Macedonia and came from collection at that time. The leaves of the two herbal drugs look very similar, though the build of the plants was completely different. Herbalists collected the plantain, dried it under undefined conditions and brought the dried material to collecting points. From there it was delivered to the customer. The complete dried material supplied was assembled under one batch number. Random samples were taken and blended to produce a representative homogeneous laboratory sample for quality control. The statistical sampling described was not able to detect the adulteration caused somewhere in the product chain.

Detection of the Plantain Adulteration with Visual Test

Adulteration of plantain with foxglove is extremely rare. It can easily be detected under the microscope. Ribwort plantain shows stomata of the diacytic type whereas *Digitalis* has stomata of the anomocytic type (Fig. 4). But only representative sampling and a large quantity of material inspected will make it possible to detect the deviating stomata in a nonhomogeneous adulterated sample.

Detection of the Plantain Adulteration with TLC test

The Ph.Eur. (edition 4.4) monograph *Ribwort Plantain* makes a note on potential adulteration of plantain with *Digitalis*. The TLC identity test for plantain refers to Aucubin and Acteosid detected in the UV light at 254 nm (Fig. 5). When evaluating the chromatogram of plantain using UV light of 365 nm a blue fluorescent zone of a *Digitalis* glycoside is visible. The limit of detection is 2% *Digitalis* leaves in plantain leaves (sample 2).

Adulteration of Chinese Star Anise with Japanese Star Anise

At the end of the year 2000 an adulteration of Chinese star anise with Japanese star anise occurred. The distinction is aggravated by the fact that other *Illicium* species such as *I. cambodianum* are used as spices and may further adulterate *I. verum*. A visual distinction of the different species is not possible.

Detection of Chinese Star Anise Adulteration with TLC Test

Chromatographic methods used for the distinction of *Illicium verum* from other *I.* species do not rely on a test for the toxic Sesquiterpencilactones Anisatin and Neoanisatin

present with 0,02% in the essential oil of *Illicium anisatum*. The reference standards of these characteristic toxic markers are not commercially available. All tests actually refer to other markers present in the essential oil. For this reason chromatographic tests incur the risk of false negative results. The TLC test presented (Fig. 6) allows the detection of 2% *Illicium anisatum* in *Illicium verum* provided that the orange zone $RF=0.7$ of the chromatogram is present in the adulterant. An additional GC test is required to exclude false negative results.

Detection of Chinese Star Anise Adulteration with GC Test

Chinese Star anise oil contains 80-90% trans-Anethole and 1-10% Limonene whereas Japanese star anise oil contains up to 10% Methyleugenol, 7% Safrol and 4% Myristicin. The presence of Myristicin is considered to be indicative for Japanese star anise (Fig. 7). A differentiation of *I.* species using these criteria allows the detection of about 2% adulteration of *Illicium verum* with other *Illicium* species. But since a specific test to exclude *I. anisatum* is desirable PL has started to produce Anisatin and Neoanisatin reference standards in order to offer them commercially.

TCM in the German Drug Codex (DAC)

The consumption of Traditional Chinese Medicine (TCM) drugs in Europe has increased considerably since 1990. The drugs are imported in small quantities, consequently a comprehensive quality control is too costly. The German Medicines Codex (DAC) has established minimum criteria to control for the identity and purity of TCM herbs.

Potential Adulteration with Aristolochic Acid

The DAC has listed 12 TCM herbs known to be potentially adulterated with *Aristolochia*. The adulteration is partly due to drug parts of *Aristolochia* with similar appearance. But confusion is mainly caused by the very similar names of different drugs, for example, Mutong, Chuan Mutong and Guan Mutong or Mu Fangji and Guang Fangji. A test for aristolochic acid is mandatory for the 12 listed drugs. Tests are also recommended for other TCM drugs.

Detection of Aristolochic Acid with LC-MS-MS

A very selective and specific detection and quantification of aristolochic acid can be achieved with LC-MS-MS. Using a triple quadrupole allows the selective separation of the precursor ion of aristolochic acid with the mass number 359 which is its ammonium adduct. After fragmentation a main product with the mass number 296 results which can be specifically quantified (Fig. 8). Depending on the plant matrix and on the type of herbal preparation 2-4 ppm aristolochic acid can be quantified in solid forms. For liquid forms the method is more sensitive by a factor of 10.

The Influence of Good Agricultural Practice (GAP) on Herbal Drug Quality

Cultivation of herbal drug plants requires intensive care and management. The soil of the cultivation site should contain appropriate amounts of nitrogen, phosphorus, potassium, minerals, organic matter and other elements to ensure optimal plant growth. The handling of these matters can be achieved, for example, by a Global Positioning System (GPS) based field inventory established by performing soil investigations utilizing a soil scanner and a vertical penetrometer.

The Effect of Nitrogen on Plant Development

Nitrogen, as all fertilising agents, should be applied sparingly and in accordance with the needs of the particular plant species. In the case of *Cynara* (artichoke) the production of leaves is increased with increased nitrogen content of the soil (Fig. 9). But this does not mean that nitrogen fertilisers can be applied in excess. Elevated nitrogen content of the soil has a detrimental effect on the formation of Caffeoyl China Acid

derivatives considered as active substances in artichoke leaf (Fig. 10). For this reason why a soil analysis is absolutely recommended when scheduling an artichoke cultivation project.

Heavy Metals in Herbal Drugs

The need for soil analysis also applies to cultures of cadmium accumulating plants, as the results will give information on the suitability of the site for the cultivation project. A list of cadmium accumulating plants with a 90% percentile (Table 1) of more than 0.5 mg and more than 1 mg cadmium per kg drug is presented.

Heavy Metals in TCM Plants

The DAC has established that TCM drugs must be tested for heavy metals. The scope of this test is determined in the Ph.Eur. The general monograph “Heavy Metals in Herbal Drugs” 2.4.27 (European Pharmacopoeia Supplement, 2003). provides test methods for the metals As, Cd, Cu, Fe, Hg, Ni, Pb and Zn but no maximum limits are given. Only the monograph *Fucus* of the Ph.Eur. (European Pharmacopoeia Supplement, 2004) indicates such limits. For other herbal drugs the limits of a draft of the German directive on contaminants, dating from 1991, are applicable (Kontaminantenempfehlung Schwermetalle, 1991). Many of the TCM drugs and some of the European drugs (Table 2) do not meet these requirements. Acceptable limits of lead and cadmium should be clarified when legislators accept the proposals of the German Pharmaceutical Manufacturers Association (BAH). However, the levels of mercury in some Traditional Chinese Medicine (TCM) herb preparations were found to be extraordinary high exceeding even the BAH standards and require corrective measures. The results can only be explained by admixtures of cinnabar (mercury sulphide) or calomel.

The Future Situation of Heavy Metals

When legislators accept the limits proposed by the BAH for heavy metals mentioned in the Ph.Eur. our current problems identifying acceptable levels of lead and cadmium levels in herbal drugs may be solved. However, new problems may arise with regard to the limits provided for As, Cu, Hg and Ni, particularly for TCM drugs. The limits proposed by the German Pharmaceutical Manufacturers Association (BAH) are based on experience with herbal drugs traditionally used in Europe (Table 3). New problems may arise because Traditional Chinese Medicinal (TCM) herbs come from non-European source and there is lack of experience in diverse non-European source drugs. Fe and Zn are nutritive elements therefore need from my point of view no restrictive limits.

Plant Protection

The GAP guidelines require that any chemicals used in the growth or protection of medicinal plants should only be used when no alternative measures are available. Approved plant protection products should be applied at the minimum effective level, in accordance with the regulations of the grower and the end-user countries. Residues of such products require intensive control. The Ph.Eur. provides a test for 34 substances or substance groups. PL tests herbal drugs for about 151 plant protection substances. The number of substances monitored increased in July of 2003 to 247. Pesticide databases compiled by members of various associations give information on the occurrence of pesticides in 711 herbal drugs and show that 86 of the mentioned pesticides are detected in herbal drugs.

Herbal Drugs and Critical Pesticide Contamination

A list of plants with frequently positive pesticide results and a list of pesticides frequently present in plants stresses the importance of pesticide monitoring (Table 4). Certain plants, like ginseng root, permanently exceed the limits set for HCH isomers, Hexachlorbenzole, Lindane, Quintozene and Tecnazen and can only be used after a decontamination by extraction.

Harvest of Plants to Be Used for Herbal Drug Production

The concentration of active constituents in herbal drugs varies with the stage of plant development (e.g. Hyperforin in St. John's Wort). This is why medicinal plants should be harvested during the appropriate time period. The harvest should be effected under the best possible conditions, avoiding dew, rain or high humidity. Contact with soil should be reduced to a minimum in order to minimise microbial contamination of harvested plant material. The material must be immediately taken to an indoor drying facility to expedite drying so that any deleterious effect due to increased moisture levels is prevented.

Impact of the Drying Process on Quality

The method and temperature used for drying may have a considerable impact on quality parameters of the plant material. Shade drying is preferred to maintain leaf color. Low temperature needs to be employed in the case of plant material containing volatile or thermolabile substances. The CCS-derivatives in artichoke e.g. are very sensitive to heat. Using a drying temperature of 45°C yields degradation of CCS-derivatives by 40%. This is the reason why artichoke leaves should be dried at low temperature.

Microbiological Aspects of the Drying Process

The moisture content of the plant material should be kept as low as possible after harvesting in order to reduce damage by moulds and other microbial infestation. Fresh plants can be dried in a number of ways: in the open air shaded from direct sun light, placed in thin layers on drying frames, in well ventilated wire screened rooms, or in drying ovens/rooms.

Studies concerning the influence of drying temperature on the microbiological count of celandine herb show that very low or very high temperatures could be applied to obtain low microbiological counts. But high temperature may yield degradation of essential drug constituents. Drying at low temperature requires intensive ventilation of the plant material in order to remove moisture as quickly as possible and to avoid promotion of growth of micro-organisms, especially of mycotoxin forming moulds.

Mycotoxins, a Neglected Problem?

The discovery of aflatoxins in the early 1960s (Blount, 1961) and the recognition that aflatoxins and other mycotoxins can cause major illness in humans and animals was the beginning of a huge effort to research mycotoxin contamination of foods and feeds. Mould growth is favored by high humidity. Many moulds grow at room temperature. The optimum temperature is around 25-30°C e.g. for *Fusarium* sp. forming fumonisin, zearalenone and trichothecenes. *Aspergillus* sp. and *Penicillium* sp. forming aflatoxins, patulin and ochratoxin grow well at 35-37°C. As herbs are generally contaminated with spores of fungi during harvest, temperature and moisture content play an important role during the storage of herbal drugs and mould growth.

Mycotoxins in Herbal Drugs

Our knowledge of the occurrence of mycotoxins in herbal drugs is rather restricted. This limited knowledge calls for a systematic screening of mycotoxin incidence. It is obvious that herbal drugs considered for a long time as quite safe may contain mycotoxins e.g. dried *Wine* fruits, *Liquorice* root, *Linden* flowers. This point requires special attention in the coming years.

Desinfestation and Storage of Herbal Drugs

The integrity of herbal drugs must be kept during storage. Fresh medicinal plant materials should be stored between 10°C and 5°C, while frozen products should be stored below -20°C. Fumigation against pest attack should be carried out only when necessary and only with registered chemical agents authorised by the regulatory authorities. The use of carbon dioxide for desinfestation is considered state of the art. When saturated steam is

used for pest control or reduction of microbial counts, the humidity of the material must be controlled after treatment to avoid that microbial growth starts again.

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Tables

Table 1. Herbal drugs with high cadmium contents (number of samples investigated).

90% percentiles 0.51-1.00 mg/kg	90% percentiles > 1.01 mg/kg
Radix Angelicae (49)	Herba Hyperici (496)
Folia Betulae (245)	Fucus vesiculosus (63)
Herba Alchemillae (109)	Cortex salicis (120)
Herba Solidaginis virg. (19)	
Radix Taraxaci cum herba (50)	
Herba Taraxaci (161)	
Flores Stoechados (7)	
Herba Violae tricoloris (47)	

Table 2. Heavy metals in herbal drugs (ppm).

Herbal Drug	Lead (ppm)	Cadmium (ppm)	Mercury (ppm)
Official Limit (BAH Proposal)	5 (10)	0.2 (1)	0.1 (0.1)
Alismatis Rhizoma	0.3	0.8	<u>0.15</u>
Angelicae dahuricae Radix	0.35	0.04	<u>0.22</u>
Angelicae pubescentis rad.	0.62	<u>0.95</u>	<u>0.05</u>
Benincasae Semen	0.49	0.01	<u>0.37</u>
Buddlejae Flos	<u>8.29</u>	0.01	<u>0.32</u>
Ecliptae Herba	<u>7.69</u>	0.19	<u>0.16</u>
Epimedii Herba	<u>7.31</u>	0.12	<u>0.3</u>
Foeniculi fructus	0.02	<u>0.4</u>	0.01
Gentianae radix	1.1	<u>1.06</u>	0.1
Lonicera Flos	<u>12.0</u>	0.05	<u>8.47</u>
Lycii Cortex	<u>7.99</u>	0.04	<u>0.39</u>
Lysimachiae Herba	<u>5.97</u>	0.19	<u>1.11</u>
Mori Folium	<u>8.32</u>	0.53	<u>0.66</u>
Persicae semen	0.3	<u>0.3</u>	0.1
Poria	0.17	0.02	<u>0.29</u>
Schizonepetae Folium	<u>7.47</u>	0.09	<u>0.27</u>

Table 3. Heavy metals in Traditional Chinese Medicine (TCM) finished products (C.T. Yap, National University of Singapore, 1987).

Heavy Metal	Official Limit (ppm)	Limit (BAH) Proposed (ppm)	n	Found in TCM	
				Mean (ppm)	Max. (ppm)
As	0.2	5.0	16	6.2	34.6
Cd		1.0		-	-
Cu		40.0	22	24.9	111.0
Fe	0.1	inapplicable, essential nutrient!			
Hg		0.1	9	1.6	792.0
Ni		10.0	18	5.5	17.2
Pb	5.0	10.0	17	7.0	56.0
Zn		inapplicable essential nutrient!			

Table 4. Pesticides in herbal drugs.

Critical plants (number of residues):	Most frequent residues (number of plants):
Ginseng root (21)	Chlordane, total (26)
Ginkgo leaves (11)	Chlorpyrifos (-ethyl) (52)
Alexandrine Senna leaves (12)	Chlorpyrifos-methyl (22)
Chinese Galangal (10)	Cypermethrin (24)
Camomile flowers (40)	DDT (215)
Citrus pericarp (35)	Dichlorvos (40)
Orange leaves (16)	Dicofol (29)
Orange flowers (30)	Dithiocarbamate (35)
Orange pericarp sweet (43)	Endosulfan (104)
Cola nuts (8)	Endrin (23)
Red Sorrel calyces (12)	Fenitrothion (22)
Fennel seed (23)	HCH-isomers without Lindane (115)
St. John's Wort herb (30)	Heptachlor & Heptachlor epoxide (31)
Peppermint leaves (37)	Hexachlorbenzole (56)
Spear Mint leaves (16)	Lindane (168)
Lemon Balm leaves (15)	Malathion & Malaoxon (45)
Linden flowers (18)	Parathion-methyl & Paraoxon-methyl (22)
East Indian Kidney tee (18)	Pentachloraniline (24)
Rose flowers (14)	Pentachloranisole (25)
Cinnamon bark (7)	Pirimiphos-methyl (53)
Black tea (19)	Quintozone (45)

Figures

Fig. 1. Variability of constituents of St. John's Wort. Dependency on origin and species. 1. Rutosid (RF=0.05), Hyperoside (RF=0.20), 2. *H. perforatum* origin China, 3. *H. perforatum*, 4. *H. maculatum*, 5. *H. inodorum*, 6. *H. elegans*, 7. *H. montanum*, 8. *H. species* (PhytoLab).

Fig. 2. Variability of constituents of *Camomile* from collection and cultivation. 1. collection Bulgaria, 2. collection Egypt, 3. cultivation Germany (Mabamille) (PhytoLab).

Fig. 3. Markers of various Hawthorn species, PhEur. 1. *C. laevigata*, 2. *C. monogyna*, 3. *C. pentagyna*, 4. *C. nigra*, 5. *C. azarolus*, 6. Vitexin derivatives, 7. Hyperoside, 8. Chlorogenic acid. Schüssler, Hölzl, DAZ (1992).

Fig. 4. Microscopic purity test of plantain. Stomata on the leaves of 1. Plantain and 2. Foxglove.

Fig. 5. Adulteration of plantain (P) with *Digitalis lanata* (D). 1. Plantain (P), 2. P + 2% D, 3. P + 5% D, 4. P + 10% D, 5. P + 20% D, 6. *Digitalis* (D), 7. Reference substance aucubin.

Fig. 6. Adulteration of star anise with shikimi fruits. 1. Reference substances Rutin, chlorogenic acid, caffeic acid; 2. Chinese star anise *I. verum*; 7. Japanese star anise shikimi fruits, *I. anisatum*; 3-6. Blend of *I. verum* and *I. anisatum*. Limit of detection 2% *I. religiosum*.

Star anise

Shikimi

Fig. 7. Adulteration of star anise with shikimi fruits.

Fig. 8. Detection of aristolochic acid by LC-MS-MS. Selective and specific test on aristolochic acid by LC-MS-MS.

Herbal Drug
Produced kg/ha

Fig. 9. Correlation between nitrogen contents and leaf growth (*Cynara*). Eich, Baier, Grün, Wagenbreth, Zimmermann, 2001.

Fig. 10. Influence of nitrogen fertiliser on the CCS contents of artichoke leaves. Eich, Baier, Wagenbreth, 1998.