Safety in Laboratory and Regulatory Services for Herbal Products
Heavy metals, mycotoxins and pesticides in herbal drugs and extracts

Dr. Michael Schwarz PhytoLab GmbH & Co. KG
Overview

- Introduction
  (Quality of medicinal products, European Pharmacopoeia; General monographs)

- Heavy metals
  (Occurrence, Limits, Study on content in herbal drugs, Other regulations)

- Mycotoxins
  (Occurrence, General monographs, Study results, GACP influence on ochratoxin A)

- Pesticides
  (General monographs, Ph. Eur. 2.8.13, EC 396/2005, Pesticide database, Allocation list, Scope of testing, Variety of methods)

- Risk management
  (Difficulties with herbal products, Quality factors, Contaminant References, Sampling)

- Other contaminants
  (Radioactivity, Anthraquinone, PAH)
Introduction

Safe usage of medicinal products is guaranteed through detailed information on quality, efficacy and safety.

Herbal medicinal products - in contrast to synthetic drugs – consist of a highly complex mixture of compounds.

Despite extensive research, only for very few plants there is a link between individual constituents and the efficacy of the product.
Introduction

A uniform basis to judge **efficacy and safety** was formed in Germany in 1982 by establishment of the **Commission E**.

Since 2006, the EMA develops – based on existing registrations - herbal monographs containing information on **production, efficacy and safety**.

These **Community Monographs** replace the Commission E monographs and form the basis for harmonisation in Europe.
Introduction

**Quality** of medicinal products is laid down in the *European Pharmacopoeia (Ph.Eur.)* or in national Pharmacopoeias.

The Pharmacopoeia describe the parameters relevant for the quality of herbal drugs and herbal drug preparations in monographs. They Pharmacopoeias always define the quality parameters based **on the latest scientific knowledge.**

The Ph.Eur. orientates itself on the Community Monographs ("the Pharmacopoeia follows the authorisation") but can set higher quality standards than originally formulated.

The Ph.Eur. focuses on **quality parameters of the starting materials**, not on finished products. Prevents **usage of bad quality raw materials**.
Latest member: Ukraine
Latest observer: Azerbaijan
Purpose of the Ph.Eur.

The common regulations on the quality of medicinal products and starting materials

- facilitate the free movement of medicinal products in Europe;
- ensure the quality of medicinal products and their components imported into or exported from Europe.

The monographs and texts of the Ph.Eur. are created in such a way that they satisfy the requirements of

- regulatory authorities;
- those engaged in the quality control of medicinal products and their constituents;
- manufacturers of starting materials and medicinal products
Where do you find information on herbal drugs and herbal drug preparations?
Ph.Eur.

Listed under General monographs are a variety of „Herbal Monographs“:

Essential oils, Herbal drug extracts (new in Ph. Eur 8.5 as of July 1st, 2015), Herbal drug preparations, Herbal drugs, Herbal teas, Herbal teas, instant, Vegetable fatty oils.

These general monographs were created to cover products and also quality parameters, which are not mentioned in the individual monographs (no need to repeat, easier to update). Therefore it is vital to apply the individual monograph always in combination with the relevent general monograph.
Ph.Eur., Monograph Herbal drugs

The monograph on HERBAL DRUGS is designed the same way as a regular drug monograph. It contains binding requirements for all herbal drugs. These may exceed the requirements valid for the individual herbal drug (but individual monographs can also contain additional, or more strict, or less strict parameters):

<table>
<thead>
<tr>
<th>DEFINITION:</th>
<th>terminology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRODUCTION:</td>
<td>good agricultural practice (GACP, EtO prohibition)</td>
</tr>
<tr>
<td>IDENTIFICATION:</td>
<td>macroscopic, microscopic, and others (e.g. TLC)</td>
</tr>
<tr>
<td>TESTS:</td>
<td>additional tests are required, not mentioned in the individual monographs (pesticides, heavy metals, aflatoxin B₁, ochratoxin A, radioactive contamination).</td>
</tr>
<tr>
<td>ASSAY:</td>
<td>„… appropriate method.“</td>
</tr>
<tr>
<td>STORAGE:</td>
<td>„Protected from light.“</td>
</tr>
</tbody>
</table>
Heavy Metals

- Occurrence and Origin
- Limits in the General Monograph
- Evolution of limits in the regulations
- Method requirements
- Exceptions for extracts
- Specific limits in individual monographs
- Variety of analytical methods
- Other relevant regulations
Heavy metals - origin

The elements lead, cadmium and mercury are natural compounds of the earth's surface and are taken up by plants from the soil.

In addition to this, the elements mentioned play a major role in technical processes and applications and, as anthropogenic contaminants, find their way into air, water and soil, and from there also into foodstuffs.

Common sources are from mining and industrial wastes; vehicle emissions; lead-acid batteries; fertilisers; paints; treated woods; aging water supply infrastructure.

Lead is the most prevalent toxic heavy metal contaminant. As a component of tetra-ethyl lead it was used extensively in gasoline.

Some plants accumulate heavy metals more than others.
General monograph on Herbal Drugs

Heavy metals (2.4.27). Unless otherwise stated in an individual monograph or unless otherwise justified and authorised:

- cadmium: maximum 1.0 ppm;
- lead: maximum 5.0 ppm;
- mercury: maximum 0.1 ppm.

Where necessary, limits for other heavy metals may be required.
Setting the limits…

- Maximum permitted levels of heavy metals have only been specified in the Ph.Eur. Monographs „Kelp“ (Pb, Cd, Hg, As) and „Linseed oil“ (Cd).
- The „Draft of a Recommendation on Heavy Metals in Herbal Drugs“ sets limits for **Lead, Cadmium and Mercury** for pharmaceutical herbs (applied by German authorities).
- Data yielding of a heavy metal screening (more than 12.000 samples tested) show that the recommended limits are not generally feasible (*Heavy metals in Herbal Drugs*, *The European Journal of Herbal Medicine, Vol 4, Issue 1, 1998*).
Setting the limits...

The publication „Heavy Metals in Herbal Drugs“ (Dr. Lothar Kabelitz, The European Journal of Herbal Medicine, Vol.4, Issue 1, 1998) presents the results from the investigation of over 12,000 samples tested for lead, cadmium and mercury.

This evaluation has been updated with datas from 6 companies (Salus-Haus, Martin Bauer, Kneipp Werke, Finzelberg, Frutarom, Wala Heilmittel) considering samples analysed in 2002 to 2005.
Study evaluation – from results to limits

Suggestions for maximum levels of heavy metals in Herbal drugs could be formulated on the basis of analytical results. If an appropriate amount of data is available, the individual establishment of a maximum level for heavy metals of medicinal or herbal drugs on the basis of a 90th percentile appears to be legitimate in view of the limited availability of many herbal drugs.
Feasible maximum limits of cadmium in herbal drugs

<table>
<thead>
<tr>
<th>Herb</th>
<th>90th perc. in mg/kg</th>
<th>90th perc. in mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birch leaves</td>
<td>0.7</td>
<td>Fumitory</td>
</tr>
<tr>
<td>Goldenrod</td>
<td>0.9</td>
<td>St. John’s wort</td>
</tr>
<tr>
<td>Kava Kava rhizome</td>
<td>0.6</td>
<td>Dandelion root and herb</td>
</tr>
<tr>
<td>Dandelion herb</td>
<td>0.7</td>
<td>Lungwort</td>
</tr>
<tr>
<td>Mallow herb</td>
<td>1.2</td>
<td>Helichrysum flower</td>
</tr>
<tr>
<td>Spinach leaves</td>
<td>0.9</td>
<td>Wild pansy</td>
</tr>
<tr>
<td>Fucus</td>
<td>1.0</td>
<td>Tormentil</td>
</tr>
<tr>
<td>Willow bark</td>
<td>1.8</td>
<td>Citronella grass</td>
</tr>
</tbody>
</table>
## Various regulatory limits in the past.

<table>
<thead>
<tr>
<th>Heavy Metal mg/kg</th>
<th>Pb</th>
<th>Cd</th>
<th>Hg</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Ministry Health (1991)(^{(1)})</td>
<td>5</td>
<td>0.2</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Ph. Eur. Monograph Fucus(^{(1)})</td>
<td>5</td>
<td>4</td>
<td>0.1</td>
<td>90</td>
</tr>
<tr>
<td>Ph. Eur. Monograph Linseed(^{(1)})</td>
<td></td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAH (2002)(^{(1)})</td>
<td>10</td>
<td>1</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>(EC) No 1881/2006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh herbs (^{(2)})</td>
<td></td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Vegetables (^{(2)})</td>
<td>0.3/0.1</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food supplements (drafted) (^{(2)})</td>
<td>1.0</td>
<td>0.50</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>German national regulation (RHmV)(^{(1)})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Tea like Products</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>
# Limits of detection AAS – ICP-MS

<table>
<thead>
<tr>
<th>Elements</th>
<th>AAS mg/kg</th>
<th>ICP-MS mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>Arsen</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Copper</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.10</td>
<td>0.006</td>
</tr>
<tr>
<td>Thallium</td>
<td>0.08</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Heavy metals – needs and exceptions

- need for feasible, harmonised maximum limits
- need for alternative method: ICP-MS
- Heavy metals are normally not extracted into extract and remain in herbal drug (e.g. infusion, decocta, alcoholic extracts), therefore the general monograph on Herbal Drug Extracts allows:
Monograph on Herbal Drug Extracts

applicable comply with the requirements of any relevant monograph in the European Pharmacopoeia. Where justified, herbal drugs used for the production of extracts may exceed the limits for heavy metals specified in the monograph Herbal drugs (1433) provided that the resulting extract satisfies the requirements for heavy metals (see Tests).

pesticide residues (2.8.13) in the extracts may be necessary. Where a test for heavy metals is carried out, the same limits for heavy metals as those given in the monograph Herbal drugs (1433) are applicable to extracts unless otherwise stated in an individual extract monograph or unless otherwise justified and authorised.
Heavy metals in Ph. Eur.

- Ph. Eur. Monograph 2.4.27 provides test methods for 7 heavy metals (Pb, Cd, Hg, As, Cu, Fe, Ni), but specifies no limits
- Limits for Pb (5.0 ppm), Cd (1.0 ppm), Hg (0.1 ppm) described in general „Herbal Drugs“ monographs
- Higher limit for Cd set in monographs for willow bark (2.0 ppm), tormentil (2.0 ppm), kelp (4.0 ppm) and fumitory (1.5 ppm)
- Lower limit for Cd set in monograph on lin seed (0.5 ppm)
- Kelp: Limit for As (90 ppm); Pb and Hg mentioned but identical
- A variety of methods is mentioned: AAS, ICP-AES, ICP-MS; deviations allowed, if validation requirements are met and SST fulfilled
- Validation criteria are described, e.g. LOQ < limit of specification
Other relevant regulations

• ICH Q3D:
  • contains harmonized limits for a total of 24 elements
  • not applicable for herbal products
  • limits based on calculation of PDE (permitted daily exposure) in contrast to limits in raw material as applied by Ph. Eur.

• EC regulation 1881/2006:
  • contains limits for Pb and Cd in e.g. vegetables, fresh herbs, fruits as well as milk, fish, meat…
  • contains limits for Hg only for fish and seafood
Mycotoxins

- Significance and occurrence
- Information from the general monographs
- Methods for mycotoxins in Ph. Eur.
- Limits in general and individual monographs
- Implications in practice
- Moulds forming mycotoxins
- Commission regulation 1881/2006
- Monitoring results
- Risk management and importance of GACP
Significance and occurrence of mycotoxins

Metabolic products of moulds, characteristic of each species, for the most part heat-stable

Acute toxicity: damage to liver, kidneys, nervous system, skin, mucous membranes, immune systems

Chronic toxicity: may provoke cancer, may cause congenital abnormalities and malformations in the embryo

UN Food and Agriculture Organisation (FAO):
- 25% of the world production of foodstuffs
- 20% of the cereal harvest in the EU is contaminated with mycotoxins.
Mycotoxins – General monographs

Vegetable fatty oils:

... REFINING

The objective of refining is to remove impurities and contaminants of the oil with the least possible damage to the triglycerides and with minimal loss of oil. The contents of the following substances are reduced:

... — pigments such as gossypol (in cottonseed oil) or mycotoxins such as aflatoxin (mainly in arachis seeds);

Allergen products

... Moulds. Biologically active contaminants such as mycotoxins in moulds must be minimised and any presence justified. Appropriate measures have to be implemented to avoid contamination by foreign mould strains.

...
Mycotoxins – General monographs

Herbal Drugs:

... Where necessary, herbal drugs comply with other tests, such as the following, for example.

... Aflatoxin B$_1$, where necessary, limits for aflatoxins may be required.

Ochratoxin A, where necessary, limits for ochratoxin A may be required.

Extracts:

... Where applicable, as a result of analysis of the herbal drug or animal matter used for production and in view of the production process, tests for microbiological quality, heavy metals, aflatoxins, (ochratoxin A) and pesticide residues in the extracts may be necessary.

Herbal Drugs for Homoeopathic Preparations:

... Aflatoxins, limits for aflatoxins may be required.

...
Determination of aflatoxin B$_1$ in herbal drugs (Ph.Eur. 2.8.18.)

Detailed description of the method.

„Aflatoxin B$_1$ is considered the most toxic.“

„Herbal drugs that are subject to contamination by aflatoxin B$_1$ are tested by a validated method.“

„The method described is cited as an example of a method that has been shown to be suitable for devil’s claw root, ginger and senna pods. Its suitability for other herbal drugs (matrix groups) must be demonstrated or another validated method used.“
General Limit of aflatoxin B\(_1\) in herbal drugs
(Ph.Eur. 2.8.18.)

„Unless otherwise indicated in the monograph, herbal drugs contain **not more than 2 µg/kg of aflatoxin B\(_1\)**.“

The competent authority may also require compliance with a limit of **4 µg/kg for the sum of aflatoxins B\(_1\), B\(_2\), G\(_1\) and G\(_2\).“

(but Germany: „Aflatoxinverbotsverordnung“ or „Verordnung zur Begrenzung von Kontaminanten in Lebensmitteln“; if aflatoxin B\(_1\) > 2 µg/kg, then this material is prohibited from further use (no processing factor!))
Determination of ochratoxin A in herbal drugs
(Ph.Eur. 2.8.22.)

Detailed description of the method.
„Herbal drugs that are subject to contamination by ochratoxin A are tested by a validated method.“

„The method described is cited as an example of a method that has been shown to be suitable for liquorice extract and liquorice root. Its suitability for other herbal drugs (matrix groups) must be demonstrated or another validated method used.“
Limits for ochratoxin A in liquorice monographs

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

**Liquorice ethanolic liquid extract, standardised**
Ochratoxin A (2.8.22): maximum 80 µg per kilogram of extract.

**Liquorice dry extract (water) for flavouring purposes**
Ochratoxin A (2.8.22): maximum 80 µg per kilogram of (undiluted) extract.
Implications in practice

Methods for determination of aflatoxins and ochratoxin A in herbal drugs are described in Ph.Eur. and are working well. Method validation of matrix groups and/or specific matrices (e.g. resins, essential oils) has to be done by laboratories themselves. Fixed limit of aflatoxin B$_1$, but limit of sum of aflatoxins could be set by national authorities.

(e.g. foodstuffs: Commission Regulation (EC) No. 1881/2006).

Limit of ochratoxin A are given only in liquorice monographs. Limits for other products are still missing.

(e.g. foodstuffs: Commission Regulation (EC) No. 1881/2006).

→Monitoring of herbal drugs is necessary.
Mycotoxin residues in herbal drugs

There are a lot of data of aflatoxin and ochratoxin A residues existing. The knowledge on the occurrence of other mycotoxins in herbal drugs is rather restricted. Mainly three species of fungus form mycotoxins to which maximum levels have been set by the European Commission.

COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs
## Moulds forming Mycotoxins

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mycotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus spec. (flavus)</td>
<td>Aflatoxin B₁, B₂, G₁, G₂, M₁</td>
</tr>
<tr>
<td>Aspergillus spec. (ochraceus)</td>
<td>Ochratoxin A, Patulin</td>
</tr>
<tr>
<td>Penicillium spec.</td>
<td></td>
</tr>
<tr>
<td>Fusarium spec.</td>
<td>Fumonisin, Zearalenone, Trichothecene</td>
</tr>
<tr>
<td></td>
<td>(Deoxynivalenol, Nivalenol, T2-Toxin, HT2-Toxin)</td>
</tr>
</tbody>
</table>
Limits in commission regulation 1881/2006

COMMISSION REGULATION (EC) No 1881/2006
of 19 December 2006
setting maximum levels for certain contaminants in foodstuffs

- Aflatoxins
- Ochratoxin A
- Patulin
- Deoxynivalenol
- Zearalenon
- Fumonisins
- T-2 and HT-2-Toxin
Mycotoxin testing program

Routine-testing (target analysis):
- Aflatoxin B$_1$, B$_2$, G$_1$, G$_2$ per HPLC (limit of detection 50 ppt)
- Aflatoxin M$_1$ per HPLC (limit of detection 50 ppt)
- Ochratoxin A per HPLC (limit of detection 50 ppt)
- Patulin per HPLC (limit of detection 50 ppt)

Screening-program:
- Zearalenone per HPLC
- Deoxynivalenol, Nivalenol and T2-, HT2-Toxin per LC-MS/MS
- Fumonisin B$_1$ and B$_2$ per HPLC
Aflatoxin $B_1$ analysis of 7250 samples shows

products with positive results in many cases (>2µg/kg):

- agnus castus fruit,
- cayenne pepper fruit,
- cacao,
- colchicum autumnale,
- devil`s claw root,
- figs,
- ginger,
- musk dana,
- nutmeg,
- nux vomica seed,
- pumpkin seed,
- senna fruit tinn.

products with sporadical positive results:

- fennel,
- (bitter) orange peels,
- mistletoe,
- liquorice root,
- valerian root,
- turmeric root,
- marshmallow root
Ochratoxin A analysis of 1978 samples shows products with positive results in many cases (>20µg/kg): angelica root, cocoa, dandelion root, figs, ginger, ginkgo leaves, liquorice root, nettle root, orange flowers, pepper, raisins, restharrow root

products with sporadical positive results: green tea, linden flowers, marshmallow root, orange peels, valerian root
Patulin analysis of 278 samples shows

2 products with positive results:
  apples, orange peels

Zearalenon analysis of 125 samples shows

products with sporadical positive results (<100µg/kg):
  nettle, rosehip, peppermint, chamomile, fennel, sage, melissa, verbena
Fumonisin analysis of 155 samples shows products with sporadical positive results: cardamom, **liquorice root**, orange peels, rosehip (all results below 200 µg/kg, liquorice root below 2000 µg/kg)

DON analysis of 69 samples shows 2 products with positive results (<1000µg/kg):

silver linden flowers, tea
Nivalenol analysis of 48 samples shows

1 positive result: silver linden flowers (47µg/kg)

T2 -toxin and HT2 -toxin analysis of 48 samples shows

products with positive results (<50µg/kg):

anis fruit, linden flowers, oat straw, sage
Risk management of mycotoxins

- only few drugs are contaminated; a 100 % quality control of all batches is only necessary for the critical products/sources
- targeted analysis of aflatoxin and ochratoxin A (based on risk assessment)
- mycotoxins are well soluble in alcoholic solvents: analyses of the extracts should be performed if possible contamination of raw material is known or suspected
- besides ochratoxin and aflatoxins other mycotoxins may be considered as well on a case-by-case basis
- develop strategies to avoid contamination in the source (e.g. during drying, storage), implement GACP!
Effect of improved GACP on Ochratoxin A levels in Liquorice root (ppb)

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>Maximum value analysed</th>
<th>90(^{th}) Percentil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>50</td>
<td>425</td>
<td>234</td>
</tr>
<tr>
<td>2000</td>
<td>159</td>
<td>904</td>
<td>145</td>
</tr>
<tr>
<td>2001</td>
<td>192</td>
<td>337</td>
<td>41</td>
</tr>
<tr>
<td>2002</td>
<td>152</td>
<td>423</td>
<td>34</td>
</tr>
<tr>
<td>2003</td>
<td>60</td>
<td>72</td>
<td>20</td>
</tr>
<tr>
<td>2010</td>
<td>257</td>
<td>117</td>
<td>26</td>
</tr>
<tr>
<td>2011</td>
<td>82</td>
<td>65</td>
<td>19</td>
</tr>
</tbody>
</table>
Pesticides

- General monographs
- Pesticide residues 2.8.13 Ph. Eur.
- Limits and calculation
- EC 396/2005
- EU Pesticide database
- EHIA Allocation List
- Scope of testing
- Methodology and analytical requirements
Introduction

Raw products of herbal origin are naturally subjected to a considerable variation. Of the roughly 400 plants and parts of plants on the herbal market, 30 to 40 plants are cultivated on a large scale. Only about 25 % of the total amount of herbal drugs, but nevertheless the vast majority of plant species (70 - 80%), are gathered from the wild.

It is therefore important, when addressing the problem of pesticide residue analysis in herbal drugs, to take into account the diversity of different matrices as well as the particular circumstances in the countries of origin.
General monographs

Herbal Drugs:

**Pesticides (2.8.13)**. Herbal drugs comply with the requirements for pesticide residues. The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant.

Extracts:

**TESTS**

Where applicable, as a result of analysis of the herbal drug or animal matter used for production and in view of the production process, tests for microbiological quality, heavy metals, aflatoxins and pesticide residues in the extracts may be necessary.
Homoeopathic preparations

Herbal Drugs for Homoeopathic Preparations

**TESTS** …

**Pesticides** *(2.8.13)*. Herbal drugs for homoeopathic preparations comply with the requirements for pesticide residues.

…

Where justified, the test for pesticides may be performed on the mother tincture according to the requirements of the general monograph *Mother tinctures for homoeopathic preparations* *(2029)*.

Mother Tinctures for Homoeopathic Preparations

**Pesticides** *(2.8.13)*. Where applicable, the mother tincture for homoeopathic preparations complies with the test. This requirement is met if the herbal drug has been shown to comply with the test.

Justification is provided in cases where the test for pesticides is performed on the mother tincture, instead of on the herbal drug according to the requirements of the general monograph *Herbal drugs for homoeopathic preparations* *(2045)*.
Pesticide residues 2.8.13

The monograph pesticide residues 2.8.13 had been introduced to Ph.Eur. 1997 (USP 24 <561>, 2000)

In June 2006 the Ph.Eur. Pesticide Expert group has been mandated to update the Ph.Eur monograph 2.8.13 referring to the publication „Pesticide residues in medicinal drugs and their preparations (PHARMEUROPA Vol.17 No. 1, Jan. 2005).

Maximum limits for frequently found pesticides based on positive findings, 90th percentiles and quantitation limits have been proposed.
PESTICIDE RESIDUES
IN MEDICINAL DRUGS AND THEIR PREPARATIONS

Lothar Kabelitz(*)

ABSTRACT

Regulations for pesticide residues in herbal medicinal drugs are included in the Monograph “Pesticide Residues” of the European Pharmacopoeia (Ph. Eur.). According to the monograph limits applying to pesticides not listed in the table of the monograph comply with the limits set by the EC directives 76/895 and 90/642 including their annexes and successive updates. The limits indicated in the mentioned EC directives that have been adopted in German legislation within the Maximum Residue Level Regulation (Rückstands-Höchstmengenverordnung, RhmV) are not appropriate. They refer to certain product groups only, they are generally too low and therefore they are not practicable.

According to the Contaminants Working Group of the Federal Association of Pharmaceutical Product Manufacturers (BAH) 67 pesticides respectively substance groups are relevant to a selection of 205 herbal medicinal drugs which were given a positive assessment by the E Commission or ESCOP or a monograph by the Ph. Eur.

... Product Manufacturers (BAH) in Germany, comprising members of the scientific staff of Martin Bauer GmbH & Co KG, Finzelberg GmbH & Co KG, Emil Flachsmann AG, Gehrlicher GmbH & Co KG, Chemische Fabrik Dr. Hetterich KG, Kneipp Werke GmbH & Co KG, PhytoLab GmbH & Co KG and Salus-Haus GmbH & Co KG, collated extensive information and data concerning more than 200 pesticides and in excess of 400 drugs for the period between 1st January 1998 and 31st December 2002.
General monograph on Herbal Drugs/Extracts

Pesticides (2.8.13). Herbal drugs comply with the requirements for pesticide residues. The requirements take into account the nature of the plant, where necessary the preparation in which the plant might be used, and where available the knowledge of the complete record of treatment of the batch of the plant.

Where applicable, as a result of analysis of the herbal drug used for production and in view of the production process, tests for microbiological quality (5.1.4 or 5.1.8), heavy metals (2.4.27), aflatoxins (2.8.18), ochratoxin A (2.8.22) and pesticide residues (2.8.13) in the extracts may be necessary.
Pesticide Residues 2.8.13 - Definition

**Definition.** For the purposes of the Pharmacopoeia, 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. Pesticide residues can be present and are controlled in herbal drugs and herbal drug preparations.
Types of pesticides

Herbizides - Plants (Algicides, Graminicides, Arboricides)
Fungizides - moulds and funghi
Insecticides - insects
Acaricides - mites
Molluscicides - slugs and snails
Nematicides - nematodes
Rodenticides - rodents
Avicides - birds
Pesticide Residues 2.8.13 - Limits

**Limits.** Unless otherwise indicated in the monograph, the herbal drug to be examined at least complies with the limits indicated in Table 2.8.13.-1. The limits applying to pesticides that are not listed in Table 2.8.13.-1 and whose presence is suspected for any reason comply with the limits (levels) cross referred to by Regulation (EC) No. 396/2005, including annexes and successive updates. Limits for pesticides that are not listed in Table 2.8.13.-1 nor in European Union texts are calculated using the following expression:
Pesticide Residues 2.8.13 - Calculation

\[
\frac{ADI \times M}{MDD_{HD} \times 100}
\]

\(ADI\) = acceptable daily intake, as published by FAO-WHO, in milligrams per kilogram of body mass;

\(M\) = body mass in kilograms (60 kg);

\(MDD_{HD}\) = daily dose of the herbal drug, in kilograms.
Pesticide Residues 2.8.13 – Calculation in Extracts

The limits for pesticides in herbal drug preparations are calculated using the following expressions:

If $DER \leq 10$: 
\[ MRL_{HD} \times DER \]

If $DER > 10$: 
\[ \frac{ADI \times M}{MDD_{HP} \times 100} \]

$MRL_{HD}$ = maximum residue limit of the pesticide in the herbal drug as given in Table 2.8.13.1 or in EU texts or calculated using the expression mentioned above;

$DER$ = drug/extract ratio, i.e. the ratio between the quantity of herbal drug used in the manufacture of a herbal drug preparation and the quantity of herbal drug preparation obtained;

$MDD_{HP}$ = daily dose of the herbal drug preparation, in kilograms.
Pesticide Residues 2.8.13 – Exemption

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).
Pesticide Residues 2.8.13 – Method requirements

Qualitative and quantitative analysis of pesticide residues. The analytical procedures used are validated (e.g. according to Document N° SANCO/10232/2006). In particular, they satisfy the following criteria:

- the chosen method, especially the purification steps, is suitable for the combination pesticide residue/substance to be examined, and not susceptible to interference from co-extractives;

- natural occurrence of some constituents is considered in the interpretation of results (e.g. disulfide from Cruciferaceae);
Pesticide residues 2.8.13 (summary)

- Expanding number of substances in table 2.8.13-1 to 115 pesticides (70 MRL`s)
- Cross reference to new European Food Law
- Formula for calculation of residues in herbal drug preparations
- Sampling according Ph.Eur. 2.8.20.
- Method for determination of pesticides has been deleted
- Method validation criteria acc. to SANCO/10232/2006 (updated SANCO/12571/2013)
- Considering of natural occurring constituents by interpretation of results (e.g. disulfide)
Advantages in practice

No fixed method in Ph.Eur. (different methods are used in pesticide residue laboratories depending on substances and instruments).

Harmonised validation procedures for methods used:
Method validation and quality control procedures for pesticide residue analyses in food and feed: SANCO/12571/2013 (update).

List of frequently found pesticides expanded (34 to 115 substances).

Simple evaluation of pesticide residues in herbal drug preparations (extracts).

REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 23 February 2005

on maximum residue levels of pesticides in or on food and feed of plant and animal origin and
EC 396/2005 - Motivations

This Regulation directly concerns public health and is relevant to the functioning of the internal market. Differences in national maximum residue levels for pesticides can pose barriers to trade in products included in Annex I to the Treaty and products derived therefrom between Member States and trade between third countries and the Community. Accordingly, in the interest of free movement of goods, equal competition conditions among the Member States, as well as a high level of consumer protection, it is appropriate that maximum residue levels (MRLs) for products of plant and animal origin be set at Community level, taking into account good agricultural practice.
EC 396/2005 - Motivations

The production and consumption of plant and animal products play a very important role in the Community. The yield from plant production is continually being affected by harmful organisms. It is essential to protect plants and plant products against such organisms in order to prevent a reduction in yield or damage to them, and ensure both the quality of the products harvested and high agricultural productivity. To this end, different methods are available, including non-chemical methods, practices such as using resistant varieties, crop rotation, mechanical weeding, biological control and chemical methods such as the use of plant protection products.
EC 396/2005 - Motivations

It is also important to carry out further work to develop a methodology to take into account cumulative and synergistic effects. In view of human exposure to combinations of active substances and their cumulative and possible aggregate and synergistic effects on human health, MRLs should be set after consultation of the European Food Safety Authority established by Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (2) (hereinafter the Authority).
EC 396/2005 - Motivations

MRLs for pesticides should be continually monitored and should be changed to take account of new information and data. MRLs should be set at the lower level of analytical determination where authorised uses of plant protection products do not result in detectable levels of pesticide residues. Where uses of pesticides are not authorised at Community level, MRLs should be set at an appropriately low level to protect the consumer from the intake of unauthorised or excessive levels of pesticides.
residues. In order to facilitate control of residues of pesticides, a default value is to be set for pesticide residues present in products or groups of products covered by Annex I for which no MRLs have been established in Annexes II or III, unless the active substance in question is listed in Annex IV. It is appropriate to set the default value at 0,01 mg/kg and to provide for the possibility of setting it at a different level for active substances covered by Annex V, taking into account the routine analytical methods available and/or consumer protection.
Pesticide residues 2.8.13

What Maximum Residue Limits (MRLs) are applying to pesticides that are not listed in Ph.Eur. Table 2.8.13.-1?

Commission Regulation (EC) No. 396/2005, including annexes and successive updates

Pesticides not listed in European Union texts apply to Default MRL of 0,01mg/kg. Calculation with ADI-value is possible (fao/who).
Regulation (EC) No. 396/2005

Harmonised pesticide MRLs in Europe (number of existing MRL`s could be reduced from 500.000 national to 145.000 EU-harmonised).

old EU directives 396/2005

EU-MRLs: about 250 pesticides about 550 p.
national MRLs: about 850 pesticides none
default MRL none 0,01 mg/kg
# Regulation (EC) No. 396/2005

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Annex IV</td>
<td>Substances for which no MRLs are required</td>
<td></td>
</tr>
</tbody>
</table>

Establishment of Pesticide MRLs

- MRL for pesticide are derived from field trials conducted under GAP at the same time toxicological studies in vitro/in vivo are performed to establish ADI and ARfD values
- Results from field trials + potential intake calculation would only be used if not exceeding ADI/ARfD
- Hazard characterisation is thus disconnected from potential exposure assessment, the values are less transparent to the public
- Residual levels > MRL need to be analyzed on a case-by-case basis to determine if the intake would exceed the health-based limits
- Product withdrawal usually only if RL > ARfD
EU - Pesticides database

ACTIVE SUBSTANCES
Regulation (EC) No 1107/2009

PESTICIDES EU-MRLs
Regulation (EC) No 396/2005

Latest active substance updates
- Update of the classification of active substances according to Regulation (EC) No 1272/2008.
- Directive 67/548/EEC will be repealed on 1 June 2015.
- Insertion of the latest publications in the EU Official Journal
- Correction of the AOEL for 1-methylcyclopropene

Latest MRL updates

http://ec.europa.eu/sanco_pesticides/public/index.cfm
Regulation (EC) No. 396/2005

In the context of this regulation MRLs have been fixed (145,000) for all pesticides in each commodity listed in Annex I (315).
Diverse herbal drugs may be potentially allocated to different categories, depending on its usage:

e.g. peppermint  \rightarrow  fresh herb  
     (\rightarrow  herbal infusion ?)

e.g. fennel  \rightarrow  spices  
     (\rightarrow  herbal infusion ?)

But only one single MRL for one product is allowed.
### Allocation of products (396/2005 Annex I)

#### 1. FRUIT FRESH OR FROZEN

<table>
<thead>
<tr>
<th>(d) Other small fruit &amp; berries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blueberries (Bilberries cowberries (red bilberries))</td>
</tr>
<tr>
<td>Cranberries</td>
</tr>
<tr>
<td>Currants (red, black and white)</td>
</tr>
<tr>
<td>Gooseberries (Including hybrids with other ribes species)</td>
</tr>
<tr>
<td>Rose hips</td>
</tr>
</tbody>
</table>

#### 2. VEGETABLES FRESH OR FROZEN

<table>
<thead>
<tr>
<th>(f) Herbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chervil</td>
</tr>
</tbody>
</table>
Parsley |
|**Sage** (Winter savory, summer savory, ) |
|**Rosemary** |
|**Thyme** (marjoram, oregano) |
## Allocation of products (396/2005, Annex I)

### 6. TEA, COFFEE, HERBAL INFUSIONS, COCOA

<table>
<thead>
<tr>
<th>(iii) Herbal infusions (dried)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Flowers</td>
</tr>
<tr>
<td>Camomille flowers</td>
</tr>
<tr>
<td>Hybiscus flowers</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>(b) Leaves</td>
</tr>
<tr>
<td>(c) Roots</td>
</tr>
<tr>
<td>(d) Other herbal infusions</td>
</tr>
</tbody>
</table>

### 7. SPICES

### 8. HOPS

### 9. SUGAR PLANTS

- Sugar beet (root)
- Sugar cane
- Chicory roots
- Others
There are 315 products listed in Annex I of Regulation 396/2005 but not all herbal drugs being currently on the market are named.

In many categories the subcategory „others“ is added. Allocation of products which are not listed in Annex I (e.g. nettle, St. johnswort) is becoming difficult.

The European Herbal Infusion Association (EHIA) published an allocation list for about 400 plants and parts of plants. Based on the inventory list of EHIA all 400 products are allocated to the categories of Annex I considering the main use of the product, too.
# EUROPEAN HERBAL INFUSIONS ASSOCIATION

## ALLOCATION LIST

**EHIA INVENTORY LIST OF HERBALs CONSIDERED AS FOOD**

<table>
<thead>
<tr>
<th>English Name of the plant</th>
<th>Latin Name of the plant</th>
<th>German Name of the plant</th>
<th>Plant part used</th>
<th>Allocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrimony</td>
<td>Agrimonia eupatonia L.</td>
<td>Odenmennig</td>
<td>Kraut</td>
<td>tea, coffee, herbal infusions and cocoa</td>
</tr>
<tr>
<td>Althaea / Lucerne</td>
<td>Medicagia sativa L.</td>
<td>Althaea / Luzerne</td>
<td>Kraut</td>
<td>tea, coffee, herbal infusions and cocoa</td>
</tr>
<tr>
<td>Allspice</td>
<td>Pimenta dioica (L.) Merr. / Pimenta officinale Lindl.</td>
<td>Piment</td>
<td>Friichte</td>
<td>spices</td>
</tr>
<tr>
<td>Almond</td>
<td>Prunus dulcis var. dulcis</td>
<td>Mandel</td>
<td>Blätten</td>
<td>tea, coffee, herbal infusions and cocoa</td>
</tr>
<tr>
<td>Almond</td>
<td>Prunus dulcis var. dulcis</td>
<td>Mandel</td>
<td>Samen</td>
<td>fruit fresh and frozen; nuts</td>
</tr>
<tr>
<td>Aloe vera</td>
<td>Aloe barbadensis Mill. / Aloe vera (L.) Burm. f.</td>
<td>Aloe vera</td>
<td>Blattgel</td>
<td>tea, coffee, herbal infusions and cocoa</td>
</tr>
<tr>
<td>Alpine ladies mantle</td>
<td>Achernillo alpina L.</td>
<td>Silbermantel</td>
<td>Kraut</td>
<td>tea, coffee, herbal infusions and cocoa</td>
</tr>
<tr>
<td>Angelica</td>
<td>Angelica archangelica L.</td>
<td>Engelwurz</td>
<td>Wurzeln</td>
<td>vegetables fresh or frozen</td>
</tr>
<tr>
<td>Angelica</td>
<td>Angelica archangelica L.</td>
<td>Engelwurz</td>
<td>Stengel</td>
<td>vegetables fresh or frozen</td>
</tr>
<tr>
<td>Anise</td>
<td>Pimpinella axium L.</td>
<td>Anis</td>
<td>Fruchte</td>
<td>spices</td>
</tr>
<tr>
<td>Annato</td>
<td>Bixa orellana L.</td>
<td>Annatto</td>
<td>Samen</td>
<td>spices</td>
</tr>
</tbody>
</table>

**Allocation according to Reg. (EU) No 600/2010**

<table>
<thead>
<tr>
<th>Category</th>
<th>Group</th>
<th>Subgroup</th>
<th>Code number</th>
</tr>
</thead>
<tbody>
<tr>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
</tr>
<tr>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
</tr>
<tr>
<td>spices</td>
<td>spices</td>
<td>allspice</td>
<td>620010</td>
</tr>
<tr>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions, flowers, others</td>
<td></td>
<td>631900</td>
</tr>
<tr>
<td>fruit fresh and frozen; nuts</td>
<td>nuts</td>
<td>almonds</td>
<td>120010</td>
</tr>
<tr>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions, other herbal infusions</td>
<td></td>
<td>639000</td>
</tr>
<tr>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions, other herbal infusions</td>
<td></td>
<td>639000</td>
</tr>
<tr>
<td>vegetables fresh or frozen</td>
<td>root and tuber vegetables</td>
<td>other root and tuber vegetables except sugar beet</td>
<td>213040</td>
</tr>
<tr>
<td>vegetables fresh or frozen</td>
<td>leaf, vegetables and fresh herbs</td>
<td>celery leaves</td>
<td>256030</td>
</tr>
<tr>
<td>spices</td>
<td>spices</td>
<td>anis</td>
<td>810010</td>
</tr>
<tr>
<td>spices</td>
<td>spices</td>
<td>seeds, others</td>
<td>810890</td>
</tr>
</tbody>
</table>
Regulatory aspects

The different allocations of herbal drugs are causing different MRLs in many cases. For example:

<table>
<thead>
<tr>
<th>Herbal drugs</th>
<th>Acetamiprid MRL</th>
<th>Endosulfan MRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange peels</td>
<td>5 mg/kg (DF 5)</td>
<td>0,25 mg/kg (DF 5)</td>
</tr>
<tr>
<td>Rosehips</td>
<td>0,05 mg/kg (DF 5)</td>
<td>0,25 mg/kg (DF 5)</td>
</tr>
<tr>
<td>Peppermint</td>
<td>15 mg/kg (DF 5)</td>
<td>0,25 mg/kg (DF 5)</td>
</tr>
<tr>
<td>Chamomille</td>
<td>0,1 mg/kg</td>
<td>0,5 mg/kg</td>
</tr>
<tr>
<td>Fennel</td>
<td>0,1 mg/kg</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Liquorice</td>
<td>0,1 mg/kg</td>
<td>0,5 mg/kg</td>
</tr>
<tr>
<td>Tea</td>
<td>0,1 mg/kg</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Organic products</td>
<td>0,01 mg/kg</td>
<td>0,01 mg/kg</td>
</tr>
</tbody>
</table>

This has to be considered developing a method of analysis.
Scope of testing

In Ph.Eur. 2.8.13. a reduced testing is allowed if the herbal drug has been cultivated according to good agricultural and collection practices (GACP).

Residue analysis on a regular basis of used pesticides is recommended.

For all other herbal drug the following recommendation on pesticide analysis could be given:

1. Analysis of frequently found pesticides (multi methods)
2. Analysis of pesticides whose presence is suspected for any reason (group specific and single methods)
Development of methods for pesticide testing

There are **550 compounds and metabolites** listed in EU 396/2005, but totally existing **about 1,650 chemical pesticides** (Pesticide Manual 2003).

In analytical laboratories **400-600 analytes** could be detected with:

- multi methods
- group specific methods
- single residue methods

There is an analytical gap of more than 1000 substances.
Development of methods for pesticide testing

**Multi methods:**
GC-methods (e.g. DFG S19)
LC-MS/MS (e.g. Quechers)

**Group specific methods:**
Dithiocarbamates, Phenoxyalkancarboxic acids, Phenylureas, Carbamates, …

**Single methods:**
Pyridat, Chlormequat, Glyphosate, Paraquat, Nicotine …

→ about 200-300 substances

→ about 200-300 substances

→ about 60 substances

→ about 20 substances
Method overview

Multi-methods would usually allow the analysis of pesticides which are found frequently in herbal drugs and extracts.

The classical GC multi-method can detect substances from Ph. Eur. table 2.8.13 – (except dithiocarbamates) and a broad spectrum of chlorine and phosphorous.

With the LC-MS/MS-multi method modern and polar pesticides such as Carbendazim, 2,4-D, Neo-Nicotinoids, and others) are detected.

With the group and single methods compounds which are not detectable with the multi methods can be analyzed.
Influence of concentration and naturally occurring compounds

Herbal drugs are herbal raw materials which have been dried for conservation. The remaining water content of herbal drugs is less than 12%. For this reason the processing causes a concentration of pesticide residues with a factor of approximately 5.

In the same way chemical substances in the plant are concentrated. This has two effects on pesticide analysis:

- On the one hand the matrix effects are also enriched and disturb analytical methods,
- on the other hand genuine occurrence of some substances in the plant leads to false positive results in some cases.
Requirements for an effective control of pesticides in herbal products:

- detailed information about the legal requirements
- experience and knowledge which pesticides are used for which plants and in which country respectively
- Experience and knowledge about risks of contamination (data base)
- Choice of the most suitable test method(s)
- monitoring and target analyses of the herbal drug
Pesticides – risk assessment

- high sensitivity in the population

- it is not possible to guarantee products without any pesticide residue

- in quality control not all pesticides can be tested every time because of the number of substances (analytical gap) and the number of different methods used (→ risk based analysis )

→ combination of detailed information about supply chain (e.g. controlled cultivation) and analytical control is necessary
Risk Management

- Difficulties with herbal products
- Factors influencing the quality
- Basis for risk management
- References to contaminants information
- Sampling
Difficulties in quality control of herbal products

• Considerable variability in terms of their constituents
• Large number of products commercially available (about 400 plants and parts of plants)
• About 30 to 40 medicinal plants are cultivated on a large-scale
• About 30 % of the total amount of herbal drugs are gathered from the wild
• But 80 % to 90 % of medicinal plant species are collected from the wild
• → Large numbers of small tonnages of collected products
Factors influencing the quality

- genetic factors (resistancies, content of actives)
- environmental factors (climate, soil, air)
- economic factors (subsidies, political situation)
- production of the drug (time of harvest, harvesting, drying, processing, transport, storage)
- cultivation vs. wild collection
Dried Plant material

**Taking samples**
- Appearance
- Smell/aroma
- Homogeneity
- Sieve
- Fluidity
- Volume/bulk density

**Sensoric**
- Taste
- Infusion
- Ash
- Foreign matter
- Loss on drying

**Monograph - requirements**
- Identity
- Pest
- Ingredients

**Contaminants**
- Pesticides
- Gassing agents
- Heavy metals
- PCP
- PCB, PAH, Dioxins
- Mycotoxins
- Microbiology
- Radioactivity
- Acrylamide
- ?

**Technical parameter**
- Volume/bulk density
Basis for Risk management of contaminants

- screening of existing regulations and publications
- definition of possible contaminants
- development of suitable methods of analysis (or search for a suitable laboratory)
- perform analyses (monitor products, target analyses)
- evaluation of results (data base) and risk assessment
- create risk based schemes of testing (herbal drug is normally tested)
- Improve quality (GACP, processing)
References to Contaminants

• **European Pharmacopoeia** (monograph „Herbal Drugs“, pesticides, heavy metals, aflatoxins, ochratoxin A and microbiology).

• **EC regulations for foodstuffs** (e.g. pesticides, mycotoxins, heavy metals, PAHs, radioactivity, nitrates).

• **National regulations** (e.g. German aflatoxin regulations, German regulation for contaminants).

• **Publications**
  (e.g. Codex Alimentarius, Food and Agriculture Organization (FAO/UN);
Sampling, transport to the lab and sample preparation influence the accuracy of analytical results in majority of cases.

Effective and precise analytical methods as well as careful evaluation of data cannot correct errors made before.
Sampling acc. to Ph.Eur.

In Ph.Eur. 2.8.20 „HERBAL DRUGS: SAMPLING AND SAMPLE PREPARATION”

the number of samples:

<table>
<thead>
<tr>
<th>Number of containers in batch (N)</th>
<th>Number of containers to be sampled (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 3</td>
<td>all</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>$n^* = \sqrt{N} + 1$</td>
</tr>
</tbody>
</table>

* round $n$ up to the next integer

and the minimum mass of samples are described:

<table>
<thead>
<tr>
<th>Mass of herbal drug in the batch (kg)</th>
<th>Minimum mass of samples as a percentage of the mass of the batch of herbal drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>1.00*</td>
</tr>
<tr>
<td>50 - 100</td>
<td>0.50</td>
</tr>
<tr>
<td>&gt; 100 - 250</td>
<td>0.25</td>
</tr>
<tr>
<td>&gt; 250 - 500</td>
<td>0.20</td>
</tr>
<tr>
<td>&gt; 500 - 1000</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Example 1:
Batch of 1.000 kg, 40 containers à 25 kg

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Weight of mixed sample</th>
<th>Weight of single sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph.Eur.</td>
<td>8</td>
<td>1.800 g</td>
<td>225 g</td>
</tr>
<tr>
<td>(EU) 401/2006 Mykotoxins</td>
<td>30</td>
<td>3.000 g</td>
<td>100 g</td>
</tr>
<tr>
<td>(EU) 63/2002 Pesticides</td>
<td>10</td>
<td>2.000 g</td>
<td>200 g</td>
</tr>
<tr>
<td>(EU) 333/2007 Heavy metals</td>
<td>10</td>
<td>1.000 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>
**Example 2:**
Batch of 5500 kg, 220 containers à 25 kg

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Weight of mixed sample</th>
<th>Weight of single sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph.Eur.</td>
<td>16</td>
<td>4.400 g</td>
<td>275 g</td>
</tr>
<tr>
<td>(EU) 401/2006 Mykotoxins</td>
<td>80</td>
<td>8.000 g</td>
<td>100 g</td>
</tr>
<tr>
<td>(EU) 63/2002 Pesticides</td>
<td>10</td>
<td>2.000 g</td>
<td>200 g</td>
</tr>
<tr>
<td>(EU) 333/2007 Heavy metals</td>
<td>10</td>
<td>1.000 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>
Example 3:
Batch of 10,000 kg, 400 containers à 25 kg

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Number of samples</th>
<th>Weight of mixed sample</th>
<th>Weight of single sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph.Eur.</td>
<td>21</td>
<td>8,000 g</td>
<td>381 g</td>
</tr>
<tr>
<td>(EU) 401/2006 Mykotoxins</td>
<td>100</td>
<td>10,000 g</td>
<td>100 g</td>
</tr>
<tr>
<td>(EU) 63/2002 Pesticides</td>
<td>10</td>
<td>2,000 g</td>
<td>200 g</td>
</tr>
<tr>
<td>(EU) 333/2007 Heavy metals</td>
<td>10</td>
<td>1,000 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>
How to do sampling

- different sampling methods for one batch but different analytes is not feasible.

- Sampling according to Ph.Eur. 2.8.20 fulfills most requirements as laid down in official sampling requirements for food analysis, except for mycotoxins. A much larger number of samples is required (ca. 4 times higher) in this case, while total sample weight is only slightly higher (factor 1.25-1.8).

- the laboratory sample is only a subsample made from the bulk sample. Sample preparation is normally done with 1 to 10 g only. For that reason the bulk sample has to be homogenized very well.

→ for analysis of contaminants in herbal drugs the sampling procedure of Ph.Eur. 2.8.20 should be taken.
Other contaminants

- Radioactivity
- Anthraquinone
- PAH – Polycyclic aromatic hydrocarbons
Radioactive contamination

• Ph.Eur. Monograph „Herbal Drugs“: *In some specific circumstances, the risk of radioactive contamination is to be considered.*

• EU Council Regulation 737/90 after accident of Tschernobyl (updated by EU 616/2000 of 03/2000)

• EU Council Regulation 322/2014 after accident of Fukushima
  - Positive list of the products to be examined
  - Caesium 134/137 maximum limits:

  Infant foods, milk       max. 50 Bq / kg
  mineral water, tea      max. 10 Bq / kg
  other food              max 100 Bq / kg
Radioactive contamination

Positive samples
Herbal products where radioactive contamination of more than 370 Bq/kg have been found:

- Ash leaves
- Blueberry fruits
- Iceland moss
- mushrooms
- Erica flowers
- Blueberry leaves
- Peppermint
- Greater Celandine herb
Anthraquinone

- component of paper (paper packing?)
- component of smoke (anthracene → anthraquinone)
- high risk in smoked products
- as the toxicological properties of anthraquinone remain unknown and a potential carcinogenic effect cannot be excluded low MRLs have been set (lower limit of analytical determination)
- analyses with pesticide multimethod (GC-MS) in 0.01 mg/kg

Collection of occurrence data on PAH in food has been performed in 2004. High levels of PAH were found in dried fruits, olive pomace oil, grape seed oil, spices/sauces and condiments. Maximum levels are necessary for benzo(a)pyrene in certain foods:

- containing fats and oils or where smoking or drying processes or environmental pollution may cause contamination
- Foods for infants and young children Maximum level 1,0 µg/kg wet weight according to (EC) No 1881/2006
Muito obrigado!

Your contact person:

Dr. Michael Schwarz
PhytoLab GmbH & Co. KG
Dutendorfer Straße 5-7
91487 Vestenbergsgreuth
Germany
Tel.: +49 9163 88-327
Fax.: +49 9163 88-456
michael.schwarz@phytolab.de
Copyright and liability

We emphasise that the contents of this presentation constitute works subject to protection under the laws of copyright. Any reproduction, dissemination, further processing or other use of the presentation, of the information and contents thereof, or of corresponding excerpts thereof, shall be subject to our express consent.

The presentation was drawn up to the best of its authors’ knowledge and belief and is offered for information purposes. Absent further agreement, any information or contents found therein shall serve as non-binding indications only and shall represent no promise or pledge. The authors of the presentation cannot accept liability for damage that may arise as a result of utilisation of the information and contents of which the presentation consists, unless information and contents of which the presentation consists have been made part of a concrete agreement as between our customer and us.