Pesticide Testing According to the European Pharmacopoeia (Ph.Eur.) – Legal Requirements and Practical Approach

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Introduction

Pesticide residues, Ph.Eur. 7th edition

Limits (EU 396/2005, allocation of products)

Methods and scope of testing
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Summary
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 vast diversity of different matrices as well as the particular circumstances in the countries of origin, which are mostly outside the EU.
Pesticide residues,
Ph.Eur. 7th edition
Introduction

In the European Pharmacopoeia (Ph.Eur.) pesticide residues are described under chapter 02 „Methods of analysis“. The Ph.Eur. Monograph „2.8.13. Pesticide residues“ contains „Definition“, „Limits“, „Sampling“ and „Qualitative and quantitative analysis of pesticide residues“ of herbal drugs.

The requirements for “Herbal Drugs” and “Extracts”, as well as “Herbal Drugs for Homoeopathic Preparations” and “Mother Tinctures for Homoeopathic Preparations” are referred within the scope of their monographs in chapter 05 „General texts“:
General monographs

Herbal Drugs:
TESTS ...

**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.*
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, Ph.Eur. 6.2

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 refering 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 2.8.13, Ph.Eur. 6.2

Definition of pesticide
(in herbal drugs and herbal drug preparations)

Limits for herbal drugs in table 2.8.13.-1
(calculation for herbal drug preparations)

Sampling and sample preparation of herbal drugs 2.8.20
(general chapter)

Qualitative and quantitative analysis of pesticide residues
(validated analytical procedures)
Pesticide residue 2.8.13 (new)

- 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
- Considering of natural occurring constituents by interpretation of results (e.g. disulfide)
Advantages in practise

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/10684/2009 (update).

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

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

Limits
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 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

(Text with EEA relevance)
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).

<table>
<thead>
<tr>
<th></th>
<th>old EU directives</th>
<th>396/2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU-MRLs:</td>
<td>about 250 pesticides</td>
<td>about 550 p.</td>
</tr>
<tr>
<td>national MRLs:</td>
<td>about 850 pesticides</td>
<td>none</td>
</tr>
<tr>
<td>default MRL</td>
<td>none</td>
<td>0,01 mg/kg</td>
</tr>
</tbody>
</table>

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### Regulation (EC) No. 396/2005

<table>
<thead>
<tr>
<th>Annex</th>
<th>Description</th>
<th>Regulation (EC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>List of commodities</td>
<td>No 178/2006</td>
</tr>
<tr>
<td>II</td>
<td>Existing EU MRLs</td>
<td>No 149/2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(March 2008)</td>
</tr>
<tr>
<td>III</td>
<td>Temporary MRLs</td>
<td>No 839/2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Update, 30 August 2008)</td>
</tr>
<tr>
<td>IV</td>
<td>Substances for which no MRLs are required</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Fumigants</td>
<td>No 260/2008</td>
</tr>
</tbody>
</table>

**Application date of Regulation (EC) 396/2005 including annexes:**
1 September 2008.
Allocation of herbal products
Public database on EU-MRLs and active substances

Active substances

Directive 91/414/EEC

Pesticide EU-MRLs

Regulation (EC) No 396/2005

Active substance updated on 16/03/2009

Directorate General for Health & Consumers

Products

Pesticides

MRLs updated on 18/02/2009

Disclaimer

This database is made available solely for the purpose of information. It has no legal value. The Commission declines all responsibility or liability whatsoever for errors or deficiencies in this database. Neither the Commission nor any person acting on behalf of the Commission is responsible with regard to the improper use of the document and its contents. The official MRLs are those published in the Official Journal of the European Union (Plant Protection - Pesticide Residues - Community Legislation).

http://ec.europa.eu/sanco_pesticides/public/index.cfm

In the context of this regulation MRLs for all pesticides in each commodity listed in Annex I (315) have been fixed (145.000).

Allocation of many products to several categories seems to be possible depending on its usage:

e.g. peppermint \(\rightarrow\) fresh herb

(\(\rightarrow\) 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>
<tr>
<td>Parsley</td>
</tr>
<tr>
<td>Sage (Winter savory, summer savory, )</td>
</tr>
<tr>
<td>Rosemary</td>
</tr>
<tr>
<td>Thyme (marjoram, oregano)</td>
</tr>
</tbody>
</table>
### 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

Sugar beet (root)

Sugar cane

Chicory roots

Others

#### 9. SUGAR PLANTS

Sugar beet (root)

Sugar cane

Chicory roots

Others
Allocation List of EHIA

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, hypericum, …) 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:
# Allocation List

**European Herbal Infusions Association**

**Updating January 2009**

## Ehia Inventory List of Herbals Considered as Food

<table>
<thead>
<tr>
<th>English name</th>
<th>Latin name of the plant</th>
<th>German name of the plant</th>
<th>Allocation</th>
<th>Category</th>
<th>Group</th>
<th>Subgroup</th>
<th>Code-Number</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrimony</td>
<td>herb</td>
<td>Oudemarrig</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
<td>maybe subgroup leaves 0632000</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>herb</td>
<td>Alfalfa</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
<td>maybe subgroup leaves 0632000</td>
<td></td>
</tr>
<tr>
<td>Allspice</td>
<td>fruits</td>
<td>Piment officinalis</td>
<td>spices</td>
<td>spices</td>
<td>allspice</td>
<td>820010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>flowers</td>
<td>Prunus dulcis var. dulcis</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>flowers, others</td>
<td>631590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Almond</td>
<td>seeds</td>
<td>Prunus dulcis var. dulcis</td>
<td>fruit fresh and frozen; nuts</td>
<td>nuts</td>
<td>almonds</td>
<td>120010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aloe Vera</td>
<td>leaf cap</td>
<td>Aloë barbadensis / Aloë vera</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpine Lady's Mantle</td>
<td>herb</td>
<td>Alchemilla alpina</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>other herbal infusions</td>
<td>639000</td>
<td>maybe subgroup leaves 0632000</td>
<td></td>
</tr>
<tr>
<td>Angelica</td>
<td>roots</td>
<td>Angelica archangelica</td>
<td>tea, coffee, herbal infusions and cocoa</td>
<td>herbal infusions</td>
<td>roots, others</td>
<td>633990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angelica</td>
<td>stems</td>
<td>Angelica archangelica</td>
<td>vegetables fresh or frozen</td>
<td>leaf, vegetables and fresh herbs</td>
<td>celery leaves</td>
<td>256000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anise</td>
<td>fruits</td>
<td>Pimpinella anisum</td>
<td>spices</td>
<td>spices</td>
<td>anis</td>
<td>910010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annatto</td>
<td>seeds</td>
<td>Bixa orellana</td>
<td>spices</td>
<td>spices</td>
<td>seeds, others</td>
<td>810590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>fruits</td>
<td>Malus domestica</td>
<td>fruit fresh and frozen; nuts</td>
<td>pome fruit</td>
<td>apples</td>
<td>130010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Mint</td>
<td>leaves</td>
<td>Mentha x stoechas</td>
<td>vegetables fresh or frozen</td>
<td>leaf, vegetables and fresh herbs</td>
<td>mint</td>
<td>256080</td>
<td>regrouping herbal infusions 0632000</td>
<td></td>
</tr>
</tbody>
</table>

**EHIA European Herbal Infusions Association**

Sonnenallee 26 • D-22507 Hamburg City Sud
Tel: +49/40/23 60 16-33-27 • Fax: +49/40/23 60 16-10-11 • E-Mail: ehia@wge-hh.de • [http://www.ehia-online.org](http://www.ehia-online.org)

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Methods of analysis and scope of testing
Scope of testing

According to Ph.Eur. monographs all pesticides in herbal drugs must comply, but which pesticides must be tested?

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.
Scope of testing

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

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

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)
Scope of testing, methods

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

**Group specific methods:**
- Dithiocarbamates, Phenoxyalkancarbonic acids, Phenylureas, Carbamates, …

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

→ about 200-300 substances
→ about 60 substances
→ about 20 substances
Scope of testing, methods

Analysis of all pesticides with one (multi-) method (e.g. LC-MS/MS) is not possible.

For example: 550 compounds of EU 396/2005
- 88% analysed
- 38% only LC-MS/MS
- 16% only GC-MS/MS
- 34% both possible

As herbal drugs consist of a complex matrix methods of analysis must be validated accurately and the suitability of multimethods should be tested (false positive / false negative).
Matrix effects

Matrix effects cause changes in signal intensity:
(G. Kempe, 6th International Fresenius conference, 24th May 2011)
Measurement of matrix effects in LC-MS/MS

Determination of matrix effect profiles with postcolumn infusion
(G. Kempe, 6th International Fresenius conference, 24th May 2011)

with continuous measurement the matrix load in the entire chromatogram could be made visible.
Requirements on multimethods

• in LC-MS/MS there are mainly peak suppressions observed:
  → in LC-MS/MS each sample has to be calibrated necessarily in \textit{the same} matrix or use of internal (labelled) standard

• in the GC-MS/MS both, suppression or fortification of the signal, is possible

• unclear findings should be confirmed by standard addition

• but false positive results caused by matrix substances („same“ transitions, „same“ Rt):
  → change chromatography or \textbf{HRMS}
Prospects - HRMS

1. Confirmation of positive results

2. Analysis of „known unknowns“
   (closing the analytical gap?)
Isobaric Pesticides

Thiamethoxam: $[M+H]^+ = C_8H_{11}ClN_5O_3S$ (292.02656)

![Thiamethoxam structure](image1)

Parathion-ethyl: $[M+H]^+ = C_{10}H_{15}NO_5PS$ (292.04031)

![Parathion-ethyl structure](image2)
Simulated Resolution = 15,000 (Mix 1:3)

Resolution 15,000

m/z

Relative Abundance

292.04031
C_{10}H_{15}OsNPS

292.02656
C_{8}H_{11}OsN_{5}ClS

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Simulated Resolution = 20,000 (Mix 1:3)

Resolution 20,000

292.04031
C_{10}H_{15}O_5NPS

292.02656
C_8H_{11}O_3N_5ClS

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Simulated Resolution = 25,000 (Mix 1:3)

Resolution 25,000

292.04031
C<sub>10</sub>H<sub>15</sub>O<sub>5</sub>NPS

292.02656
C<sub>8</sub>H<sub>11</sub>O<sub>5</sub>N<sub>5</sub>ClS

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Simulated Resolution = 35,000 (Mix 1:3)

Resolution 35,000

Folie 38

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Simulated Resolution = 50,000 (Mix 1:3)

Resolution 50,000

Folie 39
Simulated Resolution $= 100,000$ (Mix 1:3)

Resolution 100,000

- $m/z = 292.04031$: \(\text{C}_{10}\text{H}_{15}\text{O}_5\text{N}_5\text{P}_5\text{S}\)
- $m/z = 292.02656$: \(\text{C}_8\text{H}_{11}\text{O}_5\text{N}_5\text{Cl}\text{S}\)
Summary
Summary

There is no fixed pesticide method prescribed in Ph.Eur. but method validation requirements.

MRLs of 115 pesticides are listed in Table 2.8.13.-1. With cross reference to Regulation (EC) 396/2005 there are harmonised limits given for estimation of pesticide residues in medicinal herbs.

Unambiguous allocation is possible with EHIA Allocation List.

Scope of testing depends on origin of material and method of analysis.

Strong matrixeffects in herbal drugs multimethod analysis lead to false negative and/or false positive results.
Thank you for your interest!