

# EDQM Symposium

## Herbal Drugs & Herbal Drug Preparations

25 September 2009  
Vienna, Austria



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## Second Session

### Introduction of new assay methods in the European Pharmacopoeia and regulatory implications



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# New analytical methods, regulatory implications - an update from EDQM

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## Contents

- New assay methods to be introduced in Ph.Eur. monographs
- Assay method/marker for “other extracts”
- Newly adopted texts:
  - General limit for heavy metals in herbal drugs
  - Aristolochic acids
  - Ochratoxin A
- Cooperation with HMPC



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## Why new assay methods ?

- Modern instrumental methods are more specific
- Provide better reproducibility
- Provide meaningful information in terms of stability
- Example: Colorimetric assays in
  - Aloe dry extract, standardised or
  - Horse-chestnut dry extract, standardised



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## Assay methods - problem statement

- Approved MAA dossiers refer to existing assay methods and content specifications
- HMPC monographs refers to literature and dosages which may have been obtained by methods that are nowadays outdated
- Need to replace assay methods in Ph.Eur. may result in **different** assay results



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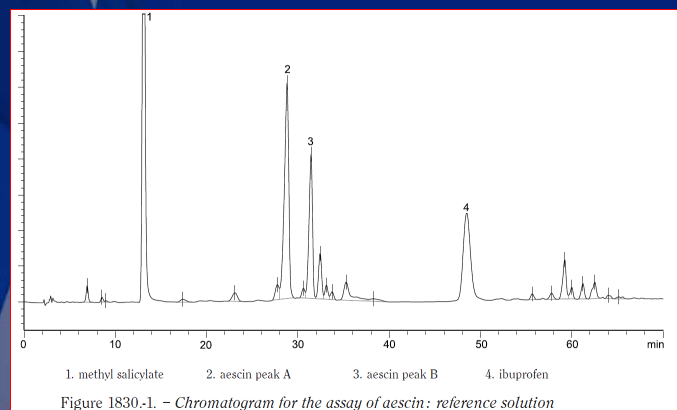


# Horse-chestnut (1)

## Current situation

- The colorimetric method as published in the German Pharmacopoeia is widely recognised.
- The applied limits are the following:
  - Horse-chestnut: minimum 3.0 % of triterpene glycosides, expressed as aescin (dried drug);
  - Horse-chestnut dry extract, standardised: 16.0 %-20.0 % of triterpene glycosides, expressed as aescin (dried extract).

# Horse-chestnut (2): HPLC method



## Horse-chestnut (3)

- A changeover from the colorimetric method to a more specific and precise HPLC method is envisaged.
- Difficulty:  
Different content values are obtained with the more specific HPLC method in comparison to the colorimetric method.



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## Some results ...

Batch	UV %	HPLC %	Ratio
#1	17.6.	10.5	1.7
#2	18.9	12.0	1.6
#3	18.1	11.1	1.6
#4	16.1	9.4	1.7
#5	17.6	11.2	1.6
#6	18.6	12.4	1.5
Average	17.8	11.1	1.6



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## Assay – transition (1)

- A close collaboration with assessors and industry is necessary:
  - for the determination of the HPLC content values;
  - for the change in labelling of products.
  - Publish new method as non-mandatory method B in addition to the existing method and collect data using both in parallel
  - Review data when adopting new content specification



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## Assay – transition (2)

- For more details see Pharmeuropa: 20.3
- Apply the same principle to the respective herbal drug as well to keep consistency between herbal drug and herbal drug preparation
- Under discussion: Choice of reference standard



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## Similar projects ongoing

- Rhubarb
- Senna



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## Rhubarb (1)

- Current situation:
- Photometric determination of hydroxyanthracene derivatives
- The total of hydroxyanthracene derivatives is determined after hydrolysis in the form of the aglycones. Anthraquinone glycosides and anthraquinone aglycones are **not determined separately**.



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## Rhubarb (2)

- Anthraquinone aglycones
  - They are absorbed in the intestine
  - They are concerns as regards their toxicity
  - ➔ proposal: Anthraquinone aglycones should be limited.

## Rhubarb (3)

- A changeover from the photometric determination of hydroxyanthracene derivatives, in the form of the aglycones, to a HPLC method, determining the content of hydroxyanthracene glycosides in parallel to the aglycones, is envisaged.

## Extract classification in Ph.Eur.

- **Standardised Extracts**
  - ▲ Constituents of known therapeutic activity
- **Quantified extracts**
  - ▲ Active markers
- **Other Extracts**
  - ▲ Analytical markers



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## Assay methods for “other extracts” (1)

HMPC reflection paper on markers:

- Marker suitable for herbal substances may not be suitable for herbal preparation or finished product
- Analytical markers serve for analytical purposes only



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## “Other extracts” (2)

- Consequently, the analytical marker described by a Ph.Eur. extract monograph may not be useful for all applications
- Analytical interference with other ingredients
- Already authorised products broadly documented using other markers would need to be updated (variations), when the monograph is published
- Revision of the General monograph is envisaged



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## Other extracts (3)

- Revision proposal: State that the use of the given analytical marker is not mandatory but may be replaced by other suitable markers with limits to be justified by the applicant
- Monograph could still serve as a validated analytical platform for regulatory submissions
- Consider renaming this class e.g. “Characterised extracts”



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## Recently adopted texts



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## Aristolochic acids

- The general method “Test for aristolochic acids in herbal drugs (2.8.21)” is divided in 3 subsections:
  - Method A: Screening test for aristolochic acids (HPTLC);
  - Method B: Limit test for Aristolochic acid I (HPLC)
  - Method C: Confirmation test for aristolochic acid I (LC-MS)



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## Aristolochic acids

The limit for all 3 methods is set at  
2 ppm  
due to the Limit of Quantification



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## Aristolochic acids

The general method is intended to  
detect adulteration of herbal drugs by  
aristolochic acid containing species and  
is **not** for use as an assay method for  
those species producing aristolochic  
acids as secondary metabolites.



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## Ochratoxin A

- NEW (1/2010):
- “Determination of ochratoxin A in herbal drugs (2.8.22.)”  
using HPLC with FLD



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## Ochratoxin A

- Maximum limits on ochratoxin A can be required in specific monographs:
  - Liquorice root: maximum 20µg/kg
  - under discussion:  
Liquorice extract monographs.



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## Heavy metals (1)

### General limits adopted:

- 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
  - Exemptions in specific monographs where necessary
  - (see presentation Melanie Bald, EDQM)



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## Heavy metals (2)

- General method “Heavy metals in herbal drugs and fatty oils”
- Currently only atomic absorption spectrometry
- Will be revised to embrace
- ICP-AES and ICP-MS



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## Microbiological quality

### Why change ?

- Methods changed during International Harmonisation
- Categories of chapter 5.1.4 have been interpreted divergently
- NEW ( details see presentation Dr Helliwell)
- Microbiological examination of medicinal products containing herbal drugs (2.6.31)
- Microbiological quality of medicinal products containing herbal drugs (5.1.8)



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## Interaction HMPC-EDQM

- Mutual participation of observers
- Meeting of Ph.Eur. Chairpersons and with QDG
- Early HMPC input and comments on draft Ph.Eur. Monographs



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## Items discussed

- Establishing a link between “traditional” and modern analytical assay methods
- Classification of a particular extract
- Choice of relevant “markers”
- Harmonisation of solvent ranges for extraction between Ph.Eur. and HMPC monographs



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## Conclusion

- Ph. Eur. regularly updated
  - to provide state-of-the-art methods
  - in response to public health concerns
- Regulatory issues linked to changes of analytical methods and choice of markers have been identified
- Transition mechanisms foreseen
- Close co-operation between regulators, HMPC and EDQM working parties



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**Thank you!**



## **Introduction of New Assay Methods in the European Pharmacopoeia and Regulatory Implications - Industry Viewpoint**

EDQM Symposium on Herbal Drugs  
and Herbal Drug Preparations  
**Vienna, Austria, 25 September 2009**  
Dr Barbara Steinhoff (BAH, Bonn)  
on behalf of AESGP,  
the European Self-Medication Industry



### **AESGP: Who are we?**

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- AESGP represents the manufacturers of non-prescription medicines in Europe
- Founded in 1964; offices in Brussels
- Member of WSMI (World Self-Medication Industry)

#### AESGP Mission:

- To create a favourable climate for the growth of the self-care market
- To ensure that consumers receive quality medicines by respecting state-of-the-art standards in pharmaceutical manufacturing



## **General Recommendations**

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AESGP support all initiatives improving the pharmaceutical quality of medicines

- NTA/ CTD discussions
- EMEA hearings
- EDQM projects
- WHO activities
- ICH initiatives, e.g. PDG



## **Introduction of new assay methods**

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- Modern analytical methods represent recent scientific knowledge and new technical standards
- Suitable for integration into automated/computerised processes
- Control of consistent quality of herbal drugs and herbal drug preparations
- Inclusion of new analytical methods into EP monographs desirable



## Relation between Quality and Efficacy Monographs (I)

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- HMPC and ESCOP monographs
- Established dosage recommendations (derived from clinical studies, bibliography, tradition)
- Based on the **established** and/or conventional methods
- Mostly unspecific pharmacopoeial methods



## Relation between Quality and Efficacy Monographs (II)

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- No information to which analytical method the dosage recommendations of an HMPC/ESCOP monograph refer to
- Close link between HMPC/ESCOP and EP monographs important
- Assessment by regulatory authorities
- Practical aspects for manufacturers
- Important for "standardised" extracts



## Relation between Quality and Efficacy Monographs (III)

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### Standardised extracts

Defined content of a particular substance/  
group of substances (on which the dosage  
recommendation is based), e.g.

- Senna: hydroxyanthracene glycosides expressed as sennoside B
- Horse-chestnut: triterpene glycosides expressed as aescin
- Milk-thistle: silymarin expressed as silibinin



## Companies' experiences

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- New analytical methods e.g. HPLC normally have a higher selectivity and specificity
- Typically lower assay values
- Established methods e.g. photometry: higher assay values
- Gap between standardisation and published dosage recommendations



## Companies' experiences

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- New Ph.Eur. method and established method:
  - reference to the HMPC and ESCOP monograph
  - but: additional work
- Data on the differences in assay values: modern, more selective methods (e.g. HPLC) as compared to established methods (e.g. photometry)



## Proposal for solution (I)

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Correlation between results obtained with established and new methods

→ Link between EP monographs and dosage recommendations in HMPC/ESCOP monographs

→ **Proposal to introduce conversion factors between new and established analytical method**



## **Proposal for solution (II)**

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### **Introduction of conversion factors**

- 1. Publication Gaedcke F, Stahl-Biskup E, Lang F et al. Pharmeuropa Vol. 20 No. 1 (January 2008) with examples
- 2. Laboratory comparison for a Milk Thistle extract with approx. 75 % silymarin: Gaedcke F, Hubbert M, Kurth H et al. Pharmeuropa Scientific Notes 1-2009, 5-8



## **European Herbal Industry's viewpoint**

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- Determination of a conversion factor and inclusion into EP monograph useful
- High relevance for standardised extracts
- Cases where health authorities ask for specific methods during stability testing (detection of changes in composition vs. reference to clinical studies)



## Example St. John's Wort Extract

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- DAC 1986/1991: photometric assay of dianthrones calculated as hypericin
- EP: hypericin and pseudohypericin determined by HPLC (summed up)
- By HPLC lower values obtained
- Mean factor 1.3 determined for a methanol 80 % extract\*)

\*) Pharmeuropa Vol. 20 No. 1 (January 2008)



## Example Milkthistle Extract

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- DAB 10: determination of flavanol derivatives (calculated as silymarin) by photometric method
- EP: separate determination of silibinin A and B, isosilibinin A and B, silidianin and silichristin
- EP method more selective, with lower values
- Change of labelling (from 70 % to 53.9-58.3 %, depending on the factor used)
- By recent publication\*) conversion factor of 1.3 confirmed

\*) Pharmeuropa Scientific Notes 2009-1



## ESCOP Monograph „Milkthistle Fruit“

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- Published August 2009 (Second edition, Supplement 2009, 35 new and revised monographs)
- Milk Thistle Fruit and Milk Thistle Dry Extract
- Definition according to EP „the material complies with the monograph ...“
- Determination method HPLC



## ESCOP Monograph „Milkthistle Fruit“

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### Important Note:

“All "silymarin" contents reported in this monograph were analyzed by the UV spectroscopic method of the Deutsches Arzneibuch for the herbal drug because this was the standard before a monograph for "Milk Thistle Dry Extract" (with an HPLC method) appeared in the Deutsches Arzneibuch in 2001, superseded in 2007 by the monograph of the European Pharmacopoeia.

The HPLC method of the European Pharmacopoeia usually gives lower values for silymarin (defined as the sum of the peak areas of silibinins A and B, isosilibinins A and B, and silicristin and silidianin; calculated as silibinin), amounting to only about 77 per cent of the value indicated by the UV spectroscopic method.”



## Development of Conversion Factors (I)

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- Inclusion of a range of batches of a herbal drug/herbal drug preparation (variability by nature or manufacture)
- Comparison of results obtained with both methods for each type of preparation
- Pairs of values; determination of factors; mean factor; deviation from the mean
- Applicability of a factor depends on the method



## Development of Conversion Factors (II)

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- Testing in laboratory comparisons: agreement on e.g. sample preparation necessary to avoid differences
- Certain variability exists
- **Inspite this variability the introduction of a conversion factor (mean factor) appears to be an acceptable compromise for a correlation between the results**



## **Advantages of Conversion Factors**

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- Technical and analytical progress (use of new methods)
- Reference to the dosage given in HMPC and ESCOP monograph (related to the established method)
- In addition: mention the analytical method used also in the dosage recommendations of HMPC and ESCOP monographs



## **Pharmeuropa draft monograph "Aesculus" (I)**

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- Pharmeuropa Vol. 20 No. 3 (July 2008):  
"Horse-Chestnut" and "Horse-Chestnut Dry Extract, standardised"
- Assay A (photometry) to be used to demonstrate compliance with the monograph
- Assay B (HPLC) for stability testing
- Use of both methods in parallel and collection of data on both analytical methods within a certain period



## Pharmeuropa draft monograph "Aesculus" (II)

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- Evaluation and development of new content specification based on collected data
- Switch-over to method B
- Industry appreciates option to use both methods in parallel („smooth transition“)
- Industry would like to support the EDQM with respective data once a call for submission of data has been published (2011)

AESGP 

## Conclusion

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In order to establish a correlation between EP monographs which use modern analytical methods such as HPLC, and the dosage recommendations in HMPC/ESCOP monographs which are mainly based on established methods (e.g. photometry), a conversion factor should be determined and included into the respective EP monograph.

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## New practical experience with instrumental methods recently introduced into the European Pharmacopoeia

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Germany

Vienna, September 25<sup>th</sup>, 2009



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PhytoLab

## Overview of topics

- Introduction
- New methods in pharmacognosy (Ph.Eur. 2.08.)
  - methods
  - practical experience
- New assay methods in individual monographs
  - introduction
  - examples of herbal drug and extract monographs
  - practical experience
- Conclusion

EDQM Symposium, Vienna 2009, Dr. Bernhard Klier

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## Introduction

In the last few years new analytical methods concerning herbal drugs and herbal drug preparations has been introduced to the European Pharmacopeia (Ph.Eur.) and some existing methods have been revised.

There are some **new methods in the general part** of the Ph.Eur. containing methods for determination of contaminants in herbal drugs.

At the same time modern analytical methods such as more selective LC assays replace unspecific conventional **assays in individual monographs**.

In the following some examples for new methods will be shown and the experience in practise will be discussed.



## New methods – overview

Determination of aflatoxin B<sub>1</sub> **in herbal drugs**, 2.8.18 ( Ph.Eur. 5.7)

Determination of ochratoxin A **in herbal drugs**, 2.8.22 (Ph.Eur. 6.6)

Pesticide **residues**, 2.8.13 (Ph.Eur. 6.2)

Microbiological examination of **herbal medicinal products** for oral use, 2.6.31 (Ph.Eur.6.7)

Microbiological quality of **herbal medicinal products** for oral use, 5.1.8 (Ph.Eur. 6.7)



## New methods planned – overview

Test for aristolochic acid I in herbal drugs, 2.8.21 (PHARMEUROPA 19.4)

Determination of pyrrolizidine alkaloids (TCM group)

Heavy metals in herbal drugs and fatty oils, 2.4.27 (Ph.Eur. 4.0; request of revision)

Microscopic examination of herbal drugs, 2.8.23 (draft) in combination with illustrations of microscopic pictures (individual monograph).



## New methods in pharmacognosy (Ph.Eur. 2.08.)

## Determination of aflatoxin B<sub>1</sub> in herbal drugs

The HPLC-method described is suitable for **devil's claw root, ginger and senna pods**. Its suitability for other herbal drugs (matrix groups) must be demonstrated.

Another validated method could be used.

Herbal drugs that are subject to contamination by aflatoxin B<sub>1</sub> are tested by a validated method.

Unless otherwise indicated in the monograph, herbal drugs contain **not more than 2 µg/kg of aflatoxin B1** (4 µg/kg for the sum of aflatoxins B1, B2, G1 and G2 in Germany).

## Determination of ochratoxin A<sub>1</sub> in herbal drugs

The HPLC-method described is suitable for **liquorice extract and liquorice root**. Its suitability for other herbal drugs (matrix groups) must be demonstrated.

Another validated method could be used.

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

Limits for Ochratoxin A are given in individual monographs (**liquorice root: 20µg/kg** and liquorice dry extract (DER 4:1) 80µg/kg; liquorice liquid extract (DER 2:1) 40µg/kg).



## Experience in practise

New methods for determination of aflatoxins and ochratoxin A are working well.

Method validation of matrix groups and/or specific matrices (e.g. resins, essential oils) has to be done.

Monitoring of herbal drugs is necessary.

Harmonised **Maximum Residue Limit (MRL)** of aflatoxin B<sub>1</sub>, but MRL of sum of aflatoxins could be set by national authorities  
(Commission Regulation (EC) No. 1881/2006, SANCO/06326/2009).

MRL of Ochratoxin A only in liquorice monographs. MRL`s for other products are still missing  
(Commission Regulation (EC) No. 1881/2006, SANCO/00875/2007).



## Pesticide residues (Revision of Ph.Eur. 4.0)

The monograph pesticide residues 2.8.13 had been introduced to Ph.Eur. in 1996. The monograph has been revised in Ph.Eur. 6.2:

- Sampling according Ph.Eur. 2.8.20 has been added.
- Expanding number of substances in table 2.8.13-1 to **115 pesticides (70 MRL`s)**.
- Method for determination of pesticides has been deleted.
- Method validation according to **SANCO/10232/2006**.
- Cross reference to European Food Law (**Reg. (EC) No. 396/2005**).
- Formula for calculation of residues in herbal drug preparations has been added.

## 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/2007/3131](#) (update).

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

Harmonised maximum residue limits (MRL`s) in Europe:

[Commission Regulation \(EC\) No. 396/2005](#).

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

## Microbiology

In 2009 the ICH harmonisation process of microbiological methods and microbiological acceptance criteria came into force but herbal medicinal products were out of this harmonisation process.

Publication of separate monographs in Ph.Eur. 6.7 takes into account the specific situation of herbal products and is of an essential advantage in practise:

Microbiological examination of **herbal medicinal products** for oral use, **2.6.31**

Microbiological quality of **herbal medicinal products** for oral use, **5.1.8**



## Methods in preparation/planned



## Test for aristolochic acid I in herbal drugs, 2.8.21 (PHARMEUROPA 19.4)

Test for aristolochic acid I contains three different methods:

- method A: Screening test for aristolochic acids (HPTLC)
- method B: Limit test for aristolochic acid I (HPLC-UV)
- method C: Confirmatory test for aristolochic acid I (LC-MS)

Methods A and B are intended to be used in screening of herbal drugs (TCM drugs) with the objective to detect adulterations with plant material containing aristolochic acids.

Method C is provided as a method to confirm the presence of aristolochic acid.

## Test for aristolochic acid I in herbal drugs, 2.8.21 (PHARMEUROPA 19.4)

Aristolochic acid I is an applicable marker for determination of aristolochic acids content but is not the main compound in either case (e.g. Asari rhizoma).

Method A and B have a limit of detection (LOD) of about 2 mg/kg aristolochic acid I in herbal drugs;  
Method C has a LOD of about 0,1 mg/kg in herbal drugs.

This is the first time describing a LC-MS method for analysing herbal drugs in Ph.Eur. But method C is only used for confirmation of positiv results.

## Determination of pyrrolizidine alkaloids (TCM group)

These test will be used for detection of adulterations of TCM drugs with plant material containing pyrrolizidine alkaloids (PAs) or for analysing the total content of PA containing herbal drugs (TCM).

A GC-MS method for analysis of pyrrolizidine alkaloids (PAs) and their N-oxides has been tested with Arnebia root samples.

The tested method has a limit of detection of about 1 mg/kg and gives the opportunity to analyse the total content of PAs with different chemical structures in low concentrations.

Further tests and method validation has to be done.

## Heavy metals in herbal drugs and fatty oils, 2.4.27 (Ph.Eur. 4.0; request for revision)

There is a request to describe alternative methods for determination of heavy metals in herbal drugs:

In addition to existing **AAS** method (Atomic Absorption Spectrometry)

**ICP-AES** (Inductively Coupled Plasma - Atomic Emission Spectrometry)  
and **ICP-MS** (Inductively Coupled Plasma - Mass Spectrometry)

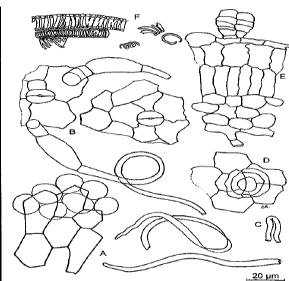
are currently be used in laboratories.

These two methods should be added to monograph 2.4.27.

## Microscopic examination of herbal drugs - illustrations of microscopic pictures

General method for microscopic examination of herbal drugs (2.8.23, drafted) in combination with illustrations in individual monographs (sukzessive indroduction) supports laboratory technicians by interpretation of microscopic pictures.

EUROPEAN PHARMACOPOEIA 5.7



A. Upper epidermis of lamina in surface view  
B. Lower epidermis of lamina in surface view showing stomata and glandular trichomes  
C. Fragment of covering trichomes  
D. Multicellular, biciliate, glandular trichome in surface view  
E. Section view of lamina showing a glandular trichome  
F. Fragments of vascular tissue  
Figure 1866-1. — Illustration for the identification of artichoke leaf (see Identification B)



## New assay methods in individual monographs



## Introduction

Since a complete qualitative and quantitative description of a herbal drugs complexity is impossible its quality is largely defined by the entire production process.

Quality control of herbal medicinal products relies on both qualitative fingerprint analyses and quantitative analyses based on **phytochemical markers**. Markers are the thread that ties together production processes and quality control.



## Definiton of Marker

- **Markers** : are chemically defined constituents or groups of constituents ... which are of interest for control purposes independent of whether they have any therapeutic activity.
- “**Active marker** : are constituents or groups of constituents which are generally accepted to contribute to the therapeutic activity.”
- “**Analytical marker** : are constituents or groups of constituents that serve for analytical purposes.

(EMA/CPMP/QWP/2819/00 Rev 1, 30.03.2006)



## Definition of extracts

In the Ph.Eur. monograph „Extracta“ three different types of extracts are defined:

- **Standardised extracts** (active marker)
- **Quantified extracts** (analytical and active marker)
- **Other extracts** (analytical marker)

The extract types are different in **definition and efficacy of constituents**, production process and quality specification. The nature of the marker affects also the assay method and definition of the monograph.

## Valerian Ph.Eur. monographs

(valerian root, valerian dry hydroalcoholic extract,  
valerian dry aqueous extract)

## Valerian monographs

**Analytical marker** in valerian monographs are sesquiterpenic acids expressed as valerenic acid

(Valerenic acid, acetoxyvalerenic acid, hydroxyvalerenic acid)

01/2008:0453  
corrected 6.0

**VALERIAN ROOT**  
Valerianae radix

**DEFINITION**  
Dried, whole or fragmented underground parts of *Valeriana officinalis* L. s.l., including the rhizome surrounded by the roots and stolons.

**Content:**

- whole or fragmented drug:
  - essential oil: minimum 4 ml/kg (dried drug);
  - sesquiterpenic acids: minimum 0.17 per cent m/m, expressed as valerenic acid ( $C_{15}H_{20}O_2$ ;  $M_r$  234.3) (dried drug);
- cut drug:
  - essential oil: minimum 3 ml/kg (dried drug);
  - sesquiterpenic acids: minimum 0.10 per cent m/m expressed as valerenic acid ( $C_{15}H_{20}O_2$ ;  $M_r$  234.3) (dried drug).

**Valerianae extractum hydroalcoholicum siccum**

**DEFINITION**

Extract produced from *Valerian root (0453)*.

*Content:* minimum 0.25 per cent *m/m* of sesquiterpenic acids, expressed as valerenic acid ( $C_{15}H_{22}O_2$ ; *M*, 234.3) (dried extract).

**Valerianae extractum aquosum siccum**

**DEFINITION**

Extract produced from *Valerian root (0453)*.

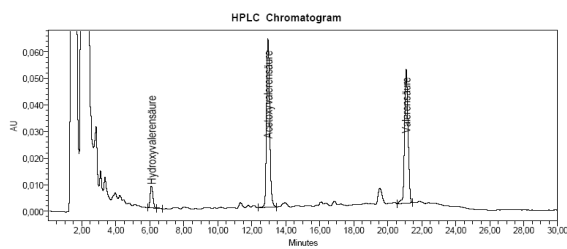
*Content:* minimum 0.02 per cent of sesquiterpenic acids expressed as valerenic acid ( $C_{15}H_{22}O_2$ ; *M*, 234.3) (anhydrous extract).

**Valerianae tinctura**

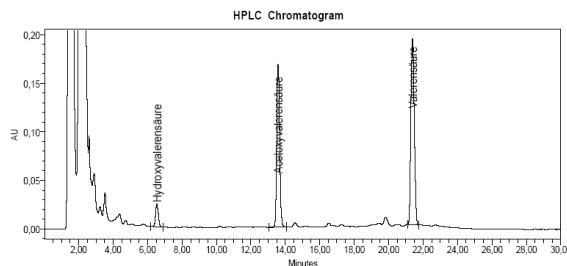
**DEFINITION**

Tincture produced from *Valerian root (0453)*.

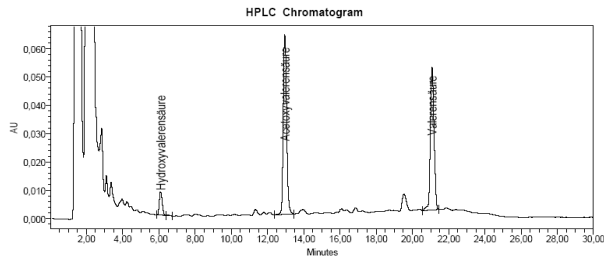
*Content:* minimum 0.015 per cent *m/m* of sesquiterpenic acids, expressed as valerenic acid ( $C_{15}H_{22}O_2$ ; *M*, 234.3).



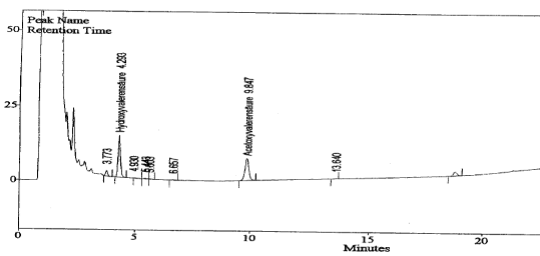
HPLC-chromatogram valerian root (Ph.Eur.)



HPLC-chromatogram valerian dry hydroalcoholic extract (Ph.Eur.)



HPLC-chromatogram valerian root (Ph.Eur.)



HPLC-chromatogram valerian dry aqueous extract (Ph.Eur.)

### Valerian monographs, definition of analytical marker

Marker	valerian root	valerian hydroalcoholic extract	valerian aqueous extract	valerian tincture
valerenic acid	x	x	x	x
acetoxyvalerenic acids	x	x	x	x
hydroxyvalerenic acid		x	x	x
content of sesquiterpenic acids	min. 0,17%	min. 0,25%	min. 0,02%	min. 0,015%

## Valerian monographs, comments

Assay method is harmonised in all valerian monographs, but content of **analytical marker** substances are variable in different herbal drug preparations.

Valerenic acid CRS should be used as reference standard in place of **valerian standardised dry extract CRS**.

Since the constituents determined for „other extracts“ were only analytical marker, other analytical marker as proposed in the specific monograph may be chosen.

## Melissa Ph.Eur. monographs (melissa leaf, melissa leaf dry extract)

## Melissa monographs

Analytical marker in melissa monographs is rosmarinic acid.

The draft monograph of Melissa leaf dry extract, quantified has not been adopted (no well established use and no traditional use described in HMPC monograph).

### MELISSA LEAF DRY EXTRACT

Melissae folii extractum siccum

**DEFINITION**

Dry extract produced from *Melissa leaf* (1447).  
Content: minimum 2.0 per cent of rosmarinic acid ( $C_{18}H_{16}O_8$ ;  $M_r$  360.33) (dried extract).

### ~~MELISSA LEAF DRY EXTRACT, QUANTIFIED~~

~~Melissae folii extractum siccum quantificatum~~

**DEFINITION**

~~Quantified dry extract produced from *Melissa leaf* (1447).  
Content: 3.0 per cent to 6.0 per cent of rosmarinic acid ( $C_{18}H_{16}O_8$ ;  $M_r$  360.33) (dried extract).~~

### ~~MELISSA LEAF~~

~~Melissae folium~~

**DEFINITION**

~~Dried leaf of *Melissa officinalis* L.  
Content: minimum 4.0 per cent of total hydroxycinnamic derivatives, expressed as rosmarinic acid ( $C_{18}H_{16}O_8$ ;  $M_r$  360.3) (dried drug).~~

04/2009:1447

### MELISSA LEAF

Melissae folium

**DEFINITION**

Dried leaf of *Melissa officinalis* L.  
Content: minimum 1.0 per cent of rosmarinic acid ( $C_{18}H_{16}O_8$ ;  $M_r$  360.3) (dried drug).

## Melissa monographs

The HPLC assay replaced the spectrophotometric assay in melissa leaf.

## Assay results

PA-Nr.	product	spectrophotometry in %	HPLC in %	factor
		calc. to dried drug	calc. to dried drug	HPLC/photometry
3594696	MELISSENBLAETTER	4,6	1,87	0,41
5466520	MELISSENBLAETTER	8,4	3,18	0,38
5490995	MELISSENBLAETTER	7,5	2,76	0,37
6847161	MELISSENBLAETTER	9,1	2,9	0,32
6162215	MELISSENBLAETTER	9,41	2,94	0,31
6881335	MELISSENBLAETTER	8,96	2,4	0,27
3600297	MELISSENBLAETTER	7,2	1,87	0,26
5374200	MELISSENBLAETTER	4,1	0,98	0,24
5571359	MELISSENBLAETTER	7,78	1,85	0,24
5509173	MELISSENBLAETTER	5,2	1,15	0,22
5841666	MELISSENBLAETTER	5,81	1,28	0,22
5952085	MELISSENBLAETTER	4,04	0,77	0,19
3593851	MELISSENBLAETTER	4,9	0,87	0,18
6476511	MELISSENBLAETTER	6,2	1,05	0,17
3759385	MELISSENBLAETTER	4,9	0,81	0,17

## Melissa monographs, comments

Assay method is harmonised in both melissa monographs; rosmarinic acid is **analytical marker** substance.

Content of **analytical marker** substance rosmarinic acid is quite different depending on method used; there is no correlation factor found between both methods:

$$\text{HPLC/photometry} = 0,2 \text{ to } 0,4 / 1$$

Since the constituents determined for „other extracts“ were only analytical marker, other analytical marker as proposed in the specific monograph may be chosen.

## Hypericum Ph.Eur. monographs (St. John`s wort, St. John`s wort dry extract, quantified)

## Hypericum monographs

### Hyperici herbae extractum siccum quantificatum

#### DEFINITION

Quantified dry extract obtained from *St. John`s wort (1438)*.

#### Content:

- *total hypericins, expressed as hypericin (C<sub>30</sub>H<sub>16</sub>O<sub>6</sub>; M<sub>r</sub> 504.5): 0.10 per cent to 0.30 per cent (dried extract);* } active marker
- *flavonoids, expressed as rutin (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>; M<sub>r</sub> 610.5): minimum 6.0 per cent (dried extract);* } analytical marker
- *hyperforin (C<sub>36</sub>H<sub>20</sub>O<sub>4</sub>; M<sub>r</sub> 536.8): maximum 6.0 per cent (dried extract).*

## Hypericum monographs

The **active marker** substance in hypericum monographs is **hypericin** (different methods in herbal drug and extract).

Additional **analytical markers** in the extract are flavonoids and hyperforin.

To adjust the content of three markers is rather difficult because quantified extracts could only be adjusted by mixing of extracts. It should be reconsidered which assay methods are necessary for the herbal drug.

### Hyperici herba

#### DEFINITION

Whole or cut, dried flowering tops of *Hypericum perforatum* L., harvested during flowering time.

*Content:* minimum 0.08 per cent of total hypericins, expressed as hypericin ( $C_{20}H_{18}O_6$ ; *M*, 504.4) (dried drug).

## Horse-chestnut, Horse-chestnut dry extract, standardised



## Horse-chestnut monographs, drafted

### HORSE-CHESTNUT

Hippocastani semen

#### DEFINITION

Whole or fragmented, dried, ripe seeds of *Aesculus hippocastanum* L.

Content: minimum 2.5-3.0 per cent of triterpene glycosides, expressed as anhydrous aescin (dried drug).

### HORSE-CHESTNUT DRY EXTRACT, STANDARDISED

Hippocastani seminis extractum siccum normatum

#### DEFINITION

Standardised dry extract produced from *Horse-chestnut* (1830).

Content: 10.0 per cent to 15.0 per cent 16.0 per cent to 20.0 per cent of total triterpene glycosides, expressed as anhydrous aescin (dried extract).

### HMPC-monograph

#### Well-established use

#### Posology

#### Adults and elderly

Extract (standardised to a content of 50 mg triterpene glycosides calculated as aescin) 2 times daily.

(PHARMEUROPA Vol. 20, No. 3, July 2008, Horse-chestnut)



## Horse-chestnut monographs, comments

Content and dosage recommendations given in the HMPC monographs usually refer to established pharmacopoeial methods (mainly conventional assay methods). It has been demonstrated that these methods are less accurate and reliable than modern LC methods.

With more selective methods (e.g. HPLC) lower assay values are expected and no linear correlation may be possible.

A transition of methods is only possible by analysing both methods in parallel over a period of time. With the collection of data a correlation factor between the two methods could be found (e.g. Milk thistle).

The monographs Horse-chestnut and Horse-chestnut dry extract are the first examples of this approach (senna, rhubarb?).



## Conclusion



## Conclusion: new methods in pharmacognosy

New methods and updates of methods in pharmacognosy on a regular basis are necessary.

New individual monographs generate the need of new analytical methods (e.g.TCM monographs).

Some important methods have been introduced into Ph.Eur. or have been revised and further methods are in preparation.

## Conclusion: new assay methods

Analytical methods of the European Pharmacopoeia must take into account the current state of current knowledge. More selective HPLC methods will replace unspecific conventional methods to ensure consistent quality of herbal drugs and herbal drug preparations and to provide meaningful data concerning their stability.

This would lead to inconsistencies with existing documents concerning regulatory affairs of herbal medicinal products (e.g. HMPC monographs, specifications, dosages, ... etc. ).

A smooth transition process has been proposed.

([PHARMEUROPA Vol. 20, No. 3, July 2008, Horse-chestnut, note on the monograph](#))

Thank you for your interest!



## **Regulatory implications of the introduction of new assay methods in the European Pharmacopoeia**

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Burt Kroes

### **Disclaimer**

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“I attend this conference as an individual, and do not represent the HMPC/MEB. The views expressed here are my personal views, and may not be understood or quoted as being made on behalf of the HMPC/MEB or reflecting the position of the HMPC/MEB”

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## Outline

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- Legal framework
    - Directives
    - Guidelines
  - Regulatory implications
  - Implementation
- 

## Directive 2001/83/EC

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*... "The monographs of the European Pharmacopoeia shall be applicable to all substances, preparations and pharmaceutical forms appearing in it"*

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## **Directive 2003/63/EC**

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... *“With respect to the quality part (chemical, pharmaceutical and biological) of the dossier, all monographs including general monographs and general chapters of the European Pharmacopoeia are applicable”*.

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## **2003/63 /EC: Herbals**

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### *Module 3*

- The provisions of Module 3, including **compliance with monograph(s) of the European Pharmacopoeia, shall apply to the authorisation of herbal medicinal products.**

The state of scientific knowledge at the time when the application is lodged shall be taken into account.

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**Guideline on summary of requirements of active substances in the quality part of the dossier**  
**CHMP/QWP/297/97 rev 1. corr**  
**EMA/CVMP/1069/02**

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For existing active substances described in the European pharmacopoeia .....each batch of these substances must comply with the current requirement of the European pharmacopoeia ....

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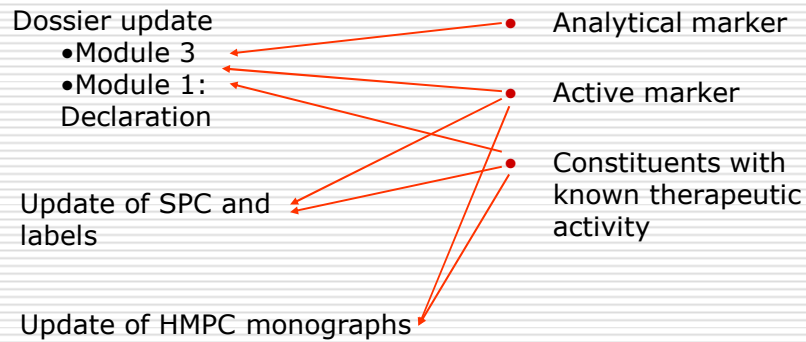
## **Outline**

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- Legal framework
    - Directives
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  - Regulatory implications
  - Implementation
-

## Regulatory implication of a change in specifications of the active substance

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## Regulatory implication: Quality

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- **Module 3: Update specifications in accordance with the Variation Guideline**
    - S- Part Specifications of starting material/ active substance =>Type 1A (nr; 25) variations
    - P-part Specifications of the finished product =>Type 1A , Type II
-

25	Change to comply with European Pharmacopoeia or with the national pharmacopoeia of a Member State	Conditions to be fulfilled	Documentation to be supplied	Procedure type
	Active substance	1, 2	1, 2	IA
<b>Conditions</b>				
1	The change is made exclusively to comply with the pharmacopoeia.			
2	Unchanged specifications (additional to the pharmacopoeia) for product specific properties (e.g. particle size profiles, polymorphic form), if applicable.			
<b>Documentation</b>				
1	Amendment to relevant section of Part IIC or equivalent in the CTD format.			
2	Comparative table of current and proposed specifications.			
3	Batch analysis data on two production batches of the relevant substance for all tests in the new specification.			
4	Data to demonstrate the suitability of the monograph to control the substance, e.g. a comparison of the potential impurities with the transparency note of the monograph.			
5	Where appropriate, batch analysis data (in a comparative tabulated format) on two production batches of the finished product containing the substance complying with the current and proposed specification and additionally, where appropriate, comparative dissolution profile data for the finished product on at least one pilot batch. For herbal medicinal products, comparative disintegration data may be acceptable.			

## Dossier update: Variation Procedures / Timeframes

- Type IA: 'Do and Tell' approach Notify within 12 months Immediate notification for certain Type IA 30 days timetable to 'review'
- Type II: An approval has to be waited for (Timeframe: 90 days)

COMMISSION REGULATION (EC) No 1234/2008  
of 24 November 2008

## Regulatory implication: Quality (2)

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- Module 3: Update of the specifications in accordance with the Variation Guideline
    - S- Part Specifications of starting material/ active substance =>Type 1A (nr; 25) variations
    - P- part Specifications of the finished product =>Type 1A , Type II
  - Implementation in ongoing stability studies (Finished products)
- 

## Different types of stability testing

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Study type	R&D study	Follow-up (commitment) Study	Ongoing studies
<b>Time point</b>	Before registration	After submission of dossier	After registration
<b>Aim</b>	Setting shelf-life	Verify registration data	Proof that conditions are still valid
<b>Background</b>	ICH	ICH	EU-GMP guideline
<b>Competent Authorities</b>	Regulatory agency	Regulatory agency	National GMP surveillance

Source <http://www.iptonline.com/articles/public/pg78nonprint.pdf>

## Ongoing stability studies GMP

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- Annual Product Quality review is compulsory for all licensed product (Chapter 1 of the EU GMP guideline). Applies to all medicinal products: **no exception for herbal medicinal product**
  - Test have to carried out continuously, usually **one batch a year for each package**
  - Studies have to be carried out under long-term conditions e.g 25 °C and 60% RH over the period of the labelled shelf life.
- 

## Regulatory implications: *Update of Module 1*

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- Declaration of the active substance
  - Change in SPC
    - Declaration of active substance (Quantified and standardized)
    - Posology (Standardized preparations)
-

**GUIDELINE ON DECLARATION OF HERBAL SUBSTANCES AND HERBAL PREPARATIONS IN HERBAL MEDICINAL PRODUCTS/TRADITIONAL HERBAL MEDICINAL PRODUCTS IN THE SPC**

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□ **Standardized herbal preparations**

*e.g..... contains 140 mg - 190 mg of extract (as dry extract) from *Aesculus hippocastanum* L., semen (Horse chestnut seed) corresponding to 38 mg triterpene glycosides, calculated as anhydrous  $\beta$ -aescin*

□ **Quantified herbal preparations**

*e.g ....contains 2.5 g of various species of genus *Salix* including *S. purpurea* L., *S. daphnoides* Vill. and *S. fragilis* L, cortex ...corresponding to 37.5 mg to 42.5 mg of total salicylic derivatives, calculated as salicin.*

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[EMEA/HMPC/CHMP/CVMP/287539/05](#) rev 1

**Regulatory implications: *HMPC monographs***

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- Section 2 . Qualitative and quantitative composition
    - active markers
    - constituents with known therapeutic activity
  - Section 4.2: Posology
    - Constituents with known therapeutic activity
-

## **Implications for HMPC monographs** ***Standardized extracts***

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- Section 2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Senna leaf consists of the dried leaflets of *Cassia senna* L. (*Cassia acutifolia* DELILE), known as Alexandrian or Khartoum senna, or *Cassia angustifolia* VAHL, known as Tinnevely senna, or a mixture of the two species. It contains not less than **2.5 per cent of hydroxyanthracene glycosides**, calculated as sennosides B (Mr 863) with reference to the dried herbal substance

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## **Implications for HMPC monographs** ***Standardized extracts***

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- Section 4.2: Posology

### **Dosage**

The maximum daily dose of hydroxyanthracene glycosides is **30 mg**. This is equivalent to ....(dose of the preparation).

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## Implications for HMPC monographs *Quantified extracts e.g. Willow bark*

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### □ Section 2 Qualitative and quantitative composition

*E.g. Dry extract (8-14:1) extraction solvent ethanol 70% V/V, 15% total salicin.*

### □ Section 4.2 posology

*Adults, elderly*

*The daily dose is 1572 mg dry extract (8-14:1)*

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## Regulatory implications: overview

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Preparation	Quality part Dossier	SPC/ Labelling	HMPC monograph
Standardized	yes	yes	yes (2 and 4.2)
Quantified	yes	yes	yes (2)
Other	yes (?)	No	No

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## Outline

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- Legal framework
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  - Implementation
- 

## Implementation

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*“The solution to use a conversion factor should also be applied to other herbal drugs and herbal preparations, especially for standardised extracts”*

**Gaedcke F**, et al., Pharmeur Sci Notes. 2009 Mar;2009(1):5-10

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## Implementation

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- Conversion factor
  - Analytical method in the HMPC monograph
- 

## Implementation HMPC monograph

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*"...Dry extracts (40-80% (v/v) ethanol) standardised to contain 16-28% triterpene glycosides, calculated as aescin (**photometric method**). ..."*

---

## Implementation

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- Conversion factor
  - Analytical method in the HMPC monograph
  - Transition period of 2-3 year: the use of both methods in parallel. New method as an annex to the monograph with an explanation that the new method is not (yet) mandatory
- 

## Implementation: issues

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- Burden on industry Analytical and financial
  - Labeling to be used during the transition phase? Difference in labels depending on the assay method used?
  - Communication with prescribers and patients
  - (Ongoing) Stability studies
  - Timeframe to revise HMPC monographs
  - Apply the specifications of the outdated method for the modern analytical method.
-



## Implementation

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Collaboration between all stakeholders  
HMPC, Ph Eur. and industry

- Setting specifications for the new assay methods
  - Conversion factor
  - Coordination of implementation
  - Classifications of active substances. Re-evaluate the role of active and analytical markers in Ph. Eur. Monographs
-

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Tack	Köszí
Muchas gracias	Ευχαριστώ
Najlepša hvala	Danke
Dziękuję	Vinaka
Obrigado	Thank you
Mersi	Bedankt
Takk	Tak mange tak
Grazzi	Děkuji
Merci	благодаря
Grazie	Kiitos

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**For your attention**

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