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Collaboration, Innovation and Scientific Excellence: the European Pharmacopoeia 11th Edition

Session 3: Herbals

Moderator: Salvador Cañigueral,

Chair of Ph. Eur. Group of Experts 13B and
Chair of the Ph. Eur. Commission

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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An alternative and simplified approach to identification and test for minimum content of TCM herbal drugs by HPTLC

Eike Reich

Member 13A, 13B, TCM WP and HOM WP

CAMAG 60 YEARS

Content

Background

- HPTLC 2.8.25
- Quantitative HPTLC
- The HPTLC fingerprint
- Comprehensive HPTLC fingerprinting

Test for minimum content by HPTLC

- Thunberg fritillary bulb
- Corydalis rhizome

- Conclusions

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Background

- Ph.Eur. 11th Ed. includes 350 monographs on herbal drugs/preparations; of those 82 are on TCM
- Suite of tests for evaluation of quality: identity, purity, content of a constituent / group of constituents
- For constituents with known therapeutic activity and active markers → an assay is relevant
- For most herbal drugs one analytical marker is assayed and a minimum content is defined
- Are there alternatives to describe the quality of TCM?

Decision of 157Th session of Ph.Eur. Commission: TCM WP to conduct a pilot phase evaluating the suitability of "semi-quantitative HPTLC" as alternative to classical assays for TCM without marketing authorization.

HPTLC 2.8.25

Preface to the 10th edition:

"The analytical performance of chromatographic identification tests in monographs on herbal drugs and herbal drug preparations has improved since the general chapter on high-performance thin-layer chromatography of herbal drugs and herbal drug preparations (HPTLC, 2.8.25) was introduced in Ph. Eur. The new method not only improves selectivity but also allows a more objective evaluation of the observed zones through the use of intensity markers. The equipment described ensures standardised plate preparation, and includes a system for electronic documentation of chromatograms."

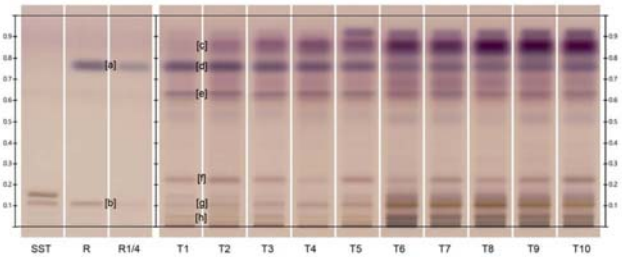
HPTLC in a nut shell

- 20x10 cm HPTLC glass plate Si 60 F_{254}
- Application: 15 tracks, 8 mm bands, 8 mm from lower edge, first track at 20 mm
- Conditioning to 33% relative humidity
- Development: 70 mm from lower edge, 20 min saturation (filter paper), 5 mm solvent level

Reproducible data, every day, everywhere ...

HPTLC in the EDQM knowledge data base

The following chromatogram is shown for information but will not be published in the European Pharmacopoeia. The zones in the chromatogram are identified by letters that correspond to the descriptions in the table above.



SST: reference solution (c)
R: reference solution (a)
R1/4: reference solution (b)
T1-T10: test solutions from different batches of forsythia fruit (ripe and unripe)

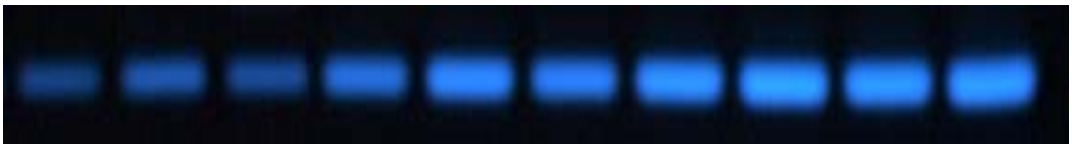
Figure 2720.-2. – *HPTLC chromatogram for identification test C of forsythia fruit*

Top of the plate	
[a] Ursolic acid: a reddish-violet zone	[c] 2 violet zones, faint to intense [d] A reddish-violet zone, faint to equivalent
	[e] A greyish-reddish-violet zone, faint
	[f] A reddish-brown zone, faint to equivalent
[b] Forsythoside A: a brownish zone	[g] A brownish or brownish-yellow zone, faint to equivalent
	[h] A brownish-yellow zone, faint to equivalent
Reference solution (a)	Test solution

There is quantitative information in the chromatogram!

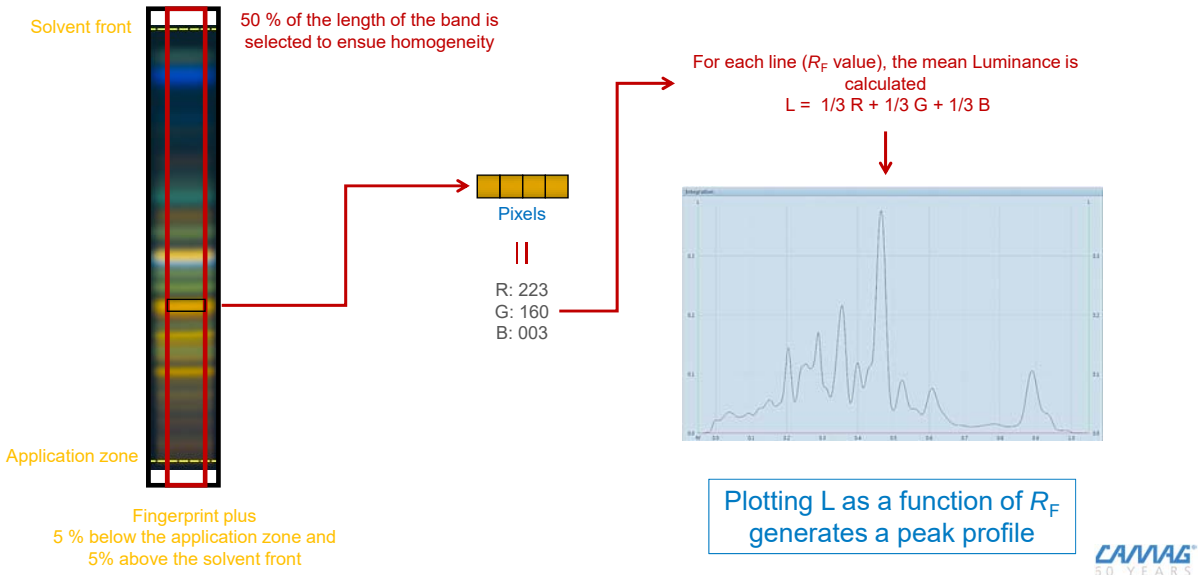
Accessing quantitative information: visual estimation of sinensetin

Calibration standards



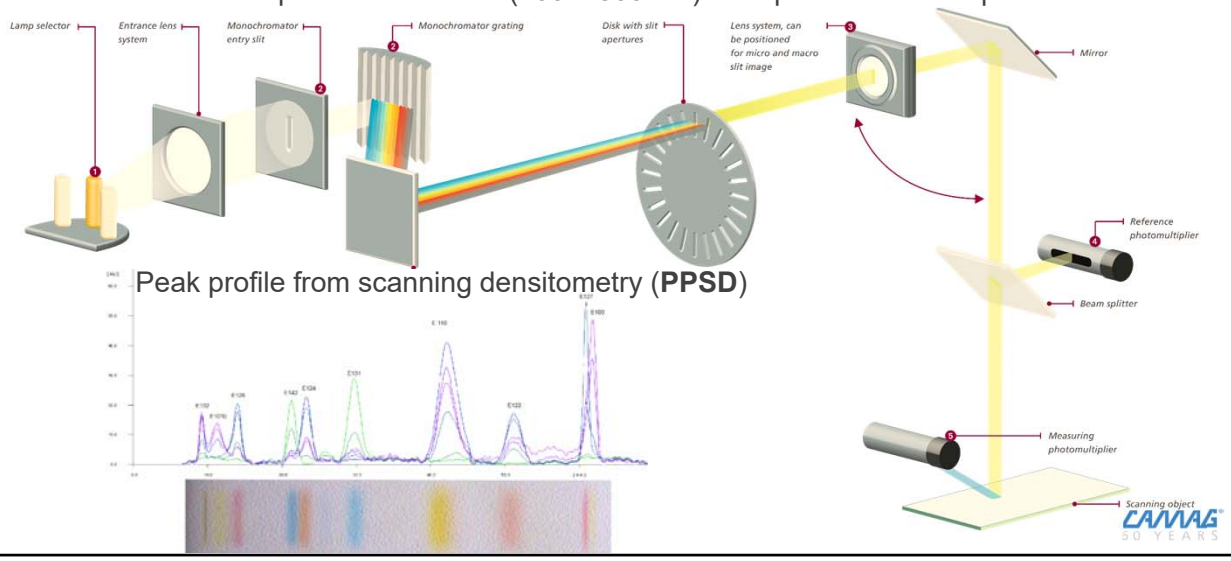
Samples

Accessing quantitative information: peak profiles from electronic images (PPI)



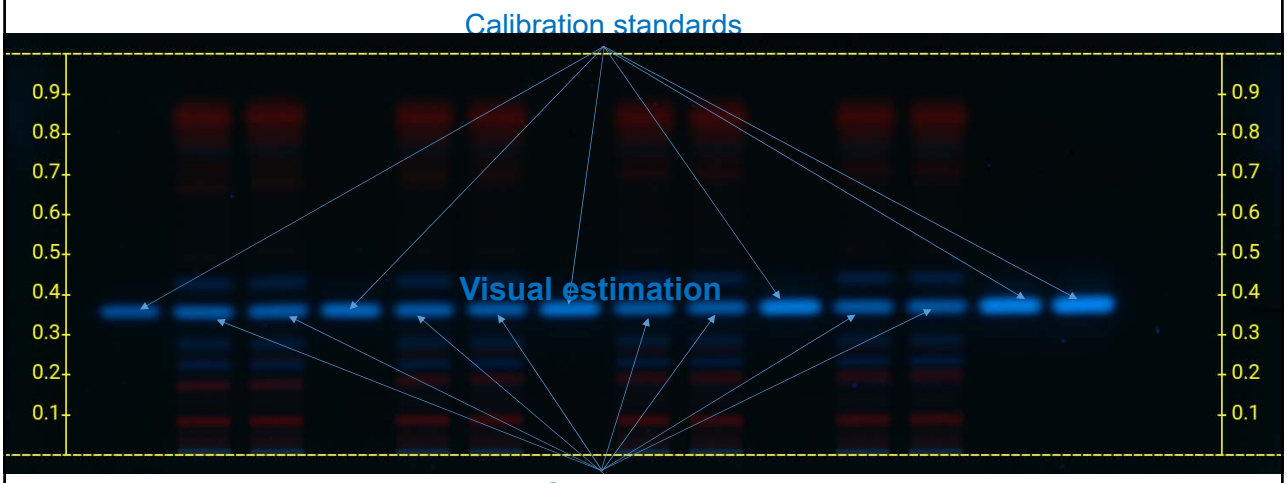
Accessing quantitative information: scanning densitometry

Monochromatic and spectral evaluation (200 – 800 nm) and quantitation of separated zones

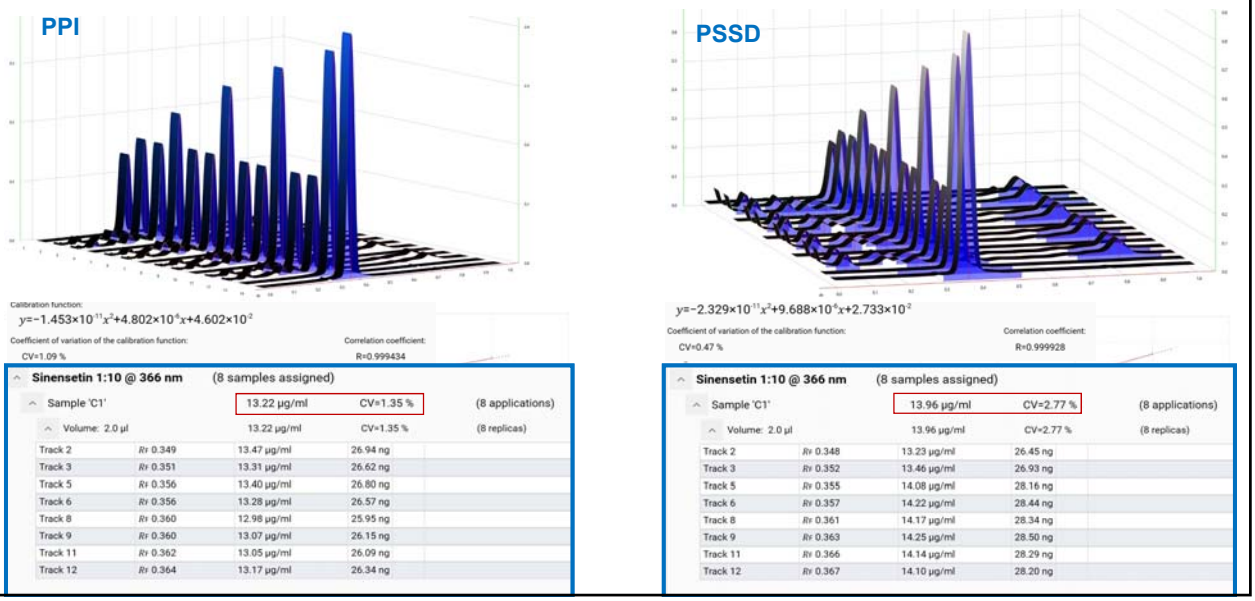


Peak profile from scanning densitometry (**PPSD**)

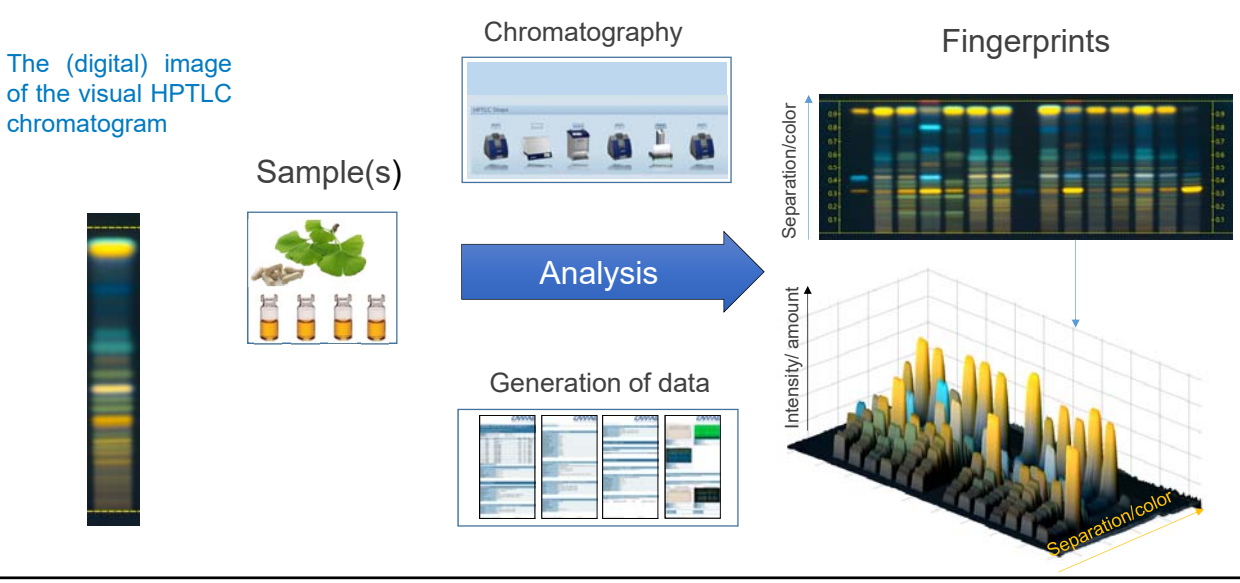
Accessing quantitative information for sinensetin in Java Tea



Quantitation of sinensetin in Java Tea



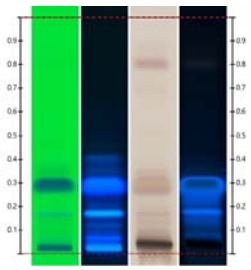
HPTLC Fingerprint



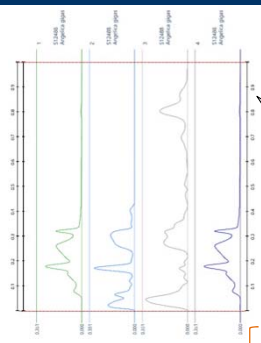
Comprehensive HPTLC Fingerprinting

- HPTLC fingerprints (images), which are used for identification, contain information beyond identity…
- **Comprehensive** HPTLC fingerprinting includes:

Images



Peak Profiles from Images (PPI)



This kind of presentation allows quantitative evaluation

This includes information about

- ✓ Identification
- ✓ Purity
- ✓ Content

Comprehensive HPTLC Fingerprinting for Quality Control of an Herbal Drug – The Case of *Angelica gigas* Root

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Supporting information available online at http://www.thieme-connect.de/products

Key words

Angelica gigas - Apiaceae, HPTLC, identity, purity, minimum content

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Bibliography

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ABSTRACT

The quality of herbal drugs is usually controlled using several tests recommended in a monograph. HPTLC is the method of choice for identification in many pharmacopoeias. If combined with a suitable reference material for comparison, HPTLC can provide information beyond identification and thus may simplify quality control. This paper describes, as a proof of concept, how HPTLC can be applied to define specifications for an herbal reference material and to control the quality of an herbal drug according to these specifications. Based on multiple batches of cultivated *Angelica gigas* root, a specific HPTLC method for identification was optimized. This method can distinguish 27 related species. It also can detect the presence of mixtures of *A. gigas* with two other *Angelica* species traded as "dang gui" and is suitable as well for quantitative assessment of samples in a test for minimum content of the sum of decursin and decursinol angelate. The new concept of "comprehensive HPTLC fingerprinting" is proposed: HPTLC fingerprints (images), which are used for identification, are converted into peak profiles and the intensities of selected zones are quantitatively compared to those of the corresponding zones of the reference material. Following a collaborative trial involving three laboratories in three countries, the method was applied to check the quality of further candidates for establishing an appropriate reference material. In conclusion, this case demonstrates that a single HPTLC analysis can provide information about identity, purity, and minimum content of markers of an herbal drug.

Introduction

To describe and assure the quality of herbal drugs, a suite of appropriate tests is recommended by regulatory agencies [1,2] and organizations [3]. Such tests, as well as specifications for compliance, are described in pharmacopoeial or other quality monographs. They include verification of identity and purity as well as determination of the amount of the active substance(s) or marker(s) [4, 5]. In order to perform all tests, different analytical techniques and expertise are needed, and together with additional experiments (e.g., test for pesticides, mycotoxins, etc.), the overall costs of quality testing can dramatically increase.

For herbal drugs, identity is still one of the central elements of quality [6]. Identity is evaluated primarily based on the morphological characteristics in comparison to a descriptive key and/or to an HRM, which is representative for the species and the corresponding plant part. Identity is also evaluated based on the chemical composition, the pattern of which may be compared to that of the HRM [2]. However, HRMs could also be used to qualify an herbal drug in a much wider sense, because the target material is

Comprehensive HPTLC Fingerprinting and test for minimum content

- Identification includes SST and intensity markers
- One marker (MC) is used as reference for minimum intensity → "minimum content" in sample

Requirements:

- Establish a (linear) calibration range based on standards
- Determine (assay) the content of marker in multiple samples
- Establish minimum content
- Adjust sample concentration/application volumes for use with single point calibration

Acceptance criteria

1. Sample meets description provided in the result table **AND**
2. Zone of the marker in the sample is at least as intense as the MC zone

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Pilot phase project 1:minimum content of peimine / peiminine in Fritillaria bulbs

"Comprehensive HPTLC Fingerprinting" → Identification
→ Test for minimum content

To prove that "semi-quantitative" HPTLC based on image evaluation is reproducible in different labs and that it can simplify the analytical process!

New ID method→ baseline separation of markers

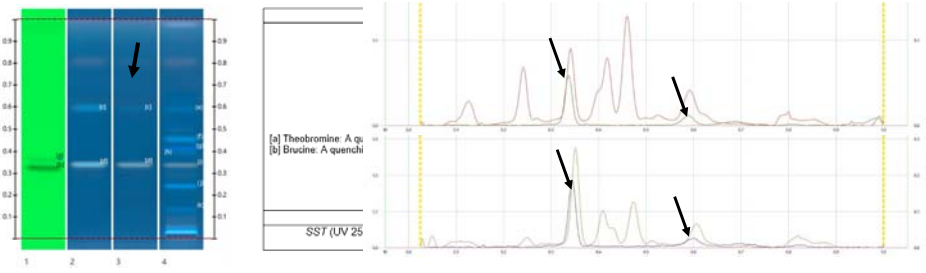
Fritillaria bulbs

Calibration curves are established for 2 markers

- The content of peimine and peiminine was assessed in 9 samples
- Acceptance criteria for minimum content were established based on the average content of each marker
- Peimine minimum content: 0.06%
- Peiminine minimum content: 0.02%

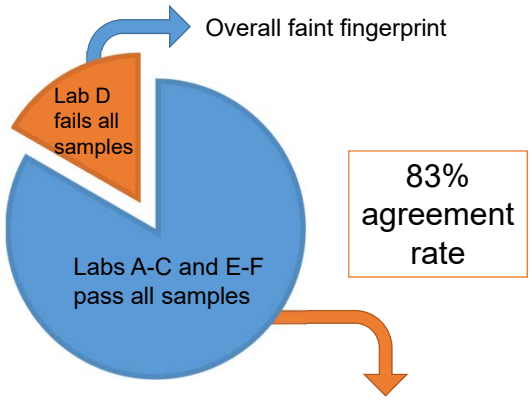
Collaborative study

- Samples and SOP were distributed to 6 laboratories for a collaborative trial
- The following parameters were evaluated:
  - Identity of the samples→ sequence of zones as described in the SOP
  - Minimum content test based on visual evaluation
  - Minimum content test based on PPI
  - Minimum content test based on scanning densitometry



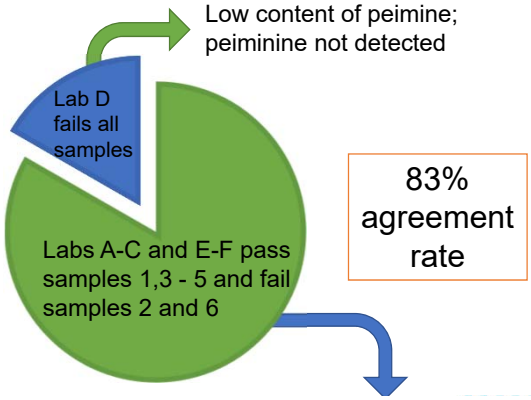
Collaborative study

- Identity of the samples: sequence of zones as described in the SOP
- 6 labs (A-F)



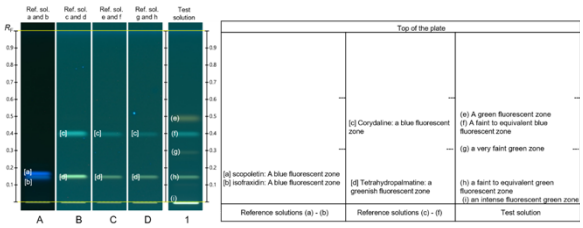
All zones described in the SOP were detected

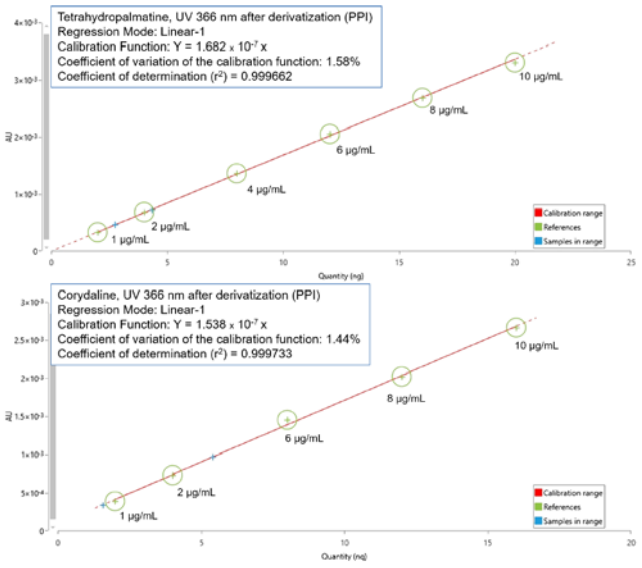
- Test for minimum content based on **visual** evaluation
- 6 labs (A-F)



The failing samples had lower content of peimine

Project 2: minimum content of corydalin / tetrahydropalmatin in Corydalis rhizome

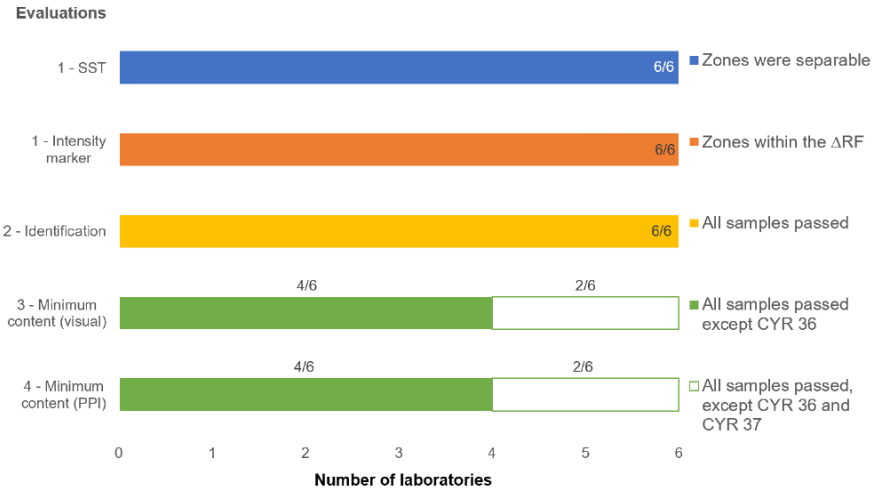




Minimum content:
Corydalin 0.04%
Tetrahydropalmatin 0.06%

Collaborative study

- 8 samples analyzed in 6 labs



Outcome of the pilot phase

The European Pharmacopoeia (Ph. Eur.) Commission has decided to accept semi-quantitative High-Performance Thin-Layer Chromatography (HPTLC) tests as an alternative quality control which could be used instead of liquid chromatography (LC) assays in monographs on Traditional Chinese Medicines (TCM). The use of the HPTLC test, which aims at maintaining the level of quality control provided by the monograph without requiring additional assays, will be limited for the time being to monographs on herbal drugs used in TCM that are not subject to marketing authorisations.

After one year of work, the pilot study was successfully completed for Thunberg fritillary bulb (*Fritillaria thunbergii* Miq.: Beimu) and Corydalis rhizome (*Corydalis yanhusuo* W. T. Wang: Yanhusuo). The participating laboratories obtained reproducible results for marker levels, identity and system suitability using HPTLC, which was also found to give the same pass/fail results as the HPLC assay. Marker levels were evaluated by visual inspection or using appropriate software which converted the HPTLC chromatogram into peak profiles.

18 JUNE 2019 TO 20 JUNE 2019
SHANGHAI, CHINA
CPhI China & CEP One-to-One Sessions

19 JUNE 2019 TO 20 JUNE 2019
STRASBOURG, FRANCE
International Conference on the 'EDQM & European Pharmaco...

Conclusions

- The results of the pilot phase have been published in Pharmeuropa Bio&SN March 2021 by Frommenwiler et.al. as: “An alternative and simplified approach to identification and test for minimum content of TCM herbal drugs”
- The monograph for *Fritillaria thunbergii* bulbs has been published in Ph.Eur. 10.6
- The EDQM Knowledge database states:

> **THUNBERG FRITILLARY BULB (2588)**
>
> This monograph is the first example describing the alternative approach for quality control by semi-quantitative HPTLC instead of the classical LC assay.

- Additional monographs for TCM drugs with this approach are under elaboration…

Conclusions

- HPTLC is always QUANTITATIVE if fingerprints obtained in accordance with 2.8.25 are suitably evaluated.
- HPTLC methods developed for identification of herbal drugs can also be used for quantitation of markers if samples and standards are prepared quantitatively.
- The concept of comprehensive fingerprinting works also for “zones” of the fingerprint which are not identified. A minimum content (intensity) can be defined with respect to any suitable reference substance.
- HPTLC can significantly simplify analysis of herbal materials during routine quality control.
- HPTLC is still underestimated, but acceptance within the Ph.Eur. is increasing ☺


Thank you for your attention!
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Collaboration, Innovation and Scientific Excellence: the European Pharmacopoeia 11th Edition
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HPLC assays for hydroxyanthracene derivatives replacing photometric assays

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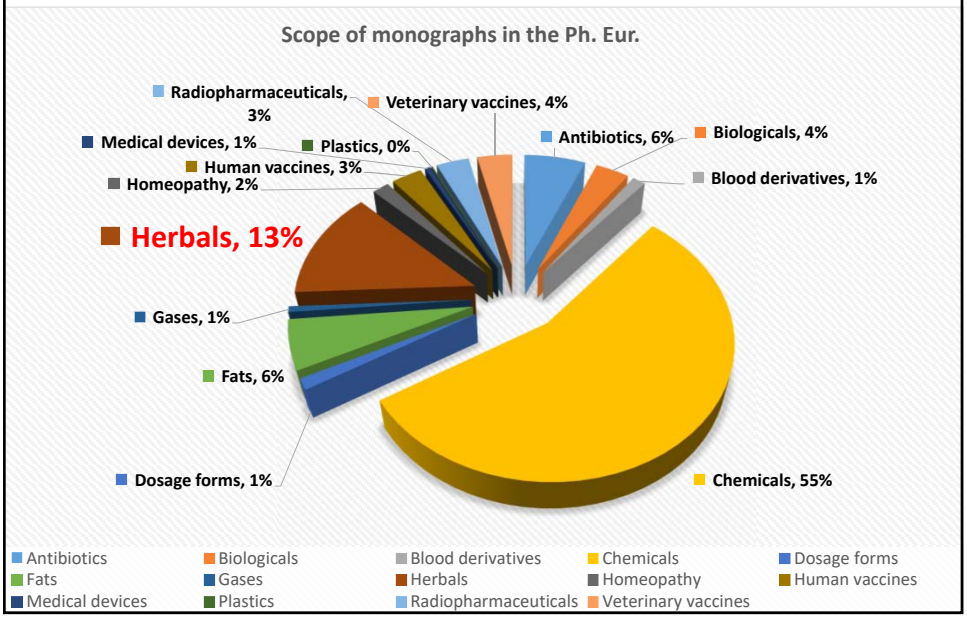
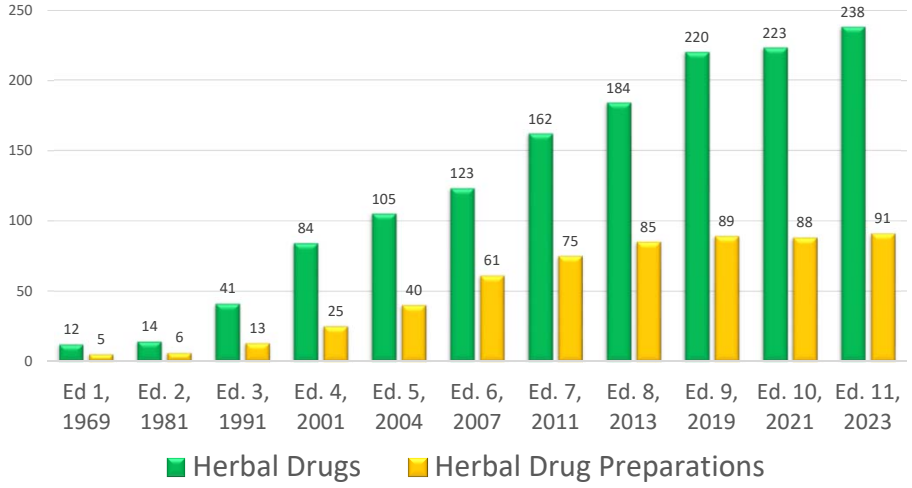
Contents of the European Pharmacopoeia:

About 2500 monographs and more than 370 general chapters



Monographs for herbal drugs and herbal drug preparations in the European Pharmacopoeia



Modernisation Program

- ✓ « Internal » harmonisation using a template
- ✓ To include recent techniques and produce a Pharmacopoeia which is scientifically state-of-the-art
- ✓ To improve existing methods to take into account recent progress in analytical technology and regulatory practice
- ✓ To suppress toxic reagents and materials
- ✓ To introduce and/or improve elements of equipment performance and qualification -> be more user-friendly
- ✓ To introduce and/or improve general system suitability tests
- ✓ International harmonisation within PDG (Pharmacopoeial Discussion Group)

Rhubarb - Rhei radix Ph.Eur. 6.0

DEFINITION

Rhubarb consists of the whole or cut, dried underground parts of *Rheum palmatum* L. or of *Rheum officinale* Baillon or of hybrids of these two species or of a mixture. The underground parts are often divided; the stem and most of the bark with the rootlets are removed.

Content: min. 2,2 % hydroxyanthracene derivatives, calc. as rhein.

http://www.bio-botanica.com/wp-content/uploads/2016/12/product_Rhubarb-Root.jpg

2004:

Request for revision by the German delegation:

Reason: Assay improvement/
determination of the hydroxyanthracene derivatives.

Suggestion
to lower the limit of 2.2 %
and to use 70 % (V/V) methanol instead of water
for extraction (110 – 150 % higher yields)

Rhubarb - Rhei radix Ph.Eur. 6.0

ASSAY

Carry out the assay protected from bright light.

Introduce 0.100 g of the powdered herbal drug (180) (*2.9.12*) into a 100 mL flask. Add 30.0 mL of *water R*, mix and weigh. Heat in a water-bath under a reflux condenser for 15 min. Allow to cool, add 50 mg of *sodium hydrogen carbonate R*, weigh and adjust to the original mass with *water R*. Centrifuge and transfer 10.0 mL of the liquid to a 100 mL round-bottomed flask with a ground-glass neck. Add 20 mL of *ferric chloride solution R1* and mix. Heat under a reflux condenser on a water-bath for 20 min, add 1 mL of *hydrochloric acid R* and heat for a further 20 min, shaking frequently. Cool, transfer to a separating funnel and shake with three quantities, each of 25 mL, of *ether R* previously used to rinse the flask. Combine the ether extracts and wash with two quantities, each of 15 mL, of *water R*. Filter the ether extracts through a plug of absorbent cotton into a volumetric flask and dilute to 100.0 mL with *ether R*. Evaporate 10.0 mL carefully to dryness on a water-bath and dissolve the residue in 10.0 mL of a 5 g/L solution of *magnesium acetate R* in *methanol R*. Measure the absorbance (*2.2.25*) at 515 nm, using *methanol R* as the compensation liquid.

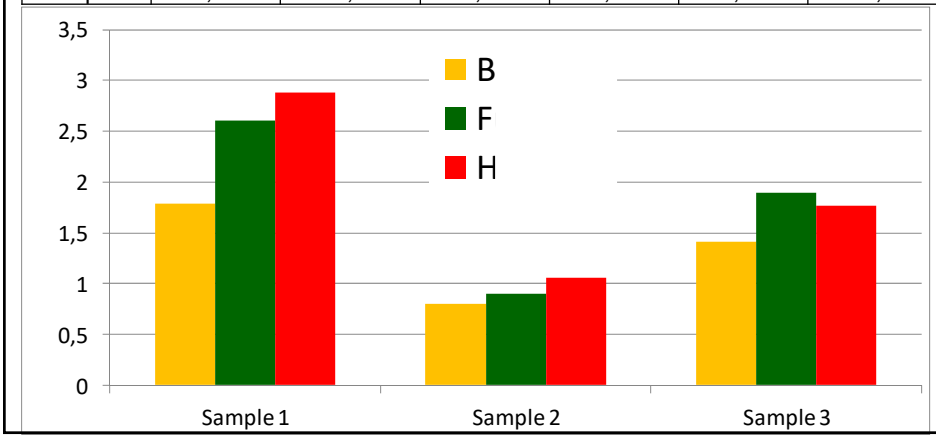
Calculate the percentage content of rhein from the expression:

$$\frac{A \times 0.64}{m}$$

i.e. taking the specific absorbance of rhein to be 468, calculated on the basis of the specific absorbance of barbaloin.

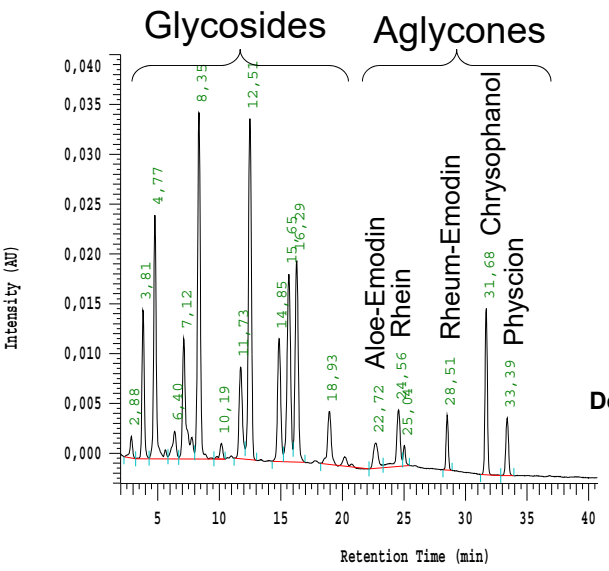
Photometric assay for Rhubarb according to Ph.Eur. 6.0

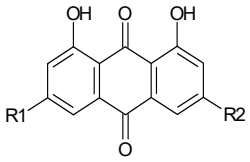
	Lab 1	Lab 2	Lab 3	Average	SD	RSD
Sample 1	1,79	2,6	2,88	2,42	0,57	23,4
Sample 2	0,80	0,9	1,06	0,92	0,13	14,3
Sample 3	1,41	1,9	1,77	1,69	0,25	15,0



HPLC method for the analysis of Rhubarb extract

R. Bauer, S. Monschein (2005)

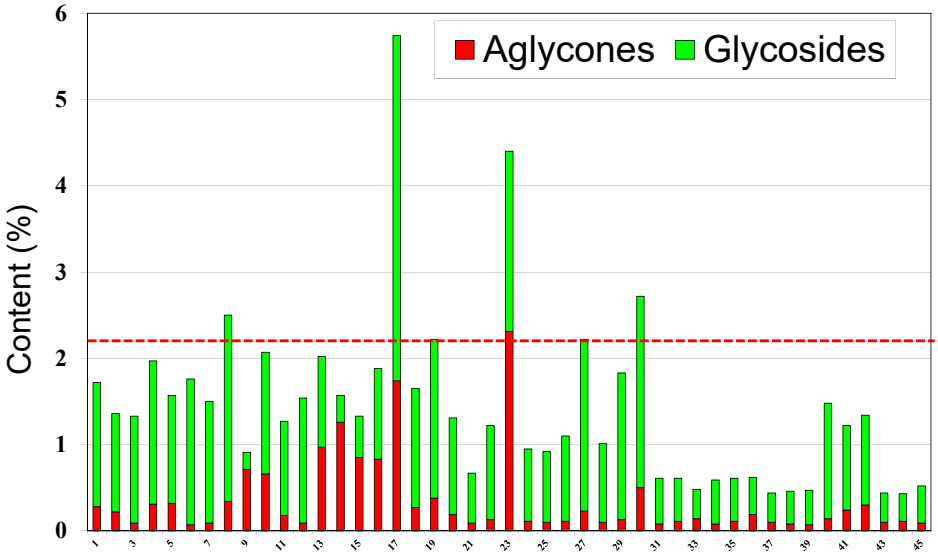




	R_1	R_2
Rheumemodin	CH_3	OH
Aloeemodin	CH_2OH	H
Rhein	COOH	H
Chrysophanol	CH_3	H
Physcion	CH_3	OCH_3

Detection wavelength: 430 nm

Determination of anthranoids in 45 batches of Rhubarb roots (calculated as Rhein)



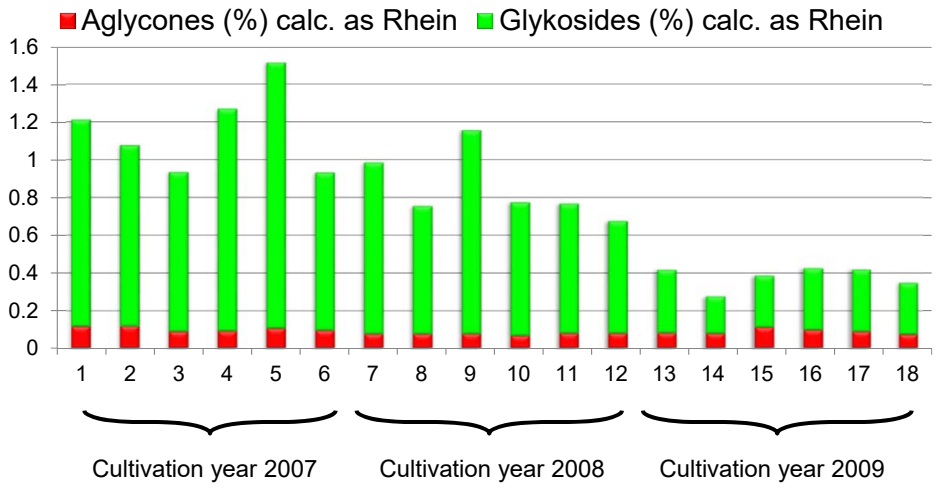
R. Bauer, H. Heuberger, S. Monschein (2005)

Determination of anthranoids in 45 batches of Rhubarb roots (calculated as Rhein)

Conclusion:

- most of the batches contained less than 2.2 % anthraquinone glycosides
- some batches contained quite a high concentration of aglycones (which previously have been neglected by the pharmacopoeia)

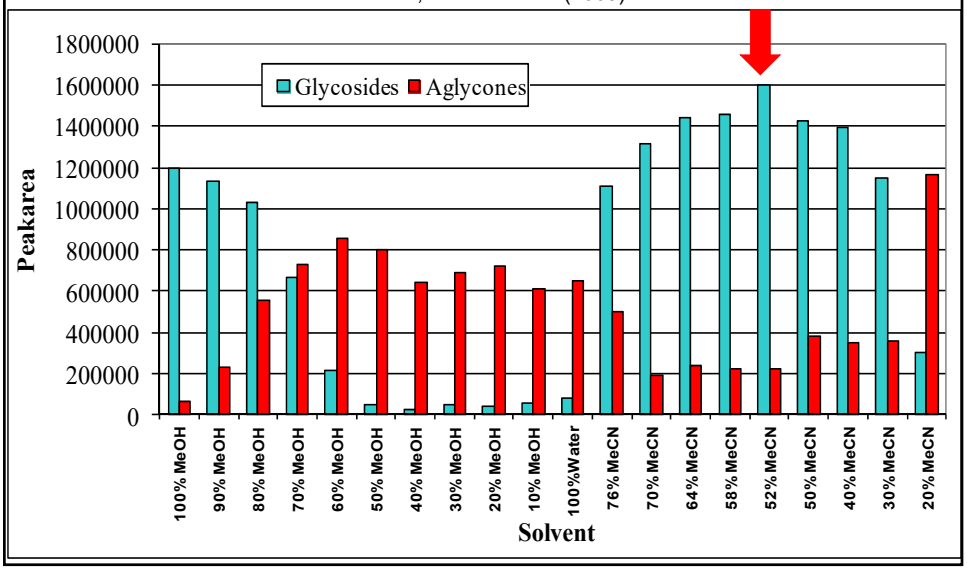
Determination of hydroxyanthracene glycosides and aglycones in cultivated rhubarb roots



R. Bauer, H. Heuberger (2010)

Extraction yield of hydroxyanthracene glycosides and aglycones with various solvents

R. Bauer, S. Monschein (2005)



Determination of hydroxyanthracene glycosides and aglycones in cultivated rhubarb roots

Conclusion:

- Content of anthranoid glycosides depends on the age of the plants and the years of cultivation
- Concentration of aglycones is independent of the age of the plants and may be an artifact
- Hydroalcoholic extraction may lead to (enzymatic?) hydrolysis of glycosides

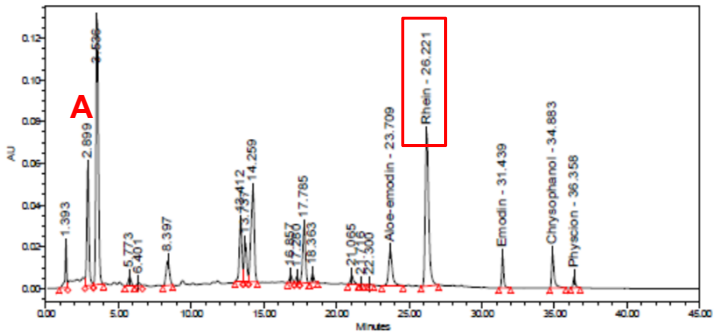
New HPLC Assay for RhubarbPh.Eur. 11.1

The assay is carried out protected from bright light.

Test Solution
In duplicate, place 1.00 g of the powdered drug (355) (*2.9.12)* and 50.0 ml solvent mixture (**1:1 V/V mixture of acetonitrile *R* and 0.2 % solution of sodium hydrogen carbonate *R* in water *R***) in a 100 ml flask, determine the weight, and **heat under reflux for 10 minutes** after the start of boiling. After cooling, adjust with solvent mixture to the previous weight and filter through a membrane filter (nominal pore size 0.45 µm).

Reference solutions:
R1) Weigh 6.0 mg of ***rhein R*** in a standard flask of 20.0 ml. Dissolve in solvent mixture and fill up. Dilute 1.0 ml to 10.0 ml with solvent mixture. Concentration of rhein in reference solution: 30 µg/ml. Prepare the reference solution just before use.
R2) Dissolve 200 mg of ***Rhei radix extract for peak identification R*** in 10.0 ml solvent mixture (1:1 V/V mixture of acetonitrile *R* and 0.2 % solution of sodium hydrogen carbonate *R* in water *R*) and filter through a membrane filter (nominal pore size 0.45 µm).

New HPLC Assay for RhubarbPh.Eur. 11.1



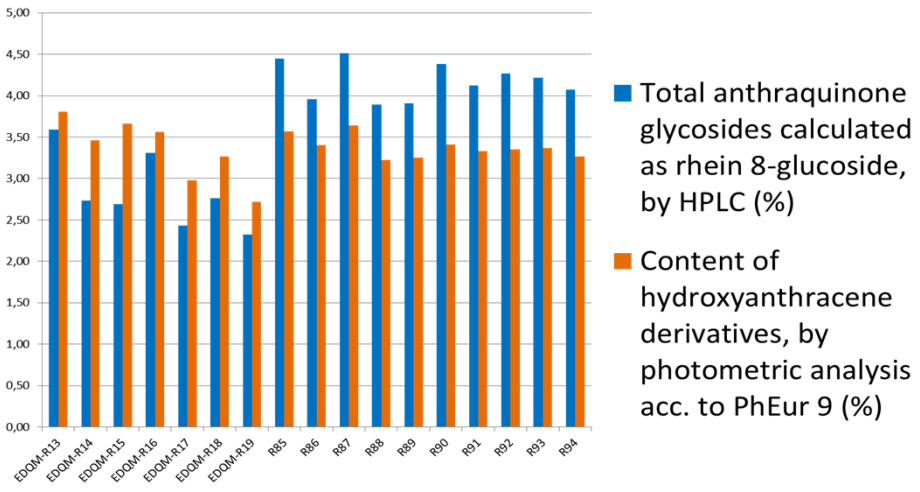
System suitability (Test solution): Resolution: minimum 1.5 between the peaks due to aloe-emodin and the peak due to rhein.
Quantification of aglycones:
Calculate the percentage content of the sum of aloe-emodin, rhein, emodin, chrysophanol and physcion with rhein as reference.
Quantification of glycosides:
Calculate the percentage content of the sum of anthraquinone glycosides eluting before the peak due to aloe-emodin (starting from peak A) as **rhein 8-glucoside** with rhein as reference, using a correction factor (calc. from the mw of rhein 8-glucoside and rhein).

Rhubarb – Specifications in Ph.Eur. 11.1

	Rhei radix	Rhei extractum siccum normatum
Assay (Content)	**Minimum 2.0 %** of the sum of hydroxyanthracene glucosides, expressed as rhein-8-glucoside (dried drug)	**4.0 % to 6 %** of the sum of hydroxyanthracene glucosides, expressed as rhein-8-glucoside (dried drug)
Test (Limit)	Sum of total anthraquinones (aloe-emodin, rhein, emodin, chrysophanol and physcion) expressed as rhein (dried drug): **maximum 20 %,** calculated with reference to the sum of hydroxyanthracene glucosides and total anthraquinones (total anthracene derivatives)	Sum of total anthraquinones (aloe-emodin, rhein, emodin, chrysophanol and physcion) expressed as rhein (dried drug): **maximum 25 %,** calculated with reference to the sum of hydroxyanthracene glucosides and total anthraquinones (total anthracene derivatives)

Comparison of new HPLC Assay for Rhei extractum siccum with Photometric Assay

M. Kranewitter, V. Wolkinger, R. Bauer (2018)



Senna leaflets and Senna pods Ph.Eur.

DEFINITION
Dried leaflets of *Senna alexandrina* Mill. (syn. *Cassia acutifolia* Delile and *Cassia angustifolia* Vahl).

Content PhEur 9:
Min. 2.5 % of total hydroxyanthracene glycosides, expressed as sennoside B.

Content PhEur 10.1:
Min. 2.0 % of total hydroxyanthracene glycosides, expressed as sennoside B.

DEFINITION
Dried fruit of *Senna alexandrina* Mill. (syn. *Cassia acutifolia* Delile) and *Cassia angustifolia* Vahl.

Content PhEur 9:
Min. 3.4/2.2 % of total hydroxyanthracene glycosides, expressed as sennoside B.

Content PhEur 10.1:
Min. 2.0 % of total hydroxyanthracene glycosides, expressed as sennoside B.

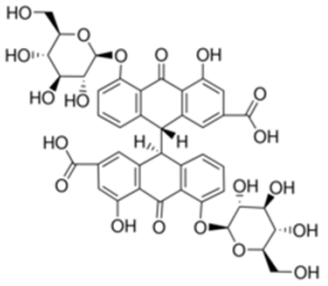
Senna leaves and pods

https://sc01.alicdn.com/kf/UTB8WekYtyDEXKJk43Oqq6Az3XXa3/817215445/UTB8WekYtyDEXKJk43Oqq6Az3XXa3.jpg

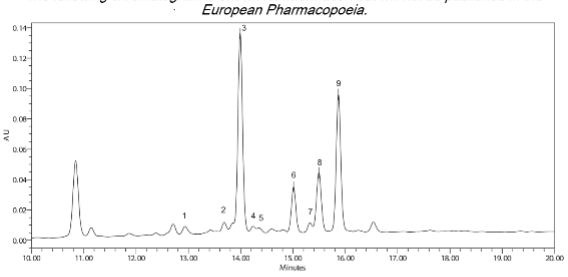
https://cdn.shopify.com/s/files/1/0077/1548/7834/products/Senna-Pods1.png?v=1568284732

Test solution. Introduce 0.500g of the powdered herbal drug (355) into a 250 mL screw-cap bottle and add 100.0 mL of the solvent mixture. Sonicate for 30 min and shake for 2h. Filter through a membrane filter (nominal pore size 0.45µm).
Reference solution (a). Dissolve10 mg of **aloe emodin R** and 10.0 mg of **rhein CRS** in tetrahydrofuran R and dilute to 50.0 mL with the same solvent. Dilute1.0 mL of the solution to 20.0 mL with the solvent mixture.
Reference solution (b). Dissolve 10 mg of **senna extract CRS** in 8 mL of the solvent mixture using sonication for 5 min and dilute to 10 mL with the solvent mixture (as light residue may remain). Filter through a membrane filter (nominal pore size 0.45µm). **Reference solution (c).** Dissolve 5.0 mg of **sennoside B CRS** in 25 mL of methanol R using sonication and dilute to 50.0 mL with water R.

Senna leaves and pods



The following chromatogram is shown for information but will not be published in the European Pharmacopoeia.



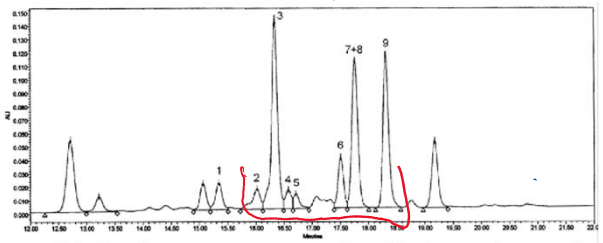
1. isorhamnetin diglucoside
2. hydroxyanthracene glycoside
3. hydroxyanthracene glycoside (sennoside B), a shoulder peak may be present
4. and 5. hydroxyanthracene glycosides, may be co-eluted or (partially) separated
6. hydroxyanthracene glycoside
7. and 8. hydroxyanthracene glycosides, may be co-eluted or (partially) separated
9. hydroxyanthracene glycoside (sennoside A)

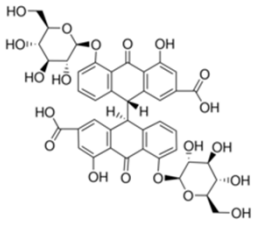
Figure 0206.-4. – *Chromatogram for the assay of senna leaflet: reference solution (b) (zoom view)*

Reference standards	**Available since**	**Cat. No.**	**Name**	**Batch No.**	**Unit Quantity**	**Price**	**SDS Product Code**
		S0400000	Senna extract CRS	1	50 mg	79 EUR	201700330

Practical Information	**Test(s)**	**Brand Name/Information**
	HPTLC	From supplement 10.1 onwards Merck HPTLC Si 60 F254
	LC	From supplement 10.1 onwards column: Synergi Polar RP D0 (dwell volume used for development of the method) = 1.7 mL

Senna leaves and pods





Calculate the percentage content of total hydroxyanthracene glycosides (peaks 2-9) (*THG*), expressed as sennoside B, using the following expression:

$$\frac{A_1 \times m_2 \times 2 \times p}{A_2 \times m_1}$$

A_1 = sum of the areas of the peaks due to hydroxyanthracene glycosides (peaks 2-9) in the chromatogram obtained with the test solution;

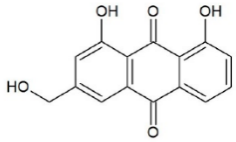
A_2 = area of the peak due to sennoside B in the chromatogram obtained with reference solution (c);

m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;

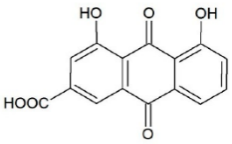
m_2 = mass of *sennoside B CRS* used to prepare reference solution (c), in grams;

p = percentage content of sennoside B in *sennoside B CRS*.

Senna leaflets Ph.Eur. and Senna pods Ph.Eur.



Aloe-emodin



Rhein

TESTS

Total anthraquinones (aloe emodin and rhein).
Liquid chromatography (2.2.29). Carry out the test protected from bright light.

Calculate the percentage content of total anthraquinones (aloe emodin and rhein) (TA), expressed as rhein.
Calculate the percentage content of total anthraquinones with reference to the sum of total hydroxyanthracene glycosides (THG, see Assay) and total anthraquinones.

Limit: **total anthraquinones (aloe emodin and rhein)** expressed as rhein: **max. 7.0 %,** calculated with reference to the sum of total hydroxyanthracene glycosides and total anthraquinones (dried drug).

Specifcation of Extracts of Senna leaflets and pods in PhEur 10.7 and PhEur 9

(total hydroxyanthracene glycosides, expressed as sennoside B

	PhEur 10.7	PhEur 9
Senna fruit dry aqueous extract, standardised Sennae fructus extractum aquosum siccum normatum	7.0 - 13.0 %	
Senna fruit dry hydroalcoholic extract, standardised Sennae fructus extractum hydroalcoholicum siccum normatum	14.0 - 22.0 %	
Senna leaflet dry extract, standardised Sennae folioli extractum siccum normatum	5.5 - 12.0 %	5.5 - 8 %

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Martin Bauer Group

Anthraquinone Contents of Senna Extracts, Comparison of Photometric to HPLC Determination

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Phytotherapy Congress 2016 *2 – 4 June 2016, Bonn; Beethovenhalle*

Extr. Sennae sicc.	Batch	Content [%] Sennoside Ph. Eur. (photom.)	Content [%] Sennoside Pharmeuropa (HPLC)	Correlation factor Photom./HPLC
e fruct. (EtOH 60% V/V)	46-15	16.7	14.4	1.16
e fruct. (EtOH 60% V/V)	47-15	21.0	19.3	1.09
e fruct. (EtOH 60% V/V)	48-15	16.9	14.2	1.19
e fruct. (EtOH 60% V/V)	49-15	18.5	16.0	1.16
e fruct. (EtOH 60% V/V)	50-15	21.2	18.2	1.16
e fruct. (EtOH 60% V/V)	92-16	20.6	16.7	1.23
e fol. (MeOH 60% V/V)	51-15	9.49	8.04	1.18

Conclusions

- In order to modernise methods in Ph.Eur., the photometric assay of hydroxyanthracene derivatives in rhubarb and senna monographs have been replaced by HPLC assays.
- The HPLC assays are better reproducible and less time consuming.
- A test for aglycones has been established. HPLC allows the determination of anthraquinone glycosides and aglycones in one run, so that assay and test can be performed together.
- An official correlation factor of the results from photometric assay and HPLC assay has not yet been established, but should be elaborated for the future.

Acknowledgement

- Members, chairmen, and secretaries of group 13A, EDQM laboratory, and contributing scientists, in particular
  - Francesco Villa, INDENA
  - Beat Meier, University of Applied Science Waedenswil
  - Sonja Monschein, Marlene Kranewitter, Volker Wolkinger, University of Graz

We work for tomorrow

UNI GRAZ

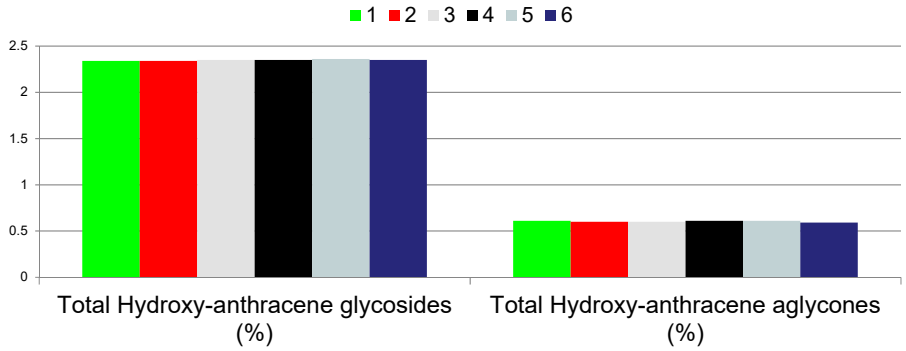
Thank you very much for your attention





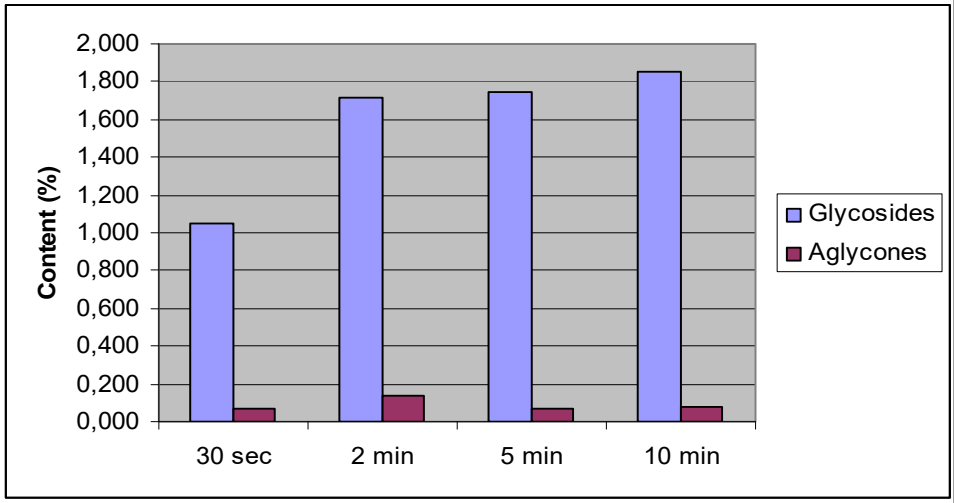

New Address: Beethovenstrasse 8, 8010 Graz, Austria, rudolf.bauer@uni-graz.at

Reproducibility of extraction method B2 (acetonitrile R and 0.2 % aqueous solution of sodium hydrogen carbonate R) in one lab



	Mean	SD	Rel. SD (%)
Total Hydroxy-anthracene glycosides (%)	2,35	0,007	0,29%
Total Hydroxy-anthracene aglycones (%)	0,60	0,008	1,24%

Optimization of the extraction procedure (time)



2022-09-20

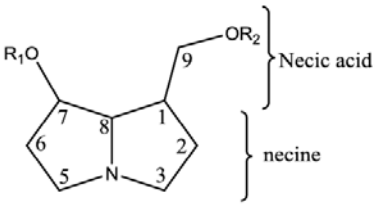
Contaminant pyrrolizidine alkaloids (new chapter 2.8.26)

Robert Burman, PhD

LÄKEMEDELSVERKET
SWEDISH MEDICAL PRODUCTS AGENCY

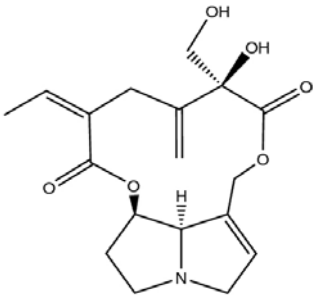
Pyrrolizidine alkaloids (PAs)

- Hundreds of different pyrrolizidine alkaloids (PAs) are known
- Found in thousands of different plant species, mainly in the families of Boraginaceae, Asteraceae and Fabaceae
- Several PAs are regarded as acute hepatotoxic and carcinogenic
- Common weeds (e.g. Senecio ssp.) containing PAs can contaminate fields of cultivated medicinal herbs



Structural requirements for toxicity*

1. A double bond in 1,2 position of a pyrrolizidine moiety
2. A hydroxymethyl substituent (C-1 position) in the pyrrolizidine moiety, preferably with a second hydroxyl group in the C-7 position
3. Esterification of the primary hydroxymethyl group with a branched mono- or dicarboxylic acid containing at least 5 C-atoms (necic acid)

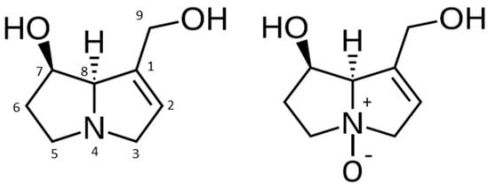


Riddelliine

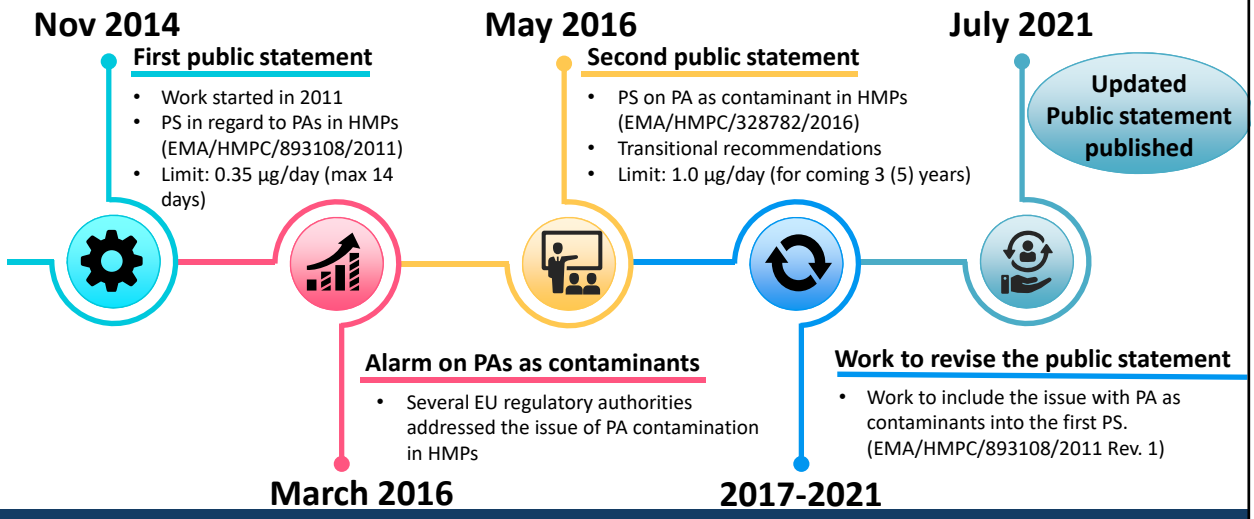
* EMA/HMPC/893108/2011 Rev. 1

Pyrrolizidine alkaloid N-Oxides (PANOs)

- PAs occur principally in two forms, namely tertiary base PAs and PA-N-oxides (PANOs)
- PANOs can easily be reduced to the corresponding tertiary PA in the alimentary tract or in experimental conditions but also within the plants
- It is necessary that both PAs and PANOs are included in the analytical determinations



EMAs public statements regarding PAs



PS on the use of HMPs containing toxic, unsaturated PAs *including recommendations regarding contamination of HMPs with PAs**

- Requirements:

1. Implementation of suitable testing procedures to ensure PA levels are controlled in line with limits agreed (i.e. Ph. Eur. 2.8.26).
   - limit 1.0 µg/day for adults (same limit for oral and cutaneous use)
   - essential oils are excluded from testing

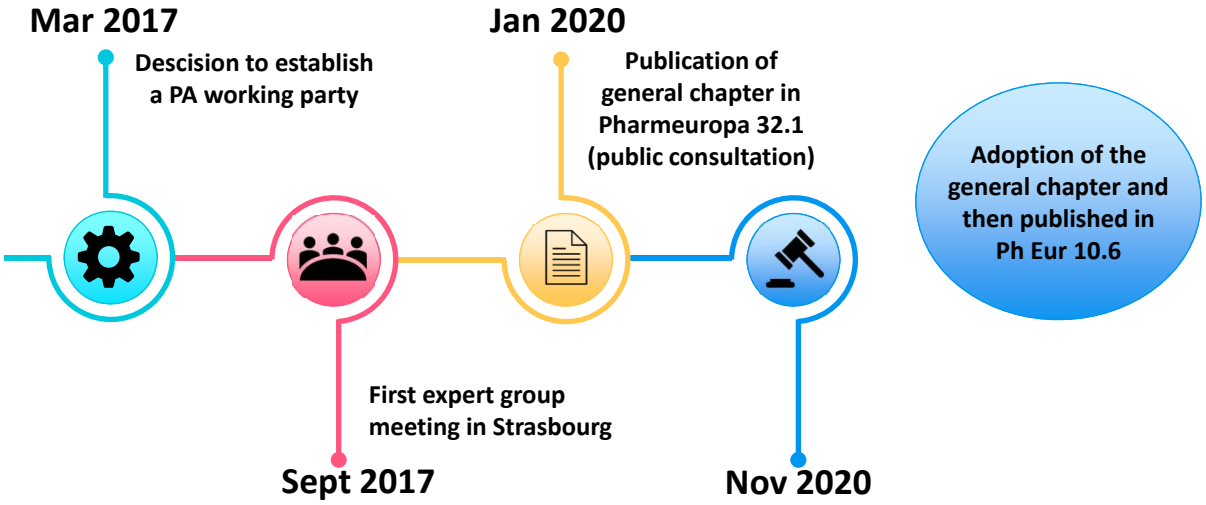
2. Implementation of measures to avoid or reduce PA contamination in HMPs.

* EMA/HMPC/893108/2011 Rev. 1

Ph Eur PA (Pyrrolizidine alkaloids) Working Party

- Terms of reference
  - Drafting of a general chapter allocated to the group by the Commission in the field of PAs.
  - Maintenance of the list of PAs which may be covered by the general chapter on PAs.

Elaboration of the general chapter 2.8.26



Analysis of PAs according to 2.8.26

- Methods should be able to detect PAs in trace amounts
- The general chapter permits the use of any procedure consisting of chromatography coupled with MS/MS or high-resolution MS
- An example method is given in this chapter that has been shown to be suitable for the determination of target PAs in a number of matrices

List of target pyrrolizidine alkaloids

1. Echimidine	11. Jacobine	21. Senecionine
2. Echimidine-N-oxide	12. Jacobine-N-oxide	22. Senecionine-N-oxide
3. Erucifoline	13. Lasiocarpine	23. Seneciphylline
4. Erucifoline-N-oxide	14. Lasiocarpine-N-oxide	24. Seneciphylline-N-oxide
5. Europine	15. Lycopsamine	25. Senecivernine
6. Europine-N-oxide	16. Lycopsamine-N-oxide	26. Senecivernine-N-oxide
7. Heliotrine	17. Monocrotaline	27. Senkirkine
8. Heliotrine-N-oxide	18. Monocrotaline-N-oxide	28. Trichodesmine
9. Intermedine	19. Retrorsine	
10. Intermedine-N-oxide	20. Retrorsine-N-oxide	

Typical chromatogram of a PA standard mix

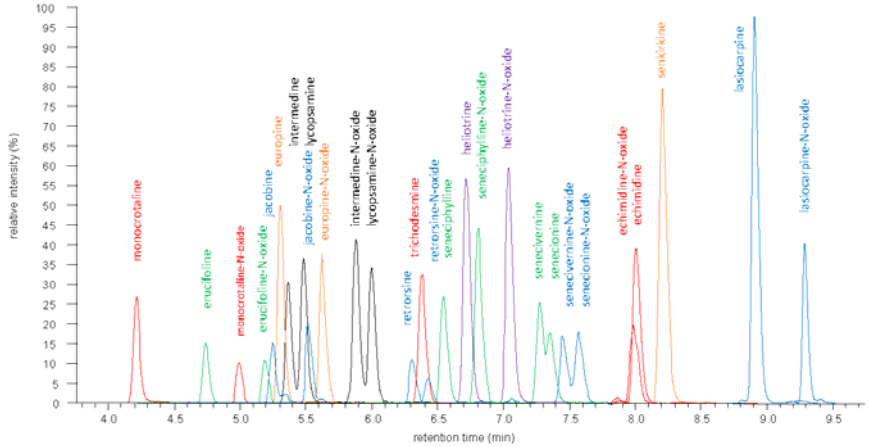
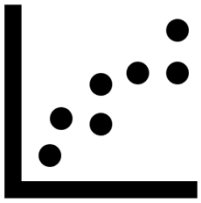


Figure 1: Typical chromatogram of a PA standard mixture (1 ng/mL), TIC of SRM-transitions

Validation

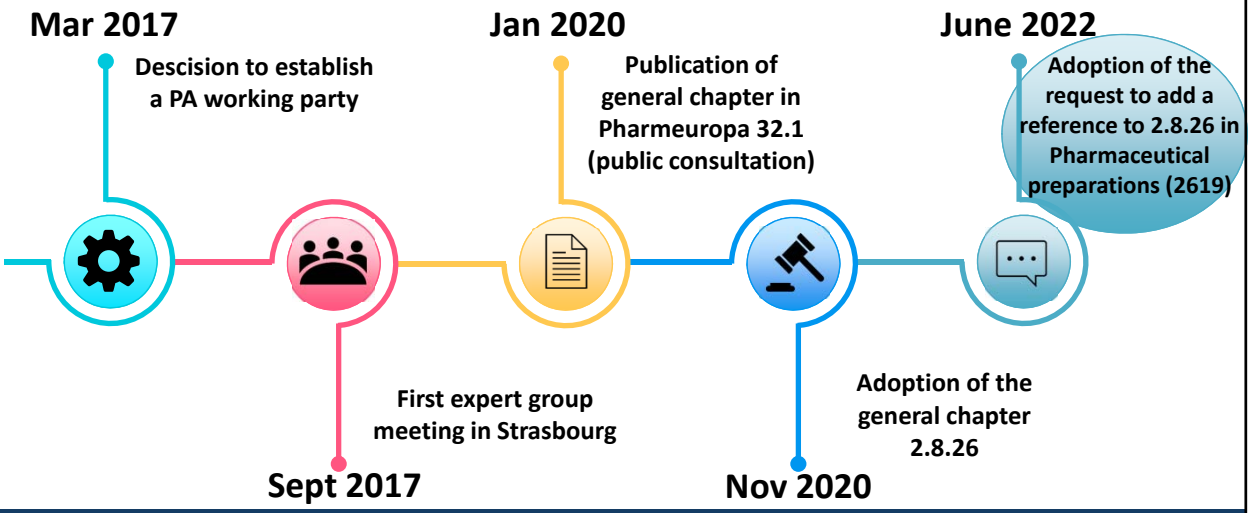
- The analytical procedure given in 2.8.26 is only given as an example method
- The analyst must confirm that the method used meet the given validation requirements in 2.8.26 using at least one representative matrix from each matrix group analysed
- After adequate validation, the procedure may be assumed to be valid for any other matrix belonging to the corresponding matrix group



Verification

- For each new sample, the analyst must perform an assessment to decide whether confirmation of validity of the analytical procedure for the new sample is necessary
- The validity is confirmed in routine analysis by performing additional measurements to demonstrate that the given verification requirements given in this chapter are met
- The assessment is subject to approval by the competent authority

Elaboration of the general chapter 2.8.26



Text proposed for Pharmaceutical preparations (2619)

- **“Contaminant pyrrolizidine alkaloids.**

  Where necessary, apply a suitable control strategy to ensure that patient exposure to pyrrolizidine alkaloids from medicinal products (e.g. herbal and homoeopathic medicinal products) does not exceed the maximum daily intake agreed by the competent authority.

  Manufacturers may refer to general chapter 2.8.26. Contaminant pyrrolizidine alkaloids for assistance with testing.”

Thank you for your time

Collaboration, Innovation and Scientific Excellence: the European Pharmacopoeia 11th Edition
International Conference
19-21 September 2022
Strasbourg, France

Essential oils

Klaus Reh
Chair Expert Group 13A

European Pharmacopoeia 10.7

Revised General monograph

Essential oils (2098)

New General text

Monographs on essential oils (information chapter) (53000)

Special characteristics of essential oils

- Essential oils are widely used as fragrances and flavourings in the cosmetic and food sectors, but usually only in small quantities as active substance in medicinal products.
- The production of essential oils is often performed by farmers or small companies with limited experience in the manufacturing of active substances for pharmaceutical use.
- The starting materials used in the production of essential oils are normally fresh herbal drugs, which are processed shortly after harvesting.
- The manufacturing processes for essential oils should be in line with the GMP Rules Part II.

Herbal drugs (1433)

Herbal drugs are mainly whole, fragmented or broken plants or parts of plants in an unprocessed state, usually in dried form but sometimes fresh.

Where used for the production of essential oils, some of the tests prescribed for dried herbal drugs may no longer be necessary.

General monograph Essential oils:

Herbal drugs used for the preparation of essential oils are of suitable quality and, where applicable, comply with the requirements of any relevant monograph in the European Pharmacopoeia.

Depending on the monograph, the herbal drug may be fresh, lightly wilted, wilted, partially dried, dried, whole, fragmented, broken or cut.

Herbal drugs (1433)

FRESH HERBAL DRUGS

A fresh herbal drug is one that is intended to be processed into a herbal drug preparation (e.g. essential oil, juice, tincture) within a relatively short period of time after harvesting. Under these circumstances, the extensive analysis prescribed for dried herbal drugs is not appropriate and the following analytical requirements, based on the provenance of the fresh herbal drug, are considered suitable, provided that processing to the herbal drug preparation takes place within a validated time period after harvesting.

Herbal drugs (1433)

(1) For a fresh herbal drug that has been cultivated from seeds, cuttings, etc., whose origin and traceability can be demonstrated, and where the complete history of the herbal drug from planting to harvesting is documented:

–macroscopic identification of the plant and plant parts to be processed;

–compliance with a suitable limit test for foreign matter.

(2) For a cultivated fresh herbal drug where the information on life cycle from seed to harvesting, as described under (1), is incomplete, ….

(3) For a fresh herbal drug that is wild-crafted, the analytical requirements should be assessed on a case-by-case basis …..

Peppermint

Peppermint leaf (0406)

Whole or cut, dried leaf of Mentha ×piperita L.

Peppermint leaf dry extract (2382)

Dry extract produced from Peppermint leaf (0406)

Peppermint oil (0405)

Essential oil obtained by steam distillation from the fresh aerial parts of Mentha × piperita L.

Essential oils (2098)

Odorous product, usually of complex composition, obtained from a botanically defined herbal drug by steam distillation, dry distillation, or a suitable mechanical process without heating. If an aqueous phase is present, the essential oils are separated from it by a physical process that does not significantly affect their composition.

Essential oils obtained from the primary production steps may be subjected to a suitable subsequent treatment in order to remove unwanted matter (e.g. insoluble matter) or remaining water, without significantly affecting their composition.

An essential oil may be subjected to further processing steps (combining, rectification, etc.), that may or may not significantly affect its composition.

Essential oils (2098)

An essential oil whose composition has been significantly modified may be known as:

–Rectified essential oil: an essential oil from which part of the constituents has been partially or totally removed by rectification;

–Deterpenated essential oil: an essential oil from which monoterpene hydrocarbons have been partially or totally removed by rectification or any other suitable process;

–Deterpenated and desesquiterpenated essential oil: an essential oil from which monoterpene and sesquiterpene hydrocarbons have been partially or totally removed by rectification or any other suitable process;

–'X'-free or partially 'x'-free essential oil: an essential oil from which one or more particular constituents have been totally or partially removed by rectification or any other suitable process.

Examples:

Eucalyptus oil (0390) rectified oil

Mint oil, partly dementholised (1838)

Essential oils (2098)

Steam distillation. The essential oil is produced from the herbal drug using steam and suitable distillation equipment. The steam may be introduced from an external source or generated by boiling water below the plant material or by boiling water in which the plant material is immersed. The steam and oil vapours are condensed. The water and essential oil are separated by decantation or any other suitable physical process.

Dry distillation. The essential oil is produced by heating the herbal drug at a high temperature in suitable equipment without adding water or steam.

Mechanical process. The essential oil, usually known as 'cold-pressed', is produced by a mechanical process without any heating. This method is used for citrus fruits and involves expressing the essential oil from the pericarp, followed by separation using suitable physical means.

Unless otherwise justified and authorised, as a minimum, water used in the production of essential oils complies with local drinking water standards or, in their absence, with World Health Organization drinking water standards.

Rectification, Rectified Essential oil

Rectification is the process of distillation of an essential oil, normally under vacuum and sometimes with the help of steam, and is performed in order to remove certain unwanted matter or to modify its composition.

Rectification is sometimes applied to essential oils to remove unwanted matter (such as water, insoluble matter or waxes etc.) without significantly changing their composition. The concept of a rectified essential oil as described in the general monograph on Essential oils (2098) does not apply to such essential oils, and the rectification step is not described in the Definition section of individual monographs on these essential oils.

Rectification can also be used to enrich an essential oil in a particular component (for example, 1,8-cineole in Eucalyptus oil (0390)), to remove a fraction of the oil (such as mono- or sesquiterpene hydrocarbons), or to remove, partially or totally, a given constituent. This type of rectification causes a significant change in the composition of the essential oil. In such cases, the Definition section of an individual monograph states that the production of the essential oil includes a rectification step.

Essential oils (2098)

Essential oils are identified by their gas chromatographic profile or by any other suitable test (e.g., high-performance thin-layer chromatography).

Example Peppermint oil: Determine the percentage content of each of the following components. The limits are within the following ranges:

–limonene: 1.0 per cent to 3.5 per cent;

–1,8-cineole: 3.5 per cent to 8.0 per cent;

–menthone: 14.0 per cent to 32.0 per cent;

–menthofuran: 1.0 per cent to 8.0 per cent;

–isomenthone: 1.5 per cent to 10.0 per cent;

–menthyl acetate: 2.8 per cent to 10.0 per cent;

–isopulegol: maximum 0.2 per cent;

–menthol: 30.0 per cent to 55.0 per cent;

–pulegone: maximum 3.0 per cent;

–carvone: maximum 1.0 per cent;

Essential oils (2098)

General tests

The essential oil complies with the prescribed limits for the following tests.

Fatty oils and resinified essential oils (2.8.7). The test applies to essential oils obtained by steam distillation or dry distillation.

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. Where justified and authorised, individual testing of every batch may not be necessary.

Pesticide residues (2.8.13). Where justified and authorised, individual testing of every batch may not be necessary.

Aflatoxin B1 (2.8.18). Where justified and authorised, individual testing of every batch may not be necessary.

Microbiological quality (5.1.4 or 5.1.8). Where justified and authorised, individual testing of every batch may not be necessary.

Essential oils (2098)

Supplementary tests

If applicable and necessary, the essential oil complies with the prescribed limits for the following tests.

Relative density (2.2.5).

Refractive index (2.2.6).

Optical rotation (2.2.7).

Freezing point (2.2.18).

Acid value (2.5.1).

Peroxide value (2.5.5).

Foreign esters (2.8.6).

Residue on evaporation (2.8.9).

Water in essential oils(2.8.5).

Solubility in alcohol (2.8.10).

Monographs on essential oils (information chapter) (53000)

Basis for elaboration of monographs on essential oils

European Pharmacopoeia (Ph. Eur.) monographs on essential oils are elaborated on the basis of essential oils used in medicinal products that have been authorised and/or registered by the competent authorities of Parties to the Convention on the Elaboration of a European Pharmacopoeia.

During the elaboration of an essential oil monograph, the group of experts or working party in charge uses data from a number of samples considered representative of the desired quality of the essential oil. Samples from different sources and different years are considered, as well as technical, state-of-the-art international standards.

Monographs on essential oils (information chapter) (53000)

Different types of certain essential oils (including chemotypes) may exist. The reason for this could be, for example, the origin of the plant material, the chemical composition or the primary processing.

- Only one type is covered
  Thyme oil, Thymol type (1374)
- Different types are covered in one specification
  Lemon oil (0620) Italian type, Argentinian type and other types
- Different types are covered in one monograph with different specifications
  Rosemary oil (1846)

Rosemary oil (1846)

	Spanish type	Maroccan/Tunisian type
α-pinene	18.0 – 26.0 %	9.0 – 14.0 %
camphene	8.0 – 12.0 %	2.5 – 6.0 %
β-pinene	2.0 – 6.0 %	4.0 – 9.0 %
β-myrcene	1.5 – 5.0 %	1.0 – 2.0 %
limonene	2.5 – 5.0 %	1.5 – 4.0 %
cineole	16.0 – 25.0 %	38.0 – 55.0 %
p-cymene	1.0 – 2.2 %	0.8 – 2.5 %
camphor	13.0 – 21.0 %	5.0 – 15.0 %
bornyl acetate	0.5 – 2.5 %	0.1 – 1.5 %
α-terpineol	1.0 – 3.5 %	1.0 – 2.6 %
borneol :	2.0 – 4.5 %	1.5 – 5.0 %
verbenone	0.7 – 2.5 %	Maximum 0.4 %

Thank you very much for your attention!

Risk assessment regarding nitrosamines in herbals: considerations from industry

EDQM Conference
Strasbourg, 19 - 21 September 2022
Dr. Barbara Steinhoff
German Medicines Manufacturers' Association

.B.A.H

Bundesverband der
Arzneimittel-Hersteller e.V.

Background: regulatory situation

EMA/CMDh Call for Review (June 2020)

- Medicinal products containing chemically synthetized or biological active substance (3 steps March 2021 to July 2023): review of processes, risk mitigation and outcome report
- Background: findings of nitrosamine impurities (N-nitroso-dimethylamine, NDMA) in active substances e.g. valsartan
- Herbal and homeopathic medicinal products excluded from Call

Risk assessment in Ph.Eur. monographs

- „Substances for pharmaceutical use" and „Pharmaceutical preparations": drafts under discussion

Background: Regulatory situation

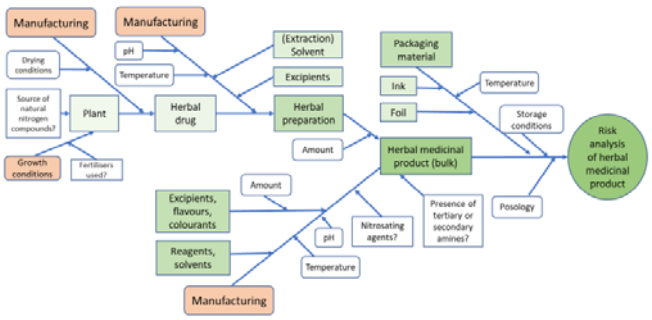
HMPC/CMDh (December 2021)

"Because there is very limited data available so far, there is no evidence that herbal medicinal products have been contaminated with nitrosamine impurities. Nevertheless, a risk evaluation is required for new marketing authorisations/registrations for (traditional) herbal medicinal products in order to prevent and mitigate the presence of nitrosamines." … "Already authorised/registered (traditional) herbal medicinal products are not in the scope of the call for review for the moment, however should an MAH suspect a risk of contamination this should be investigated and in case nitrosamines are detected, this should be reported to the relevant competent authorities together with a proposal of risk mitigation measures as necessary."

.B.A.H Bundesverband der Arzneimittel-Hersteller e.V.

Products of herbal origin: guidance for risk assessment

- BAH considerations as guidance for manufacturers of medicinal products of herbal origin on how to prepare a risk analysis
- Specific production processes → step-by-step risk analysis taking into account potential root causes as listed under Item 4 of the EMA/CMDh Q&A document (EMA/409815/2020)



Source of figure: Bruno Wagner, Finzelberg 2022

.B.A.H Bundesverband der Arzneimittel-Hersteller e.V.

Generally required reaction conditions for nitrosamine formation

Presence of amines (mainly secondary) or quaternary ammonium salt **plus** nitrosating agent and specific conditions:

- Acidic aqueous conditions (pH 2-5)
- Temperatures > 60°C
- Relevant concentrations (e.g., 1 M) of secondary amines and of a nitrosating agent
- Amount of NOx (> 150 ppb)

References:

López-Rodríguez R, McManus JA, Murphy NS, Ott MA, Burns MJ. Pathways for *N*-Nitroso Compound Formation: Secondary Amines and Beyond. Organic Process Research & Development 2020;24(9),1558-1585.

Ashworth IW, Dirat O, Teasdale A, Whiting M. Potential for the Formation of N-Nitrosamines during the Manufacture of Active Pharmaceutical Ingredients: An Assessment of the Risk Posed by Trace Nitrite in Water. Organic Process Research & Development 2020;24(9),1629-1646.

Wu Y, Levons J, Narang AS, Raghavan K, Rao VM. Reactive Impurities in Excipients: Profiling, Identification and Mitigation of Drug-Excipient Incompatibility. AAPS PharmSciTech, 2011;12(4):1248-1263.

Manufacture of the herbal preparation

Herbal drug

- For herbal drugs, the probability of a presence of the a.m. general reaction conditions for nitrosamine formation (pH, temperature, concentrations) can be regarded as low
- Use of fertilizers: *„should be applied sparingly and in accordance with the needs …“* (GACP); checked and documented by regular audits of suppliers
- Water for irrigation: compliance with standards (e.g., nitrate content)
- Drying processes normally under low temperatures (protection of instable constituents); not comparable with e.g., tobacco drying (older publications)
- Conclusion: low probability of nitrosamine formation in herbal drugs/herbal substances (discussion in individual risk assessment)

Manufacture of the herbal preparation

Extraction solvents

- Water, ethanol, methanol, acetone (no solvents bearing a risk of nitrosamine formation)
- Ph.Eur. „Water for preparation of extracts“: nitrate limit 50 ppm
- No additional risk from using recovered solvents

Manufacturing process

- Extraction process: low probability of a presence of the a.m. general reaction conditions for nitrosamine formation (pH, temperature, concentrations)
- Potential cross-contamination from other products: cleaning validation according to GMP (determination of total organic carbon, TOC)
- Packaging material for herbal preparation: normally PE bags

Manufacture of the herbal preparation

Excipients

- Inert excipients used during manufacture: mainly maltodextrin, lactose, glucose syrup, cellulose, starch; highly dispersed silica (flowability); ethanol/water; glycerine, ethylene glycol, polyethylene glycol, vegetables oils

  → reaction conditions for nitrosamine formation unlikely
- International Pharmaceutical Excipients Council (IPEC): risk by excipients in general negligible, but potential risk to be assessed by product manufacturer (template available)
- MAH should evaluate supplier‘s information

Source: https://www.ipec-europe.org/articles/questionnaire-for-excipient-nitrosamines-risk-evaluation.html

Manufacture of the medicinal product of herbal origin

Excipients used for the finished product

- Risk generally considered very low (see above)
- Only trolamine known to contain NDMA; Ph.Eur. limit 24 ppb.

Manufacturing process of the finished product

- Due to specific reaction conditions, the risk is generally low (see manufacture of herbal preparation); higher temperatures and acidic conditions are extremely rare
- Cross-contamination only relevant in case of shared facilities and in case of nitrosamines present in the product; can be excluded by cleaning validation according to GMP

Manufacture of the medicinal product of herbal origin

Packaging material

- General issues, not herbal-specific: potential risk may consist in using blister lidding foils containing nitrocellulose as printing primer or overlaquer which might react with amines in printing ink e.g., during heat sealing processes
- According to EFPIA, typical blistering processes have a low risk, for various reasons (e.g., low volatilisation, short time), no concern regarding extraction or leaching of nitrosamines from packaging material
  (https://www.efpia.eu/media/580594/workflows-for-quality-risk-management-of-nitrosamine-risks-in-medicines.pdf)
- To be considered in the individual risk assessment

Storage

- Storage of herbal substances, herbal preparations or finished herbal products is performed at room temperature or below → no relevant risk of nitrosamine formation under normal storage conditions

Conclusion

- Herbal and homeopathic medicinal products are excluded from the Call for Review of EMA/CMDh.
- No evidence of nitrosamine contamination has been observed so far.
- A risk evaluation is necessary in case of new applications for (traditional) herbal medicinal products; for already authorized/registered products, investigation in case of a suspected risk of contamination.
- The risk assessment should be performed (step by step) for the entire production process starting from cultivation/collection of the medicinal plant and ending up with the manufacture of the finished product.
- For medicinal products of herbal origin, only few potential root causes for nitrosamine formation are relevant.
- All in all, for these products the probability of nitrosamine contamination is considered low.

Thanks to the members of the BAH Working Group for their input and discussion and thank you for your attention!