DIE ROLLE DER HPTLC IM EUROPÄISCHEN ARZNEIBUCH

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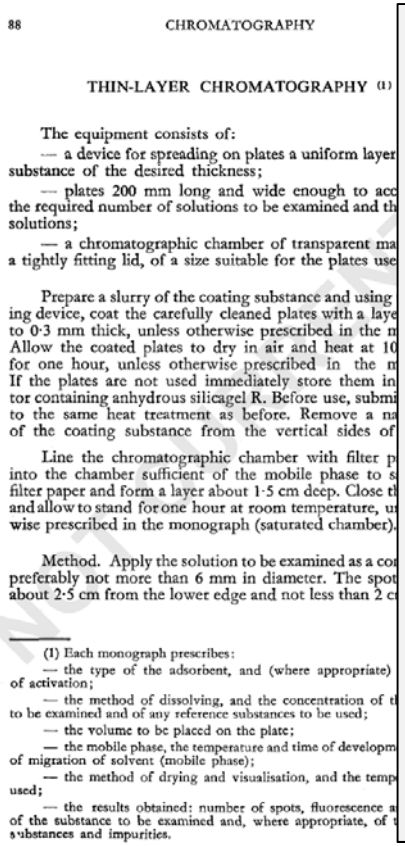
Swissmedic Expertentreffen 2018

CAMAG 60 YEARS

Worum geht es?

- Von der DC zur HPTLC
- Das allgemeine Kapitel 2.8.25
- HPTLC in der Praxis
- HPTLC Fingerprints und ihre quantitativen Aspekte
- HPTLC Fingerprints als Alternative zum HPLC Assay

Von der DC zur HPTLC – Erste Ausgabe PhEur 1969



88 CHROMATOGRAPHY

THIN-LAYER CHROMATOGRAPHY (1)

The equipment consists of:
— a device for spreading on plates a uniform layer substance of the desired thickness;
— plates 200 mm long and wide enough to acc the required number of solutions to be examined and th solutions;
— a chromatographic chamber of transparent ma a tightly fitting lid, of a size suitable for the plates use

Prepare a slurry of the coating substance and using ing device, coat the carefully cleaned plates with a laye to 0·3 mm thick, unless otherwise prescribed in the m Allow the coated plates to dry in air and heat at 10 for one hour, unless otherwise prescribed in the m If the plates are not used immediately store them in tor containing anhydrous silicagel R. Before use, submi to the same heat treatment as before. Remove a na of the coating substance from the vertical sides of

Line the chromatographic chamber with filter p into the chamber sufficient of the mobile phase to s filter paper and form a layer about 1·5 cm deep. Close th and allow to stand for one hour at room temperature, u wise prescribed in the monograph (saturated chamber).

Method. Apply the solution to be examined as a cor preferably not more than 6 mm in diameter. The spot about 2·5 cm from the lower edge and not less than 2 c

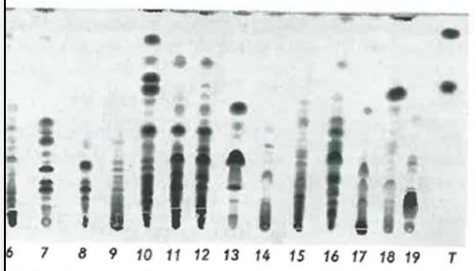
(1) Each monograph prescribes:
— the type of the adsorbent, and (where appropriate) of activation;
— the method of dissolving, and the concentration of t to be examined and of any reference substances to be used;
— the volume to be placed on the plate;
— the mobile phase, the temperature and time of developm of migration of solvent (mobile phase);
— the method of drying and visualisation, and the temp used;
— the results obtained: number of spots, fluorescence a of the substance to be examined and, where appropriate, of t substances and impurities.

- Selbstgestrichene Platten
- Gesättigte Kammer (20x20 cm)

In den Monographien definiert:

- Adsorbens/Aktivierung
- Untersuchungs- und Testlösung
- Auftragevolumen
- Mobile Phase, Temperatur, Laufstrecke
- Trocknung, Derivatisierung
- Resultate

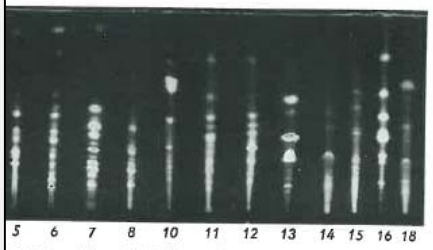
Stahl, Dünnschichtchromatographie
toriumshandbuch Springer 1967



6 7 8 9 10 11 12 13 14 15 16 17 18 19 T

htigsten Harze und Balsame auf einer 40 cm-DC-Platte. Nachweis: (I)- und -(V)-chlorid (Nr. 19, 22). Einzelheiten im Text

lubalsam
a foetida
lbanum
rrha
banum
11 Mastix
12 Dammar
13 Terebinthina
14 Colophonium
15 Canadabalsam, echt
16 Canadabalsam, künstlich
17 Sandarac
18 Copaibabalsam
19 Gutti
T Testgemisch DESAGA



5 6 7 8 10 11 12 13 14 15 16 18

nommenes Dünnschicht-Chromatogramm von Harzen und Balsamen. chnungen wie bei Abb. 108 angegeben. Sprühreagens: Antimon-(III)-chlorid (Reag.-Nr. 19)

Von der DC zur HPTLC – Vierte Ausgabe PhEur 2001, Kapitel 2.2.27.

2.2.27. Thin-layer chromatography EUROPEAN PHARMACOPOEIA 4

Method. Place in the bottom of the tank a layer 2.5 cm deep of the solvent prescribed in the monograph, close the tank and allow to stand for 24 h at 20 °C to 25 °C. Maintain the tank at this temperature throughout the subsequent procedure. Draw a fine pencil line horizontally across the paper at such a distance from one end that when this end is secured in the solvent trough and the remainder of the paper is hanging freely over the guide rod, the line is a few centimetres below the guide rod and parallel with it. Using a micro-pipette, apply on the pencil line the volume of the solution prescribed in the monograph. If the total volume to be applied would produce a spot more than 10 mm in diameter, apply the solution in portions, allowing each to dry before the next application. When more than one chromatogram is to be run on the same strip of paper, space the solutions along the pencil line at points not less than 3 cm apart. Insert the paper in the tank, close the lid, and allow to stand for 1 h 30 min. Introduce into the solvent trough, through the hole in the lid, a sufficient quantity of the mobile phase, close the tank and allow elution to proceed for the prescribed distance or time. Remove the paper from the tank and allow to dry in air. The paper should be protected from bright light during the elution process.

2.2.27. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase).

APPARATUS

Plates. The chromatography is carried out using pre-coated plates as described under *Reagents (4.1.1).*

Preconditioning of the plates. It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 100-105 °C for 1 h.

A chromatographic tank with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipettes, microsyringes, calibrated disposable capillaries or other application devices suitable for the proper application of the solutions.

Fluorescence detection device to measure direct fluorescence or the inhibition of fluorescence.

Visualisation reagents to detect the separated spots by spraying, exposure to vapour or immersion.

METHOD

Vertical development. Line the walls of the chromatographic tank with filter paper. Pour into the chromatographic tank a sufficient quantity of the mobile phase for the size of the tank to give after impregnation of the filter paper a layer of appropriate depth related to the dimension of the plate to be used. For saturation of the chromatographic tank, replace the lid and allow to stand at 20-25 °C for 1 h. Unless otherwise indicated, the chromatographic separation is performed in a saturated tank.

Apply the prescribed volume of the solutions in sufficiently small portions to obtain bands or circular spots at an appropriate distance from the lower edge and from the sides of the plate. Apply the solutions on a line parallel to the lower edge of the plate with an interval of at least 10 mm between the spots.

When the solvent has evaporated from the applied solutions, place the plate in the chromatographic tank, ensuring that the plate is as vertical as possible and that the spots or bands are above the surface of the mobile phase. Close the chromatographic tank, maintain it at 20-25 °C and protect from sunlight. Remove the plate when the mobile phase has moved over the prescribed distance. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

Horizontal development. Apply the prescribed volume of the solutions in sufficiently small portions to obtain circular spots 1 mm to 2 mm in diameter, or bands 5 mm to 10 mm by 1 mm to 2 mm, at an appropriate distance from the lower edge and from the sides of the plate. Apply the solutions on a line parallel to the lower edge of the plate with an interval of at least 5 mm between the spots. When the solvent has evaporated from the applied solutions, introduce a sufficient quantity of the mobile phase into the trough of the chamber using a syringe or pipette, place the plate horizontally in the chamber and connect the mobile phase direction device according to the manufacturer's instructions. If prescribed, develop the plate starting simultaneously at both ends. Close the chamber and maintain it at 20-25 °C. Remove the plate when the mobile phase has moved over the distance prescribed in the monograph. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

VISUAL ESTIMATION

Identification. The principal spot in the chromatogram obtained with the test solution is visually compared to the corresponding spot in the chromatogram obtained with the reference solution by comparing the colour, the size and the retention factor (R_f) of both spots.

The retention factor (R_f) is defined as the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front from the point of application.

Verification of the separating power for identification. Normally the performance given by the suitability test described in *Reagents (4.1.1)* is sufficient. Only in special cases an additional performance criterion is prescribed in the monograph.

Related substances test. The secondary spot(s) in the chromatogram obtained with the test solution is (are) visually compared to either the corresponding spot(s) in the chromatogram obtained with the reference solution containing the impurity(ies) or the spot in the chromatogram obtained with the reference solution prepared from a dilution of the test solution.

Verification of the separating power. The requirements for the verification of the separating power are prescribed in the monographs concerned.

38 *See the information section on general monographs (cover pages)*

EUROPEAN PHARMACOPOEIA 4

Verification of the detecting power. The detecting power is satisfactory if a spot or band is clearly visible in the chromatogram obtained with the most dilute reference solution.

QUANTITATIVE MEASUREMENT

The requirements for resolution and separation are prescribed in the monographs concerned.

Substances separated by thin-layer chromatography and responding to UV-Vis irradiation can be determined directly on the plate, using appropriate instrumentation. While moving the plate or the measuring device, examine the plate by measuring the reflectance or transmittance of the incident light. Similarly, fluorescence may be measured using an appropriate optical system. Substances containing radionuclides can be quantified in three ways: either directly by moving the plate alongside a suitable counter or vice versa (see *Radiopharmaceutical preparations (01/2005)*), by cutting the plates into strips and measuring the radioactivity on each individual strip using a suitable counter or by scraping off the stationary phase, dissolving it in a suitable scintillation cocktail and measuring the radioactivity using a liquid scintillation counter.

Apparatus. The apparatus for direct measurement on the plate consists of:

- a device for exact positioning and reproducible dispensing of the amount of substances onto the plate,
- a mechanical device to move the plate or the measuring device along the *x*-axis or the *y*-axis,
- a recorder and a suitable integrator or a computer,
- *for substances responding to UV-Vis irradiation*: a photometer with a source of light, an optical device able to generate monochromatic light and a photo cell of adequate sensitivity are used for the measurement of reflectance or transmittance. In the case where fluorescence is measured, a monochromatic filter is required in addition, to select a particular spectral region of the emitted light,
- *for substances containing radionuclides*: a suitable counter for radioactivity. The linearity range of the counting device is to be verified.

Method. Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph and, if necessary, prepare the reference solutions of the substance to be determined using the same solvent as in the test solution. Apply the same volume of each solution to the plate and develop.

Substances responding to UV-Vis irradiation: Prepare and apply not fewer than three reference solutions of the substance to be examined, the concentrations of which span the expected value in the test solution (about 80, 100 and 120 per cent). Spray with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with the test and reference solutions. Use the measured results for the calculation of the amount of substance in the test solution.

Substances containing radionuclides: Prepare and apply a test solution containing about 100 per cent of the expected value. Determine the radioactivity as a function of the path length and report the radioactivity in each resulting peak as a percentage of the total amount of radioactivity.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques (2.2.46).* The extent to which adjustments of

General Notices (1) apply to all monographs and other texts

- Fertigplatten
- Gesättigte Flachboden- oder Doppeltrogkammer
- Punktauftragung oder **Bandauftragung**
- 2D – Entwicklung

Neu: Horizontalentwicklung (impliziert HPTLC?)

- 1-2 mm Punkte oder 5-10 mm Bänder
- Entwicklung von gegenüberliegenden Seiten
- 2D

Neu: Quantitative Auswertung

- Densitometrie
- Absorptions- oder Fluoreszenzmessung

Von der DC zur HPTLC – Pharmeuropa 15.3, 2003

TLC for the Analysis of Herbal Drugs

A Critical Review of the Status and Proposal for Improvement of Monographs

ABSTRACT

For the analysis of medicinal plants Thin-Layer Chromatography (TLC) is well suited. Unsurpassed flexibility due to a large number of parameters, which can influence the chromatographic result, is one of the inherent advantages of the method. On the other hand, without standardisation and precise definition of those parameters, results in TLC are difficult to reproduce. As it is currently presented in the European Pharmacopoeia (Ph. Eur.), state of the art features of modern TLC are widely ignored. The following paper is an attempt to point out possible improvements in the general method descriptions as well as in individual monographs. The focus is on optimization and standardisation of experimental details, which can help to increase the reproducibility of the method. Based on theoretical discussions of individual parameters and several practical examples, the advantages of modern HPTLC (high performance TLC) are illustrated and the need for a standardised approach to TLC methodology in new pharmacopoeial monographs is explained.

presenting the chromatographic result as an image cannot be substituted [1]. Rapid analysis and low cost per sample are additional benefits.

A principal requirement for increasing the recognition and acceptance of TLC as a competitive, state of the art concerning several important experimental details (sample application, chromatogram development, derivatisation) is missing and the most important advantage of TLC, the possibility of presenting qualitative chromatographic results in form of an image, is not even mentioned.

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Even though it is described as a quantitative method in the general section there are only a couple of monographs featuring quantitative TLC. All assays are to be done by HPLC and even for identification purposes TLC is being replaced successively by other description. Alternatively, a specific performance test (system suitability test) could be included. As stipulated by the Technical Guide for the Elaboration of Monographs [2], such statement is already required for developmental work submitted to the

Although it is not permitted to specify brand names in the monographs, it should be noted that plates of different manufacturers can vary significantly with respect to the result obtained with a specific method, yet still pass the system suitability test given in the reagent section of the pharmacopoeia (Figure 2).

Because it is not feasible to design a single test that evaluates the behaviour of the stationary phase for all possible separation problems, the manufacturer of the plate should be specified in the method

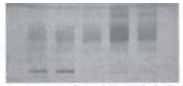

Figure 2 - Separation of several Black cohosh (Cimicifuga racemosa) samples on HPTLC Si 60 F254 plates from different manufacturers (A) Merck, (B) Macherey&Nagel. Images courtesy of E. Fluchsmann AG, Zürich.

3.2 Sample application

All identifications in TLC are primarily based on comparison of migration distances (R_f-values). The quality of the analysis therefore depends on proper positioning of the sample. For quantitative

If samples are applied as narrow bands, the visual impression of the chromatogram is improved. If such bands are sprayed, additionally a homogenous distribution of the sample over the entire length of the band is achieved. This is the basis for reliable

Figure 3 - Influence of different application techniques on the resolution of a test dye mixture in methanol (5 µL and 10 µL each) on HPTLC silica gel 60. Mobile phase: toluene. Left to right: contact spot, sprayed spot, contact band, and sprayed band. (A) Application position before chromatography. (B) Separation of dye mixture. (C) Comparison of contact spot (track 1, dashed line) to sprayed spot (track 3, solid line). (D) Comparison of contact band (track 5, solid line) to sprayed band (track 7, dotted line)

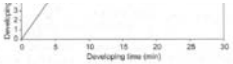

Figure 5 - Relationship of developing distance and the developing time on HPTLC silica gel 60 (experimental values). Mobile phase: ethyl acetate, toluene (5:95 V/V).

In a given chamber, keeping all other parameters constant, resolution (R_s) of 2 compounds is as

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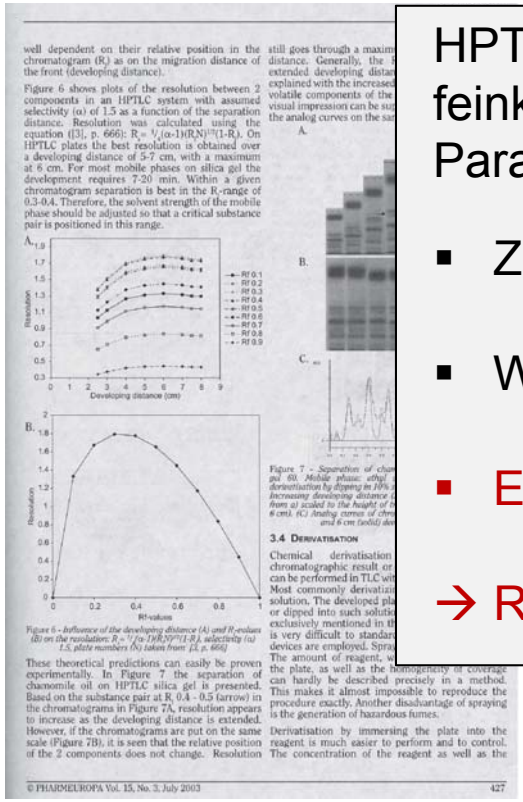
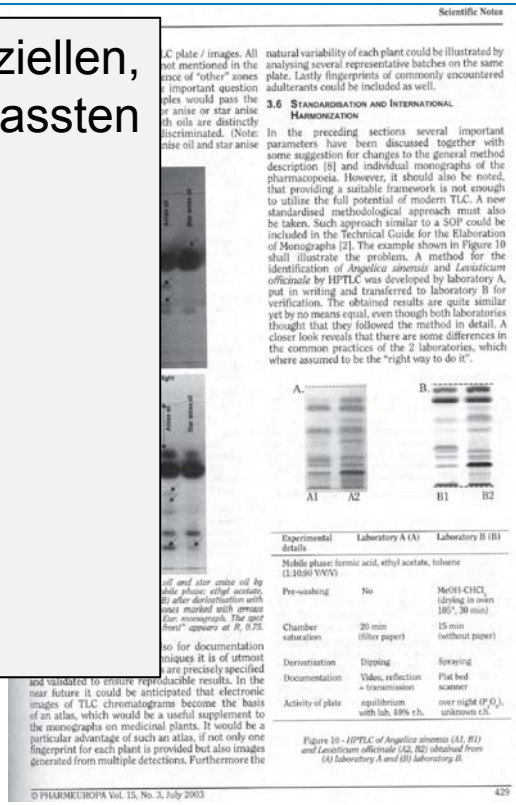
Von der DC zur HPTLC – Pharmeuropa 15.3, 2003



HPTLC ist miniaturisierte DC mit speziellen, feinkörnigen Platten und angepassten Parametern

- Zeitersparnis
- Weniger Lösungsmittel
- Einfach zu standardisieren

→ Reproduzierbare Ergebnisse



Schweiz: Antrag auf Revision des Kapitels 2.2.27

HPTLC = Miniaturisierte DC

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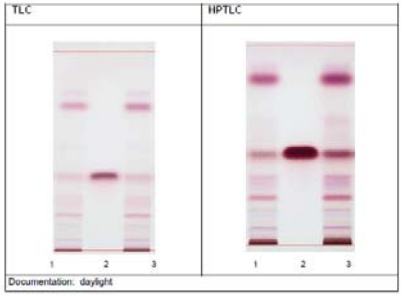
TLC versus HPTLC

Report 11/03-06/04

R. Hermann, M. Reichold, M. Goppel und G. Franz

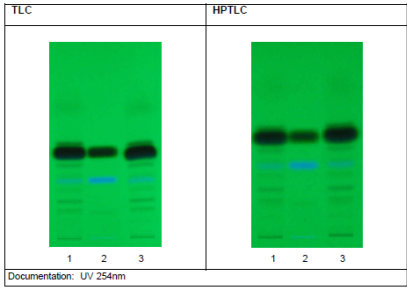


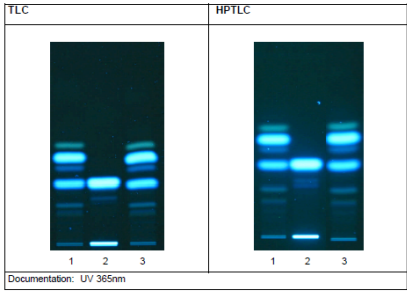
Result of HPTLC

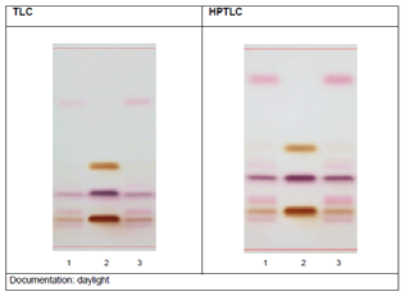
The fingerprint corresponds. Identification based on the pharmacopoeial monograph, part C, is possible.
Applied volume of the test and reference solution were reduced to 50-60%.
Rf-values of the detectable compounds are a little higher.
Gain of time 30 min.

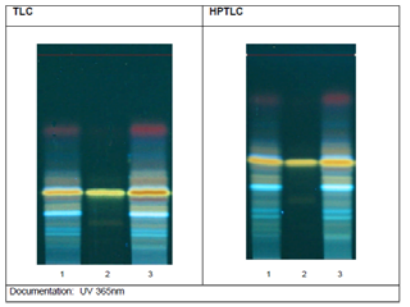
	TLC	HPTLC
Reference substance	Rf-values	
Cineole R	0.38	0.47
Development time	45min	15min
Distance	15cm	7cm

Conclusion:
Alternative utilization of HPTLC is feasible.



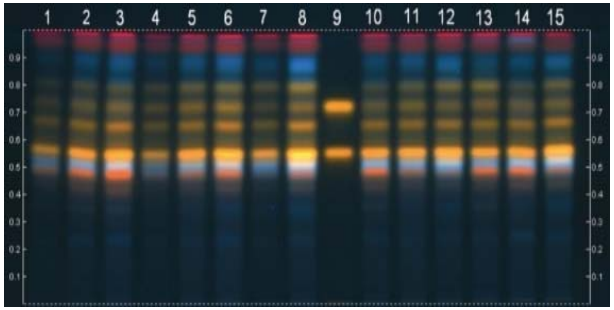




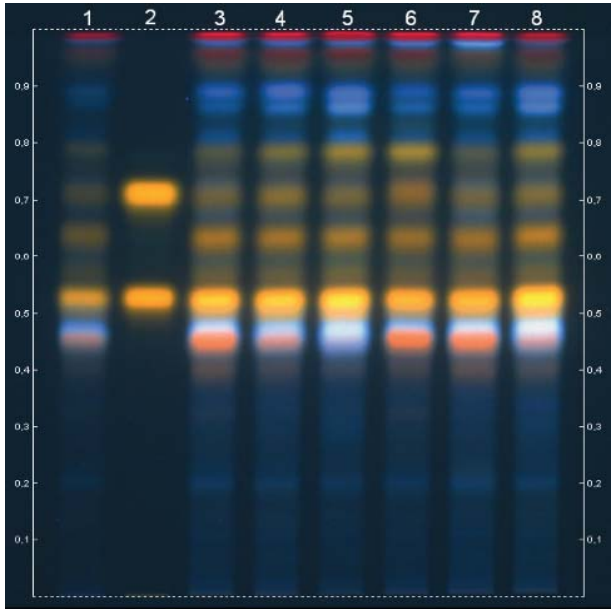


HPTLC = miniaturisierte DC

1. Die Platte



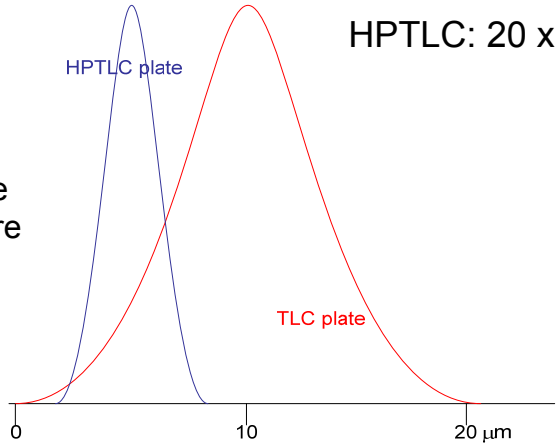
HPTLC: 20 x 10 cm



TLC: 20 x 20 cm

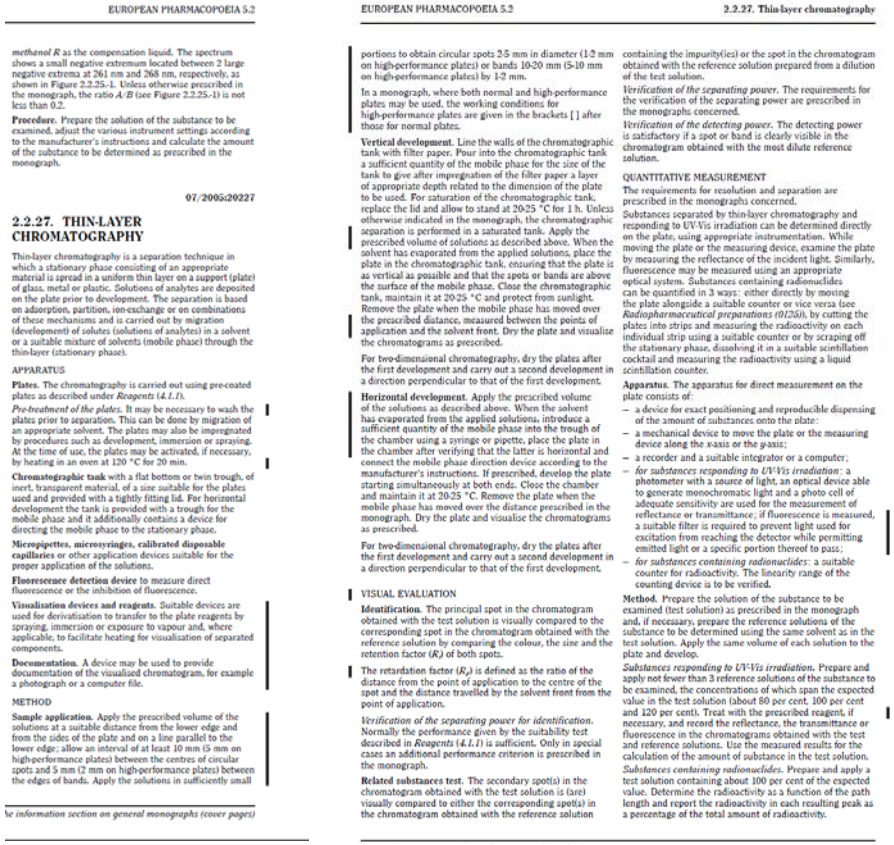
2. Die Schicht

Kleine Teilchen, enge Klassierung → höhere Trennleistung



3. Weniger Fliessmittel, kürzere Entwicklungszeit, mehr Proben

Von der DC zur HPTLC – Ausgabe 5.4 2005



EUROPEAN PHARMACOPOEIA 5.2

methanol R as the compensation liquid. The spectrum shows a small negative extremum located between 2 large negative extrema at 261 nm and 268 nm, respectively, as shown in Figure 2.2.25.-1. Unless otherwise prescribed in the monograph, the ratio *A*/*B* (see Figure 2.2.25.-1) is not less than 0.2.

Procedure. Prepare the solution of the substance to be examined, adjust the various instrument settings according to the manufacturer's instructions and calculate the amount of the substance to be determined as prescribed in the monograph.

07/2005:20227

2.2.27. THIN-LAYER CHROMATOGRAPHY

Thin-layer chromatography is a separation technique in which a stationary phase consisting of an appropriate material is spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Solutions of analytes are deposited on the plate prior to development. The separation is based on adsorption, partition, ion-exchange or on combinations of these mechanisms and is carried out by migration (development) of solutes (solutions of analytes) in a solvent or a suitable mixture of solvents (mobile phase) through the thin-layer (stationary phase).

APPARATUS

Plates. The chromatography is carried out using pre-coated plates as described under *Reagents (4.1.1)*.

Pre-treatment of the plates. It may be necessary to wash the plates prior to separation. This can be done by migration of an appropriate solvent. The plates may also be impregnated by procedures such as development, immersion or spraying. At the time of use, the plates may be activated, if necessary, by heating in an oven at 120 °C for 20 min.

Chromatographic tank with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.

Micropipettes, microsyringes, calibrated disposable capillaries or other application devices suitable for the proper application of the solutions.

Fluorescence detection device to measure direct fluorescence or the inhibition of fluorescence.

Visualisation devices and reagents. Suitable devices are used for derivatisation to transfer to the plate reagents by spraying, immersion or exposure to vapour and, where applicable, to facilitate heating for visualisation of separated components.

Documentation. A device may be used to provide documentation of the visualised chromatogram, for example a photograph or a computer file.

METHOD

Sample application. Apply the prescribed volume of the solutions at a suitable distance from the lower edge and from the sides of the plate and on a line parallel to the lower edge; allow an interval of at least 10 mm (5 mm on high-performance plates) between the centres of circular spots and 5 mm (2 mm on high-performance plates) between the edges of bands. Apply the solutions in sufficiently small

e information section on general monographs (cover pages)

EUROPEAN PHARMACOPOEIA 5.2 — 2.2.27. Thin-layer chromatography

2. Methods of analysis

portions to obtain circular spots 2-5 mm in diameter (1-2 mm on high-performance plates) or bands 10-20 mm (5-10 mm on high-performance plates) by 1-2 mm.

In a monograph, where both normal and high-performance plates may be used, the working conditions for high-performance plates are given in the brackets [ ] after those for normal plates.

Vertical development. Line the walls of the chromatographic tank with filter paper. Pour into the chromatographic tank a sufficient quantity of the mobile phase for the size of the tank to give after impregnation of the filter paper a layer of appropriate depth related to the dimension of the plate to be used. For saturation of the chromatographic tank, replace the lid and allow to stand at 20-25 °C for 1 h. Unless otherwise indicated in the monograph, the chromatographic separation is performed in a saturated tank. Apply the prescribed volume of solutions as described above. When the solvent has evaporated from the applied solutions, place the plate in the chromatographic tank, ensuring that the plate is as vertical as possible and that the spots or bands are above the surface of the mobile phase. Close the chromatographic tank, maintain it at 20-25 °C and protect from sunlight. Remove the plate when the mobile phase has moved over the prescribed distance, measured between the points of application and the solvent front. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

Horizontal development. Apply the prescribed volume of the solutions as described above. When the solvent has evaporated from the applied solutions, introduce a sufficient quantity of the mobile phase into the trough of the chamber using a syringe or pipette, place the plate in the chamber after verifying that the latter is horizontal and connect the mobile phase direction device according to the manufacturer's instructions. If prescribed, develop the plate starting simultaneously at both ends. Close the chamber and maintain it at 20-25 °C. Remove the plate when the mobile phase has moved over the distance prescribed in the monograph. Dry the plate and visualise the chromatograms as prescribed.

For two-dimensional chromatography, dry the plates after the first development and carry out a second development in a direction perpendicular to that of the first development.

VISUAL EVALUATION

Identification. The principal spot in the chromatogram obtained with the test solution is visually compared to the corresponding spot in the chromatogram obtained with the reference solution by comparing the colour, the size and the retention factor (R_f) of both spots.

The retardation factor (R_x) is defined as the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front from the point of application.

Verification of the separating power for identification. Normally the performance given by the suitability test described in *Reagents (4.1.1)* is sufficient. Only in special cases an additional performance criterion is prescribed in the monograph.

Related substances test. The secondary spot(s) in the chromatogram obtained with the test solution is (are) visually compared to either the corresponding spot(s) in the chromatogram obtained with the reference solution containing the impurity(ies) or the spot in the chromatogram obtained with the reference solution prepared from a dilution of the test solution.

Verification of the separating power. The requirements for the verification of the separating power are prescribed in the monographs concerned.

Verification of the detecting power. The detecting power is satisfactory if a spot or band is clearly visible in the chromatogram obtained with the most dilute reference solution.

QUANTITATIVE MEASUREMENT

The requirements for resolution and separation are prescribed in the monographs concerned.

Substances separated by thin-layer chromatography and responding to UV-Vis irradiation can be determined directly on the plate, using appropriate instrumentation. While moving the plate or the measuring device, examine the plate by measuring the reflectance of the incident light. Similarly, fluorescence may be measured using an appropriate optical system. Substances containing radionuclides can be quantified in 3 ways: either directly by moving the plate alongside a suitable counter or vice versa (see *Radiopharmaceutical preparations (0125)*), by cutting the plates into strips and measuring the radioactivity on each individual strip using a suitable counter or by scraping off the stationary phase, dissolving it in a suitable scintillation cocktail and measuring the radioactivity using a liquid scintillation counter.

Apparatus. The apparatus for direct measurement on the plate consists of:

- a device for exact positioning and reproducible dispensing of the amount of substances onto the plate;
- a mechanical device to move the plate or the measuring device along the *x*-axis or the *y*-axis;
- a recorder and a suitable integrator or a computer;
- *for substances responding to UV-Vis irradiation:* a photometer with a source of light, an optical device able to generate monochromatic light and a photo cell of adequate sensitivity are used for the measurement of reflectance or transmittance; if fluorescence is measured, a suitable filter is required to prevent light used for excitation from reaching the detector while permitting emitted light or a specific portion thereof to pass;
- *for substances containing radionuclides:* a suitable counter for radioactivity. The linearity range of the counting device is to be verified.

Method. Prepare the solution of the substance to be examined (test solution) as prescribed in the monograph and, if necessary, prepare the reference solutions of the substance to be determined using the same solvent as in the test solution. Apply the same volume of each solution to the plate and develop.

Substances responding to UV-Vis irradiation. Prepare and apply not fewer than 3 reference solutions of the substance to be examined, the concentrations of which span the expected value in the test solution (about 80 per cent, 100 per cent and 120 per cent). Treat with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with the test and reference solutions. Use the measured results for the calculation of the amount of substance in the test solution.

Substances containing radionuclides. Prepare and apply a test solution containing about 100 per cent of the expected value. Determine the radioactivity as a function of the path length and report the radioactivity in each resulting peak as a percentage of the total amount of radioactivity.

General Notices (1) apply to all monographs and other texts

3091

- Vorwaschen und Trocknen der Platte
- Derivatisierung («behandeln»)

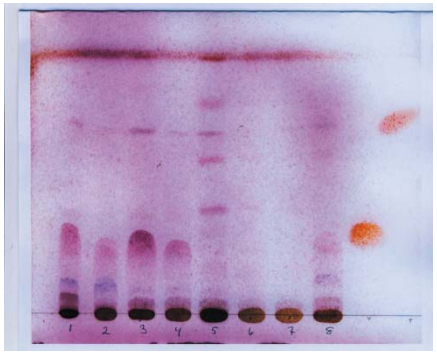
NEU: HPTLC Parameter in [Klammern]

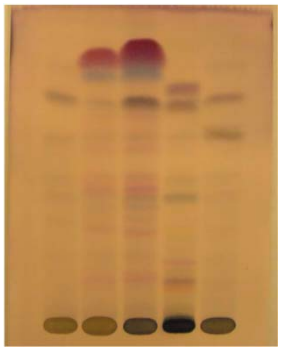
NEU: Dokumentation

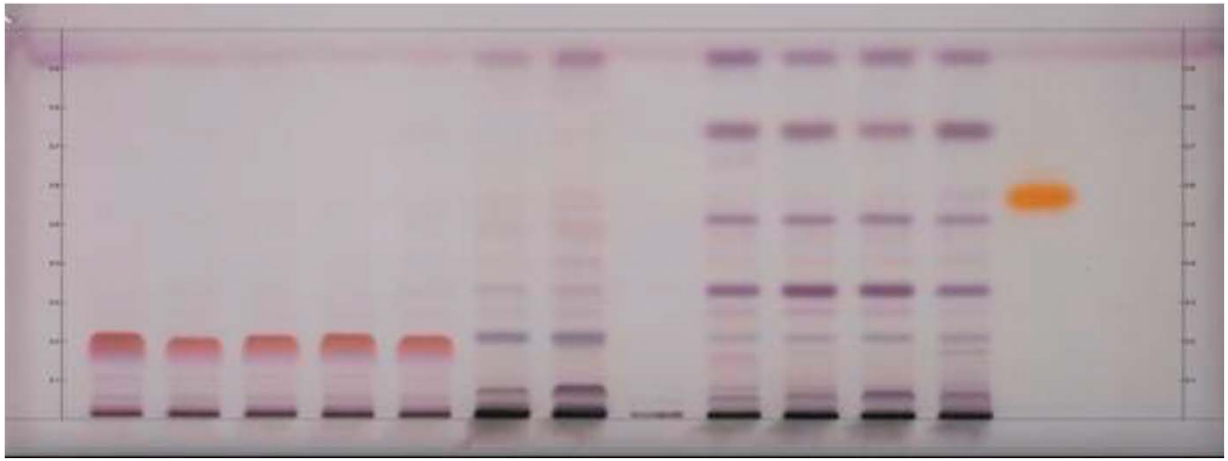
Identifizierung von Acanthopanax für PhEur

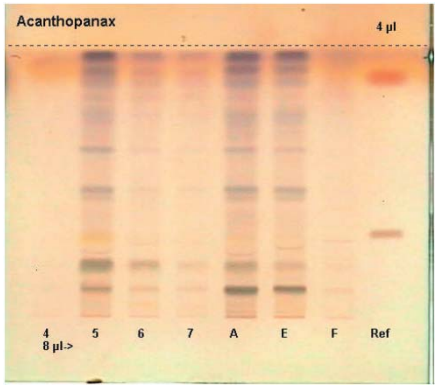
Mobile Phase: Dichlormethan, Ethylacetat 98:2 (v/v)
Platte: Kieselgel 60
Derivatisierung: Anisaldehyd-Reagenz

Welches Chromatogramm soll die Basis für die Resultatbeschreibung sein?



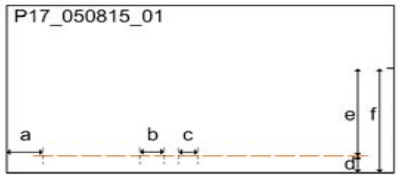




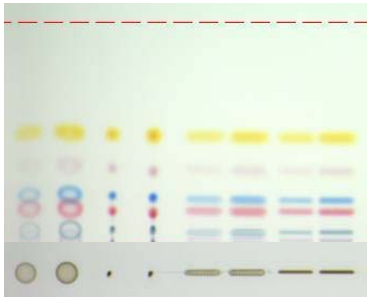


Viele Parameter sind in 2.2.27 nicht eindeutig definiert und werden in den Monographien unterschiedlich beschrieben …

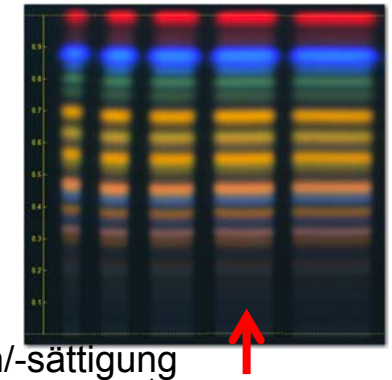
Platten Layout



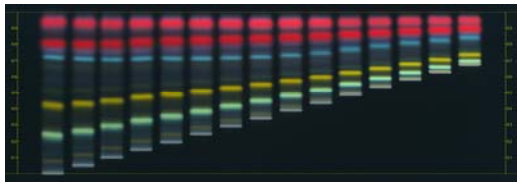
Punkt oder Band

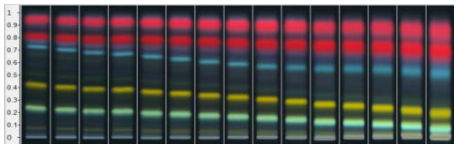


Bandlänge

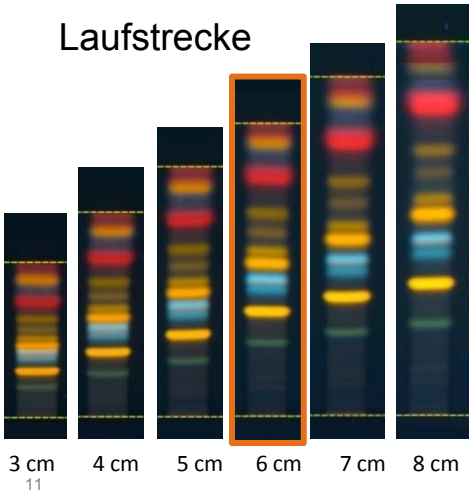


Abstand Fliessmittelpegel

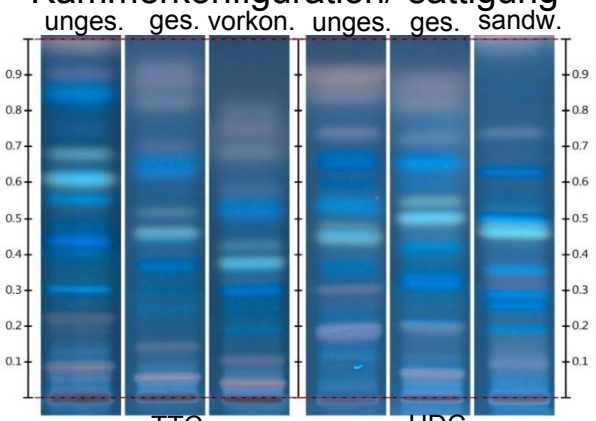




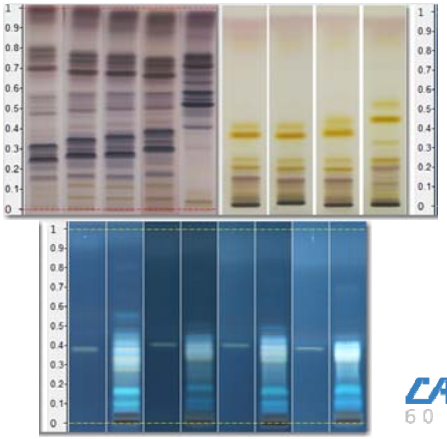
Laufstrecke



Kammerkonfiguration/-sättigung



Relative Feuchte



2012

HPTLC Association

Standard operating procedure for HPTLC

Source
B. Meier & A. Spriano, Zurich University of Applied Sciences

Purpose
This standard operating procedure (SOP) provides general guidance for analysis by high-performance thin-layer chromatography HPTLC.

Definitions
HPTLC is performed on 20x10 cm HPTLC glass plates coated with silica gel 60 F254. Suitable (preferably software-controlled) instruments must be employed for sample application, chromatogram development, derivatization, and documentation. NOTE: if no automatic developing chamber is available a 20x10 cm twin trough chamber may be used

NOTE: Record temperature and relative humidity in the laboratory

1. Preparation of plates
1.1 Obtain HPTLC plate silica gel 60 F 254 (20x10 cm). Record the batch number
1.2 Inspect plate under UV 254 for any damage of the layer. If damage is detected discard plate.
1.3 With a soft pencil label the plate in the upper right corner with: your initials – date (dd/mm/yy) - consecutive number for the day. Example ER-23/02/10-001
1.4 On the right side of the plate mark developing distance at 70 mm from lower edge of plate
Note: left handed persons may label /mark the plate on left side

2. Preparation of chamber (manual development only)
2.1 Obtain a twin trough chamber for 20x10 cm plates
2.2 Fit the rear trough of chamber with a filter paper of corresponding size
2.3 Pour 20 ml of developing solvent over the filter paper into the rear trough ensuring complete wetting. Pour 10 ml of developing solvent into the front trough
2.4 Close the lid of the chamber and allow 20 min for saturation

3. Sample application
3.1 Select the following application parameters on the application device
band length 8 mm
number of tracks 15 (20x10 cm plate) / 7 (10x10 cm plate)
first application position X: 20 mm
application position Y: 8 mm
distance between tracks: automatic (minimum 11 mm)
sample solvent type: methanol
3.2 Disable any unused tracks
3.3 Apply the application volumes as according to the Standardized procedure for selected herbal drugs

HPTLC Association

4. Plate conditioning (manual development only)
After sample application place the plate for 45 min in a suitable desiccator containing a saturated solution of $MgCl_2$

5a. Manual Development
5a.1 Slowly open the lid of the saturated chamber and insert the conditioned plate into the front trough so that the back of the plate rests against the front wall of the chamber and the layer faces the inside of the chamber. Close the lid
5a.2 Let the mobile phase ascend until it reaches the mark.
5a.3 Open the lid and remove the plate. Place it upright in a rack under a fume hood
5a.4 Dry plate with cold air from a hair dryer for 5 min

5b Automatic development
Use the following settings of the automatic chamber:
Enable pre-drying
Saturation with filter paper 20 min
Humidity control 10 min with $MgCl_2$
Migration distance 70mm
Drying time 5 min
10 ml of developing solvent
25 ml of saturation solvent
NOTE: if no humidity control is available follow step 4

6a. Derivatization by dipping
6a.1 heat the dry plate for 5 min at 100°C
6a.2 while hot dip plate for 1 sec into a solution of 0.5% NP reagent in ethyl acetate. Then, after two min of waiting, dip the plate for 1 sec into a solution of 5% macrogol 400 in dichloromethane

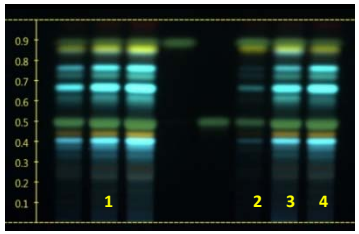
6b. Derivatization by automatic spraying
6b.1 heat the dry plate for 5 min at 100°C
6b.2.1 while hot spray the plate with 3.5 ml of a solution of 1% NP reagent in methanol then with a solution of 5% macrogol 400 in methanol
or
6b.2.2 while hot spray the plate with 3.5 ml of a solution of 0.5% NP reagent in ethyl acetate then with 3.5 ml solution of 5% macrogol 400 in dichloromethane

7. Documentation
30 min after the second derivatization step, take an image of the derivatized plate under UV 366 nm.

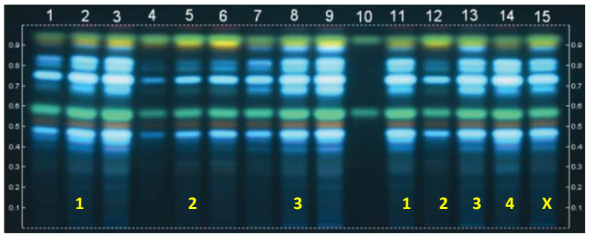
8. Reporting
Create a copy of software based report or use own reporting documents.

International Association for the Advancement of High Performance Thin Layer Chromatography
A non-profit organization dedicated to the promotion of HPTLC in plant analysis and other analytical fields • www.hptlc-association.org

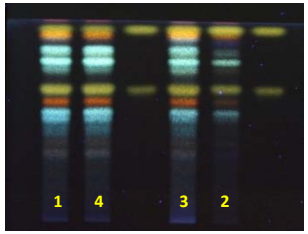
Ringversuch: HPTLC von Flavonoid-Drogen, EDQM Gruppe 13B



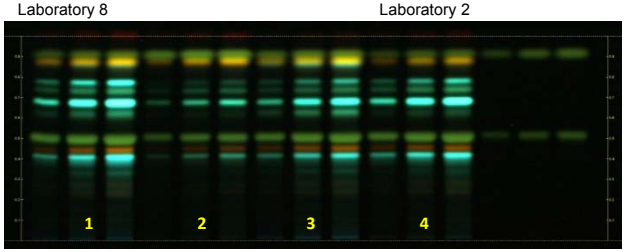
Laboratory 8



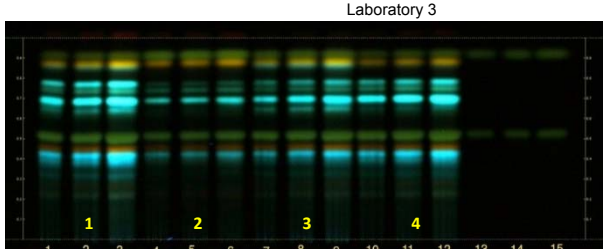
Laboratory 2



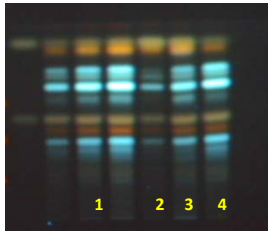
Laboratory 3



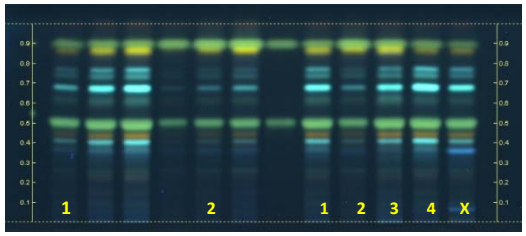
Laboratory 5 (instrumental)



Laboratory 5 (manual)



Laboratory 6



Laboratory 7

Römische Kamille, Blüten

Kapitel 2.8.25. PhEur 9.0 Januar 2017

2.8.25. HPTLC of herbal drugs and herbal drug preparations

Chloral hydrate tends to crystallise as long needles. To avoid this, proceed as follows: after heating, remove the cover slip; to the preparation add 1 drop of a 10 per cent *V/V* mixture of *chloral hydrate solution R* in *glycerol R*; place a clean cover slip on the preparation; examine under a microscope.

MOUNTING IN A 50 PER CENT *V/V* SOLUTION OF GLYCEROL

Place 2 drops of a 50 per cent *V/V* solution of *glycerol R* on a glass microscope slide. Disperse a very small quantity of the powdered drug in the liquid and cover the preparation with a cover slip. Examine under a microscope.

MOUNTING IN A 10 PER CENT *V/V* ALCOHOLIC SOLUTION OF PHLOROGLUCINOL AND HYDROCHLORIC ACID

Place a very small quantity of the powdered drug on a glass microscope slide. Add 1-2 drops of a 10 per cent *V/V* alcoholic solution of *phloroglucinol R*. Mix and allow the solvent to evaporate almost completely. Add 1-2 drops of *hydrochloric acid R* and cover the preparation with a cover slip. Examine immediately under a microscope. The red colour indicates the presence of lignin.

MOUNTING IN LACTIC REAGENT

Place 2-3 drops of *lactic reagent R* on a glass microscope slide. Disperse a very small quantity of the powdered drug in the liquid and cover the preparation with a cover slip. Heat the preparation very gently to boiling. Maintain gentle boiling for a short time. Make sure that the quantity of mounting fluid is sufficient. If necessary, add more fluid using a tapered glass pipette. Allow to cool and then examine under a microscope. Lignified structures stain bright yellow; structures containing cellulose remain colourless. Starch granules stain more or less violet; certain secretions (e.g., essential oils, resins, oleoresins) stain orange and cork stains red.

MOUNTING IN RUTHENIUM RED SOLUTION

Place 2 drops of *ruthenium red solution R* on a glass microscope slide. Disperse a very small quantity of the powdered drug in the liquid and cover the preparation with a cover slip. After about 1 minute, allow a drop of *distilled water R* to be taken up between the slide and the cover slip. Examine under a microscope. The mucilage stains violet red.

01/2017:20825

2.8.25. HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY OF HERBAL DRUGS AND HERBAL DRUG PREPARATIONS

High-performance thin-layer chromatography (HPTLC) is used for qualitative analysis of herbal drugs and herbal drug preparations. It is a thin-layer chromatographic technique (2.2.27) that, unless otherwise stated in an individual monograph, uses a glass plate coated with a uniform, porous layer (average pore size 6 nm), typically 200 µm thick, of irregular particles of silica gel between 2 µm and 10 µm in size and with an average size of 5 µm, a polymeric binder and a fluorescence indicator (F_{254}). The results are qualified using a system-specific suitability test.

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2. Methods of analysis

2.8.25. HPTLC of herbal drugs and herbal drug preparations EUROPEAN PHARMACOPOEIA 9.0

EQUIPMENT

The equipment used for qualitative HPTLC typically consists of:

- glass plates, as described above, usually 20 × 10 cm in size;
- devices suitable for the application of specified volumes of solutions as bands and allowing control of the dimensions and position of application;
- a device suitable for conditioning the stationary phase at the prescribed relative humidity;
- a suitable chromatographic tank (for example, a twin trough chamber);
- a device suitable for the reproducible drying of the developed plate;
- devices suitable for the application of reagents to, and heating of, the plate as part of the derivatisation procedure;
- a system suitable for the electronic documentation of chromatograms under 254 nm UV, 366 nm UV and white light.

NOTE: normal thin-layer chromatographic methods using glass plates or sheets coated with particles of 5-40 µm or HPTLC aluminium-backed sheets may be used, provided that the results obtained fulfil the general system suitability criteria that the bands develop perpendicular to the lower edge of the plate and the solvent front is parallel to the upper edge of the plate, and satisfy the system-specific suitability test stated in the individual monograph.

METHOD

Preparation of test solution. Unless otherwise stated in the individual monograph, the test solution is usually prepared as follows.

For dry herbal drugs or dry herbal extracts, mix 0.5 g of the powdered herbal drug or 0.1 g of the dry herbal extract with 5 mL of *methanol R* and sonicate for 15 min; filter or centrifuge and use the filtrate or supernatant as the test solution.

For essential oils, dissolve 50 µL of the essential oil in 1 mL of *toluene R* and use this solution as the test solution.

Preparation of reference solutions. Unless otherwise stated in the individual monograph, reference solutions are usually prepared as follows. Prepare a 1 mg/mL solution of suitable reagent(s) or reference standard(s) in *methanol R* or, for essential oils, in *toluene R*. Prepare a second reference solution (diluted reference solution) by mixing 1 volume of this solution and 3 volumes of the same solvent. Both solutions are used as intensity references.

Intensity marker. Use one or more of the substances in the reference solution and in the diluted reference solution as intensity marker(s) for the evaluation of the chromatogram.

Preparation of system-specific suitability solution. Prepare the solution as stated in the individual monograph.

Sample application and plate layout. Unless otherwise stated in the individual monograph, samples are applied as narrow bands of 8 mm in length at a distance of 8 mm from the lower edge of the plate. The centre of the first track, which is used for the system-specific suitability solution, is positioned 20 mm from the left edge of the plate. The minimum distance between tracks (centre to centre) is 11 mm. A maximum of 15 tracks are applied onto a standard plate. If no electronic solvent front detection device is used, the development distance is marked with a pencil close to the right or left edge of the plate.

Conditioning of the plate. Following sample application and unless otherwise stated in the individual monograph, expose the plate to air with a suitable relative humidity obtained using a saturated solution of *magnesium chloride R* (for example, by allowing the plate to stand in a closed chamber containing such a solution for 1 h or by using preconditioned air).

Preparation of the tank and development of the plate. Unless otherwise stated in the individual monograph, the chromatographic separation is performed in a saturated tank. Where a twin trough chamber is used, place a piece of filter paper in the rear trough. Load the tank with a sufficient quantity of mobile phase to wet the filter paper completely and achieve a level of 5 mm in both troughs. With the lid closed, leave the tank for 20 min for saturation. Introduce the plate in a vertical position into the front trough of the tank so that the coating layer faces the filter paper. When the mobile phase has reached 70 mm, remove the plate from the tank and dry in a vertical position in a stream of air at room temperature. Other tank configurations and developing distances may be specified in an individual monograph.

NOTE: other tanks may be employed if the results obtained fulfil all of the system suitability criteria.

Visualisation. Chromatograms on the plate are visualised as stated in the individual monograph. Where derivatisation reagents are used, typically 3.5 mL of reagent solution is homogenously sprayed onto a plate of size 20 × 10 cm, or the plate is immersed into the reagent solution, typically at a speed of 5 mm/s for a dwell time of 1 s. Observation may be performed under 254 nm UV, 366 nm UV or white light prior to and/or after derivatisation. When pictures are digitally recorded, exposure time should be adjusted based on the track with the system-specific suitability solution.

System-specific suitability test. This test is based on the separation of 2 substances that have similar retardation factors (R_F values) but that are barely separable under the specified chromatographic conditions (for example, chlorogenic acid and hyperoside in chromatographic systems used for flavonoids). The results for the test and reference solutions are only valid when the system-specific suitability solution satisfies the separation requirement stated in the individual monograph.

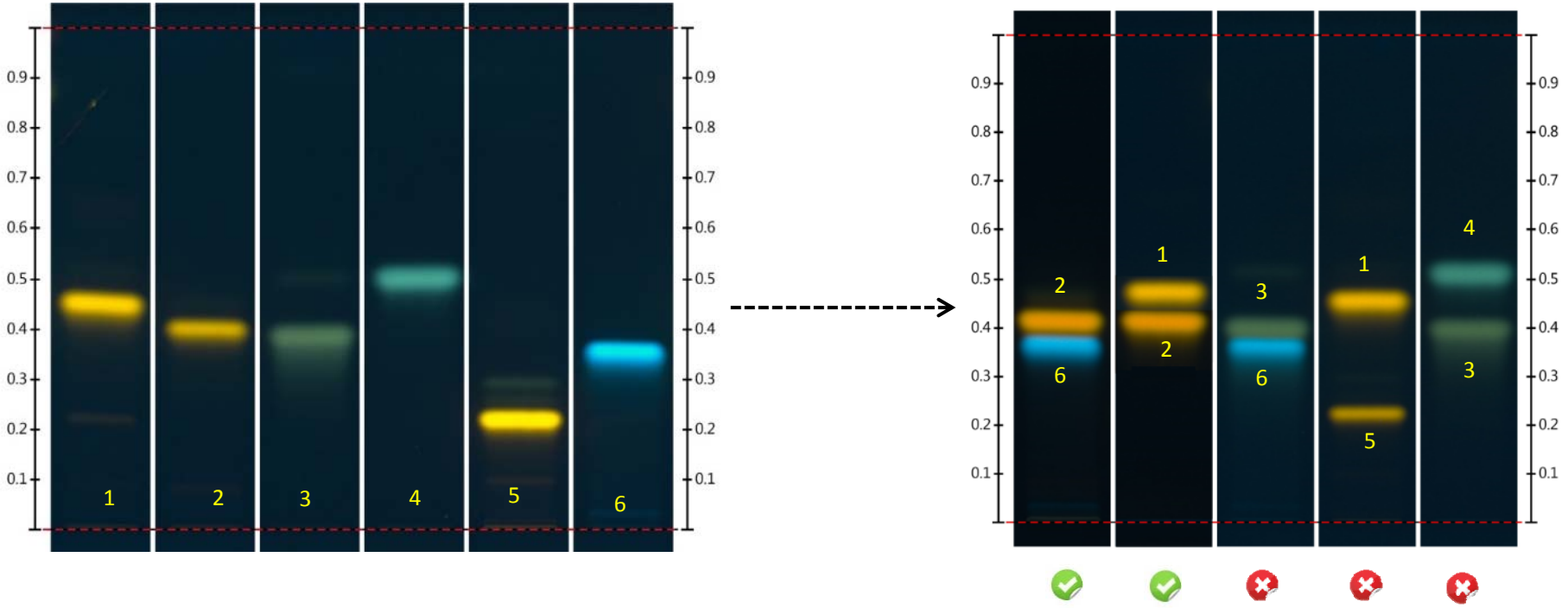
Visual evaluation. The chromatograms obtained with the test and reference solutions are compared against the description, under the results section in the individual monograph, with respect to zone position and colour, as well as intensity for the test solution. Zones of the test solution described in the results table without a descriptor have intensities similar to the zone of the intensity marker in the reference solution. Zones described as 'intense' are visually more intense than the zone of the intensity marker in the reference solution; zones described as 'faint' are visually less intense than the zone of the intensity marker in the reference solution, but equal to or more intense than the zone of the intensity marker in the diluted reference solution; zones described as 'very faint' are visually less intense than the zone of the intensity marker in the diluted reference solution.

296 *See the information section on general monographs (cover pages)*

- 20x10 cm HPTLC Glasplatte Si 60 F_{254}
- Auftragung: 15 Bahnen, 8 mm Bänder, 8 mm vom unteren Rand, erste Bahn bei 20 mm
- Konditionierung bei 33% relative Feuchte
- Entwicklung: Doppeltrogkammer, 20 min Sättigung (Filterpapier), 5 mm Fliessmittelpegel, 70 mm vom unteren Rand

Systemspezifische Eignungsprüfung (SST) Beispiel: Flavonoide

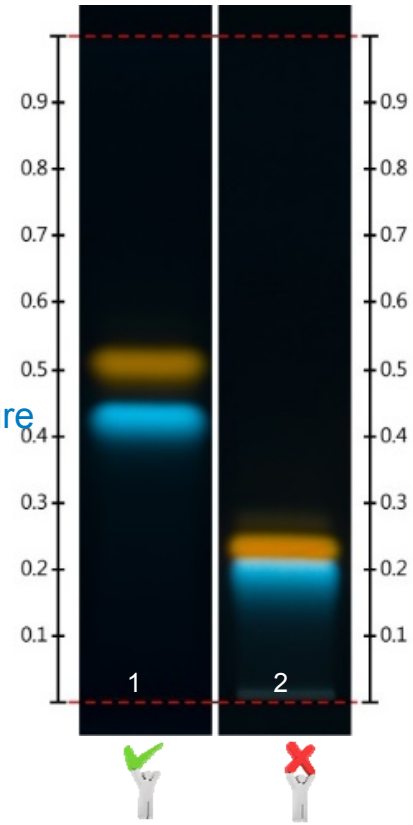
Mobile Phase: Ethylacetat, Ameisensäure, Wasser (80:10:010)



1: Isoquercitrin; 2: Hyperosid; 3: Isovitexin; 4: Apigenin; 5: Rutin; 6: Chlorogensäure

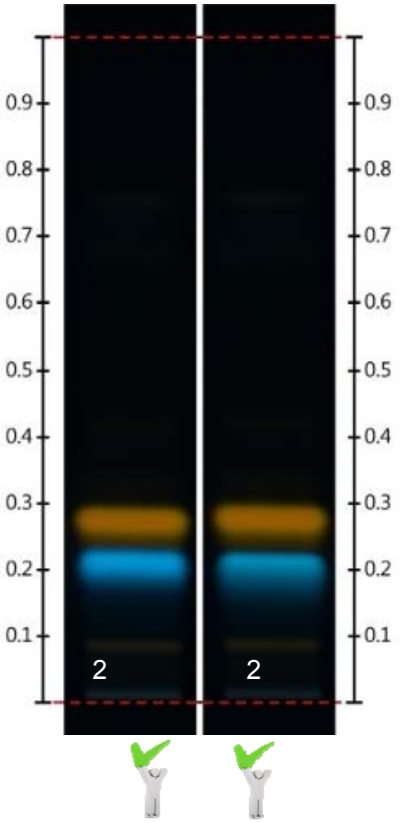
Systemspezifische Eignungsprüfung (SST) Beispiel: Flavonoide

Hyperosid
Chlorogensäure



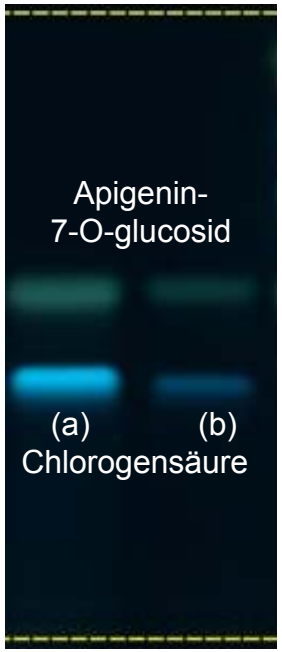
1. Ethylacetat, Essigsäure, Ameisensäure, Wasser (100:11:11:27)
2. Ethylacetat, Ameisensäure, Wasser (15:1:1)

Isoquercitrin
Caftarsäure



Isoquercitrin
Chlorogensäure

Einführung von Intensitätsmarkern – Beispiel Chlorogensäure (CA)



R R ¼

4-fach verdünnt

Zur visuellen Beurteilung

Intensive Zone:
Intensiver als CA- Zone (a)

Zone ohne Intensitätbeschreibung:
Ähnlich der Intensität der CA – Zone (a)

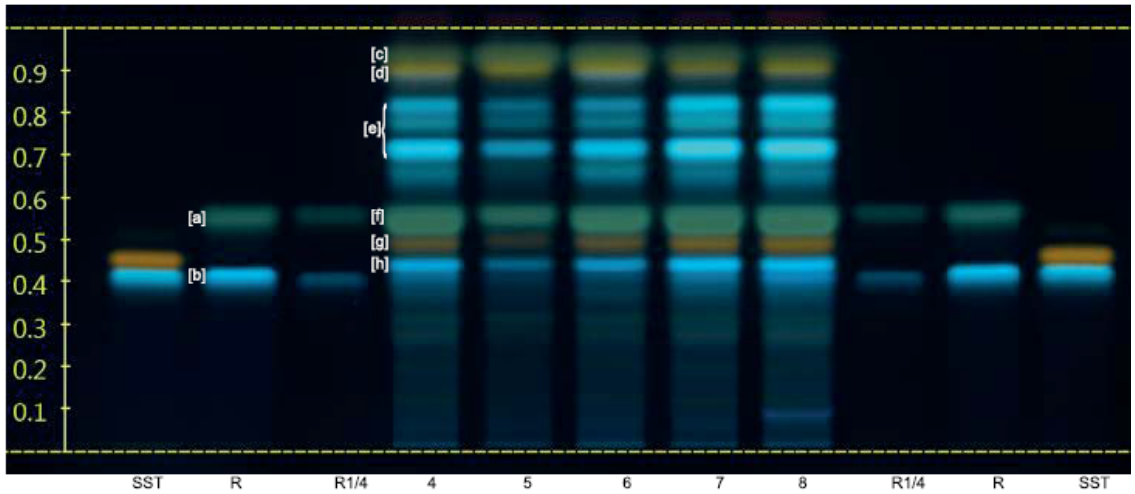
Schwache Zone:
Ähnlich der Intensität der CA – Zone (b)

Sehr schwache Zone:
Weniger intensive als CA Zone (b)

R und R¼: Referenzlösungen

HPTLC in Pharmeuropa und Knowledge Datenbank: Römische Kamillenblüten

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 which correspond to the descriptions in the table above.



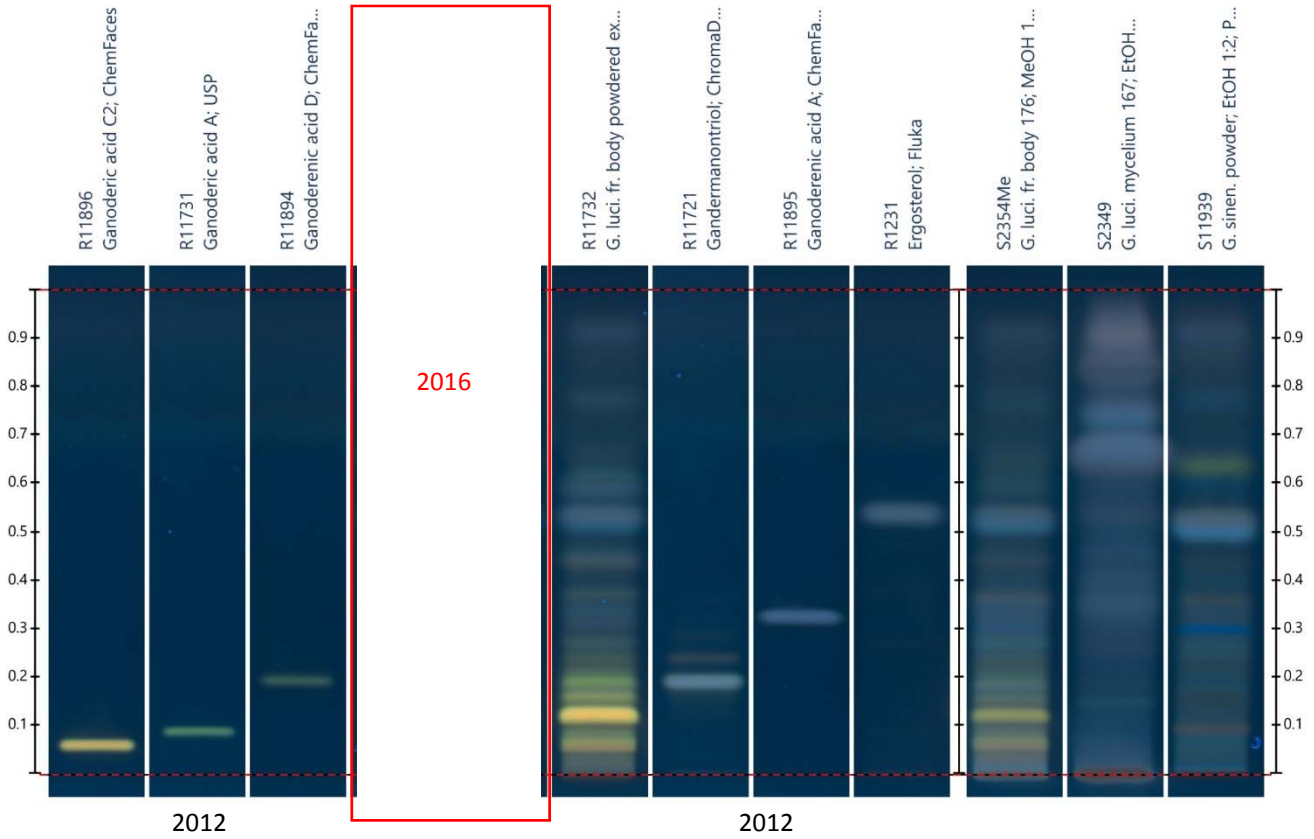
Top of the plate	
	[c] A greenish-blue fluorescent zone or a faint greenish-blue fluorescent zone [d] A brownish-yellow or orange fluorescent zone or a faint brownish-yellow or orange fluorescent zone [e] 2 light blue fluorescent zones or 2 faint light blue fluorescent zones A usually intense light blue fluorescent zone
[a] Apigenin-7-glucoside : a greenish-blue fluorescent zone	[f] A greenish-blue fluorescent zone or an intense greenish-blue fluorescent zone (apigenin-7-glucoside) [g] A brownish-yellow or orange fluorescent zone or a faint to very faint brownish-yellow or orange fluorescent zone [h] A light blue fluorescent zone or a faint light blue fluorescent zone
[b] Chlorogenic acid : a light blue fluorescent zone	
Reference solution (a)	Test solution

The letters indicating the position of the zones refer to the chromatogram shown below for information. Like the chromatogram, they will not appear in the text published in the European Pharmacopoeia. However, the table with letters will be published with the chromatogram in the Knowledge database.

SST: reference solution (c) R: reference solution (a)

R1/4: reference solution (b) 4-8: test solutions for different batches

Standardisierte Arbeitsweise - Reproduzierbarkeit qualifizierter Daten Ganoderma



HPTLC in der Praxis

- Off-line Prozess (alle Schritte sind unabhängig in Zeit und Ort)
- Trennung nach Position (gleiche Zeiten für alle Komponenten)
- Detektorsignal als Funktion des Ortes auf der Platte
- Unvollständige Trennung der Probe → fixe 2-dimensionale Anordnung auf der Platte
- Vollständige Massenbilanz!
- Mehrfachauswertung möglich
- Elektronische Bilder

HPTLC in der Praxis

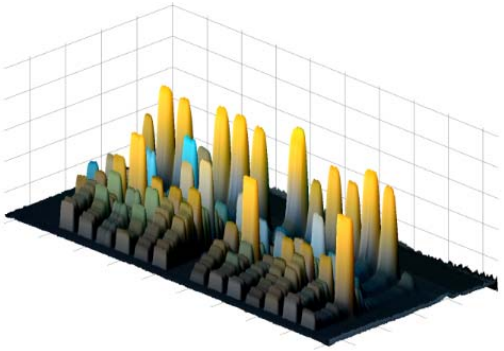
Probe(n)

Chromatographie

Analyse

Datenerzeugung

Fingerprint

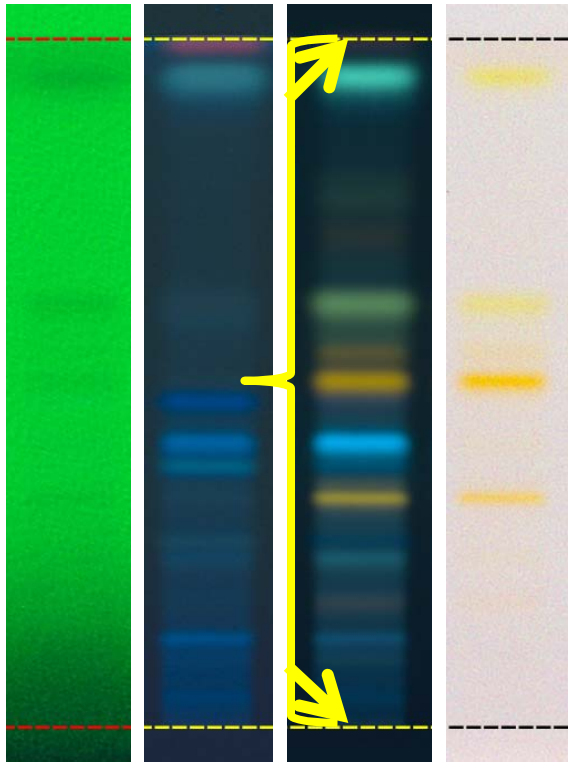


Auswertung

HPTLC Fingerprint (1): eine Bahn

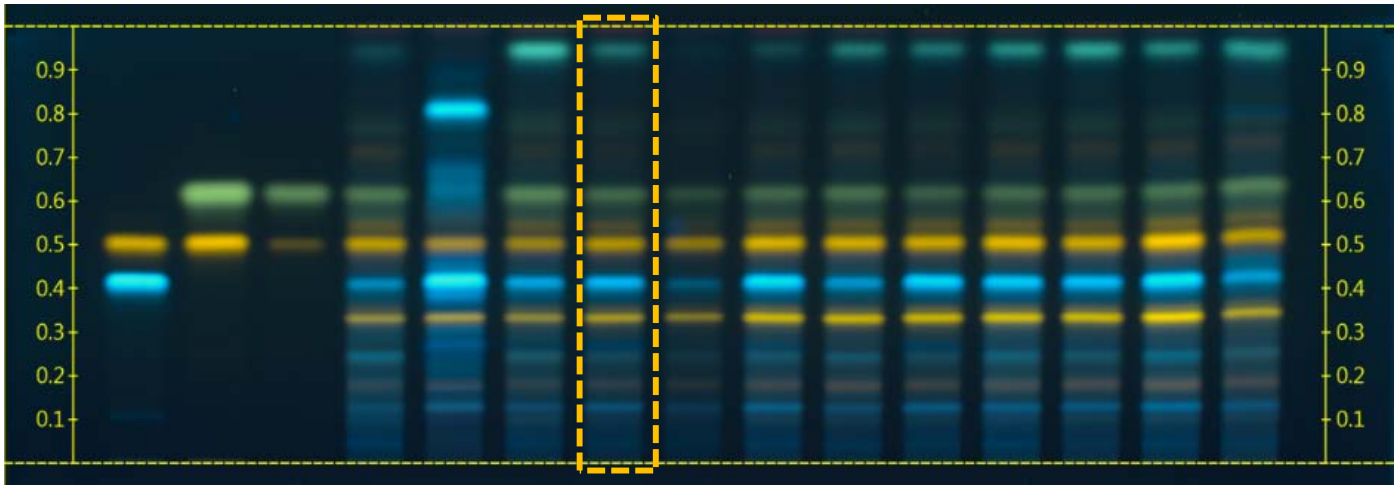
- Ein HPTLC Fingerprint:

- Ist das (elektronische) Bild des Chromatogramms
- Ist ein Abbild der Probe (Proben ID)
- Schliesst Auftrageposition und Front ein
- Besteht aus einer Sequenz (farbiger) Zonen
- Kann mehrere Bilder aus verschiedenen Detektionsmodi enthalten



HPTLC Fingerprint (2): die Platte

Ein HPTLC Fingerprint enstammt immer einer bestimmten Platte:



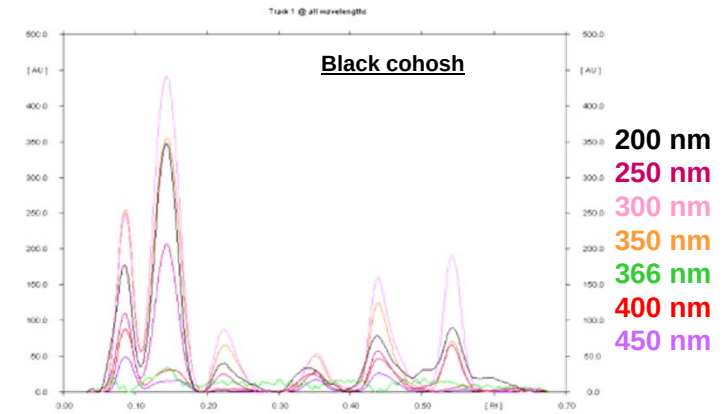
Eine Bahn (normalerweise Bahn 1) enthält den SST, die Chromatogramme sind klar strukturiert, die Zonen sind parallel und horizontal, R_F Skala!

Bis zu 14 Fingerprints pro Platte, die mit der Platten ID verbunden sind (Chromatographische Daten)

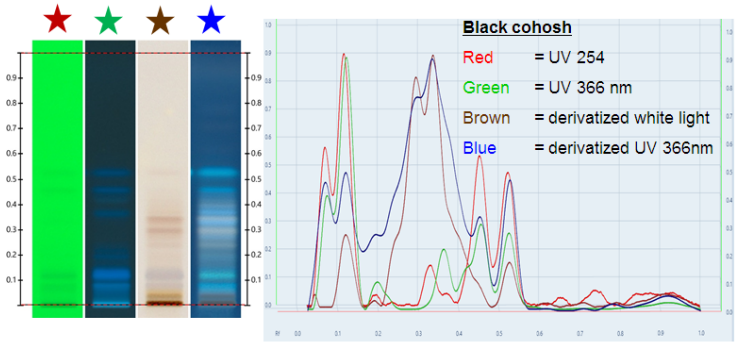
Bei gleicher Chromatographie und bestandenem SST können Fingerprints von verschiedenen Platten miteinander verglichen werden

HPTLC Fingerprint (3): Peak Profile

- Scanning-Densitometrie
  - 200 nm bis 800 nm
  - Absorptions – oder Fluoreszenzmessung



- Bildauswertung
  - UV 254 nm, UV 366 nm, (Weisslicht) vor Derivatisierung
  - (UV 254 nm), UV 366 nm, Weisslicht nach Derivatisierung



Fingerprints

Profiles obtained from images

Peakprofile aus elektronischen Bildern

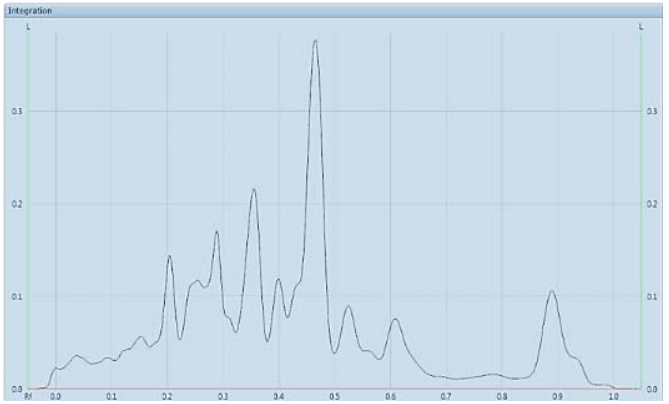
Front

50 % der Zone werden ausgewählt und pro Zeile gemittelt

Bildpunkte

R: 223
G: 160
B: 003

Für jeden R_F Wert wird die mittlere Luminanz berechnet
L = 1/3 R + 1/3 G + 1/3 B

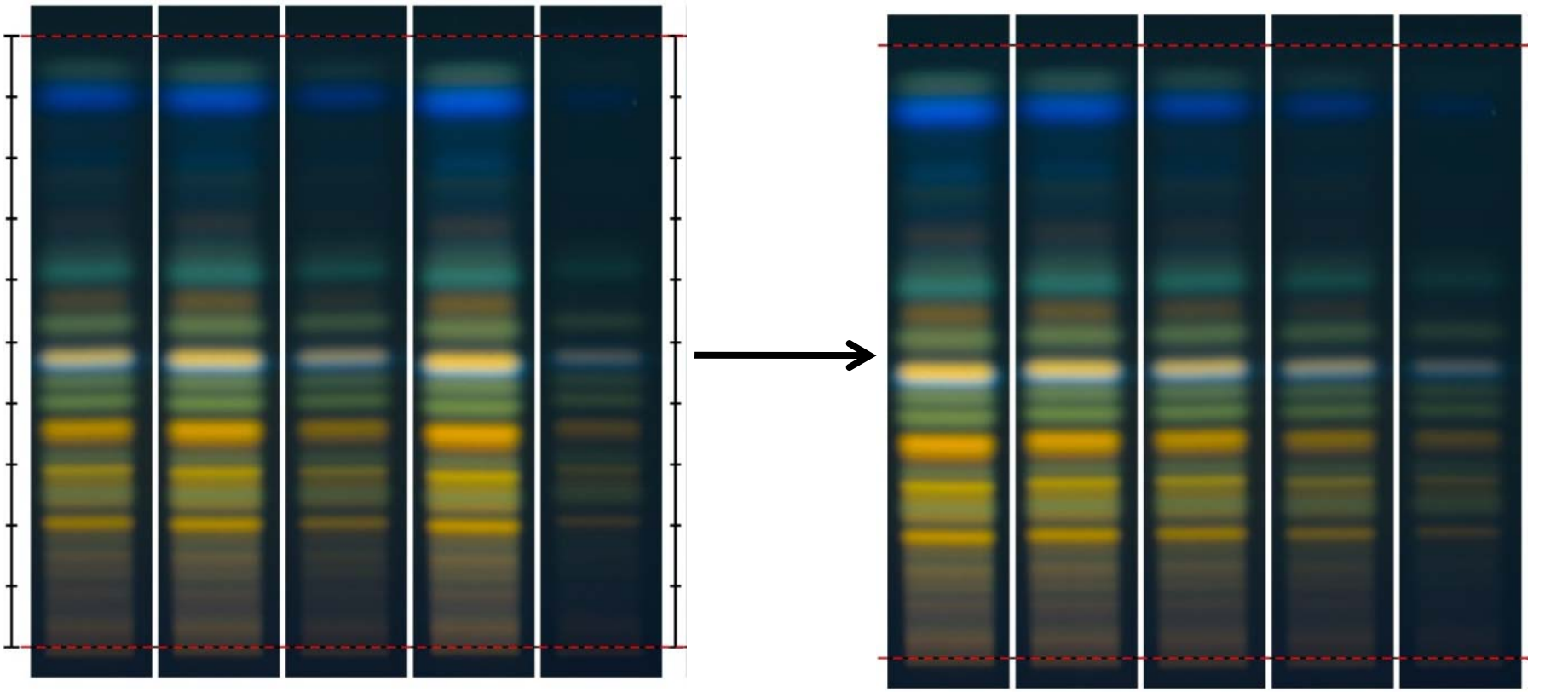


Darstellung von L als Funktion von R_F erzeugt das Peakprofil vom Bild

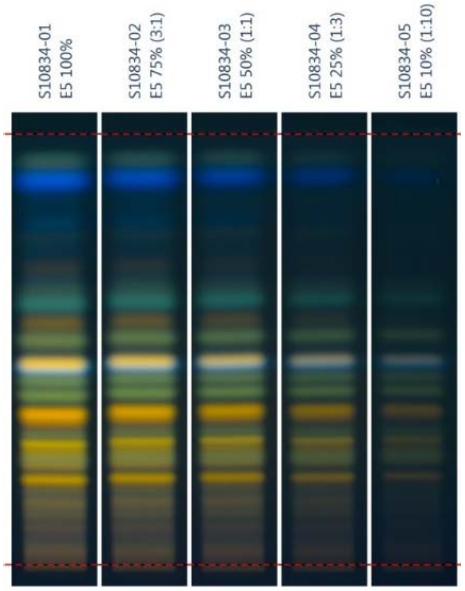
Auftrageposition

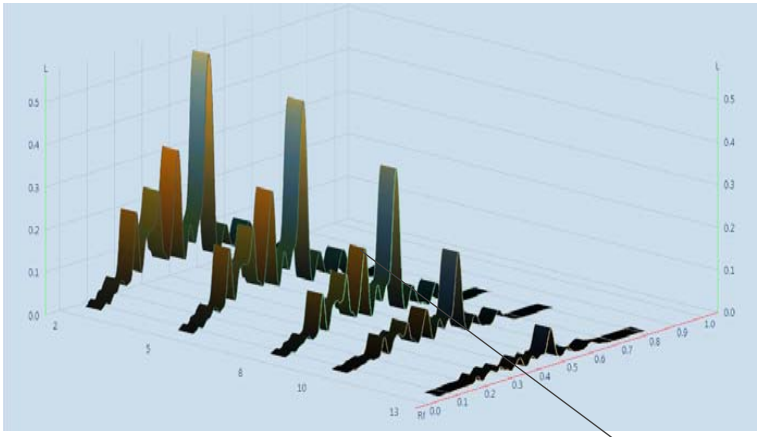
5 % unterhalb der Auftrageposition und 5% über der Front

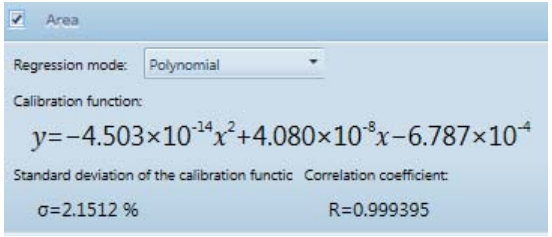
HPTLC Fingerprint (4): Quantitative Information

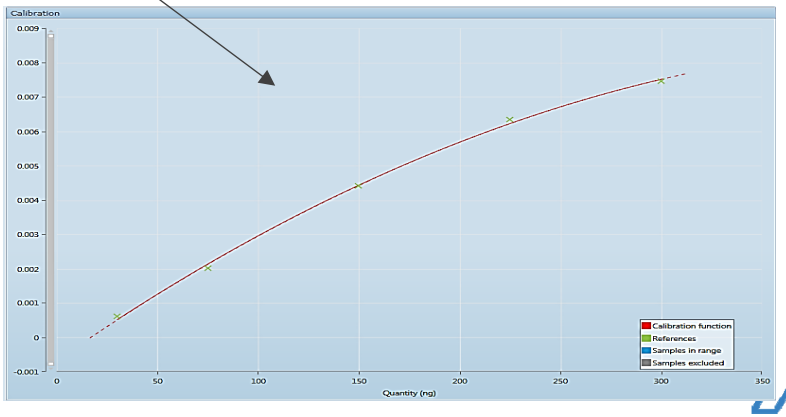


Ginkgo biloba Profil









Ginkgo – quantitativ gesehen….

Äquivalent zu 100 mg Droge
in 1 mL Probenlösung

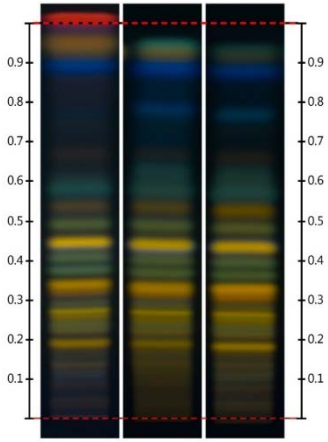
Ginkgo
Blätter

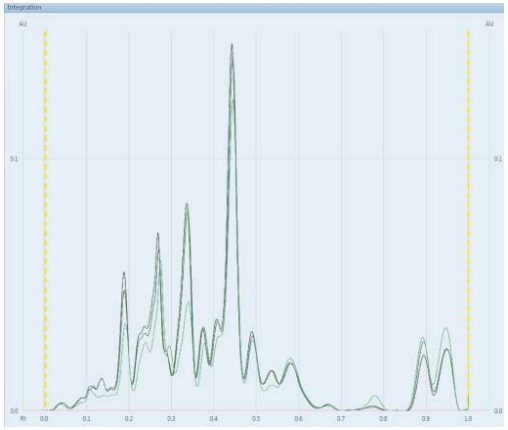
51 g Droge

1 g
Trockenextrakt
240 mg
Flavonoide

120 mg
Extrakt +
Hilfsstoffe

Produkt mit
eingestelltem Gehalt



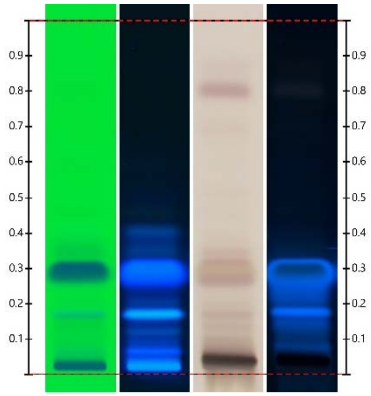


CAMAG 60 YEARS

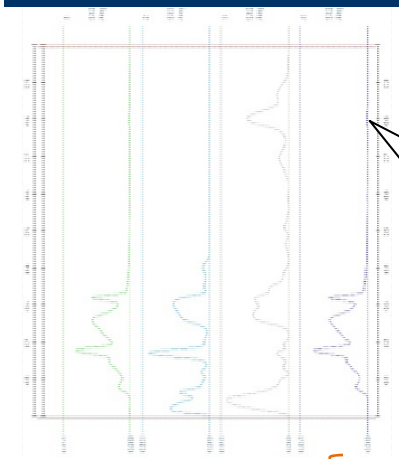
«Comprehensive HPTLC Fingerprinting»

- HPTLC Fingerprints (Bilder), die zur Identifizierung erstellt wurden, enthalten zusätzliche Information…
- **Comprehensive HPTLC fingerprinting** beinhaltet:

Bilder



Peak Profile von Bildern (PPI)



Diese Darstellung erlaubt quantitative Auswertung

Information über

- ✓ Identität
- ✓ Reinheit
- ✓ Gehalt

Original Papers — Thieme

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

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

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

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

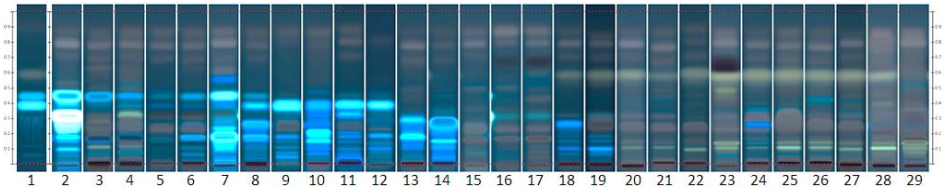
To describe and assure the quality of herbal drugs, a suite of appropriate tests is recommended by regulatory agencies [1, 2] and organizations [3]. Such tests, as well as specifications for compliance, are described in pharmacopoeial or other quality monographs. They include verification of identity 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 addition al 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

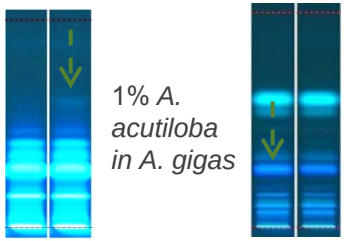
Frommenwiler DA et al. Comprehensive HPTLC Fingerprinting… Planta Med

Qualitätskontrolle einer Pflanzendroge – Fallstudie *Angelica gigas*

Identität



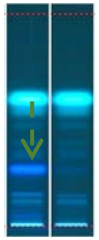
Diese Methode (PhEur *Angelica* spp.) kann 28 *Angelica* und verwandte Arten unterscheiden



1% *A. acutiloba in A. gigas*

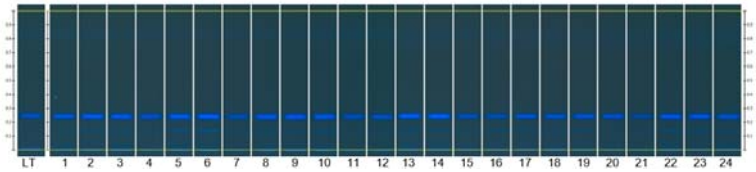
1% *A. gigas in A. acutiloba*

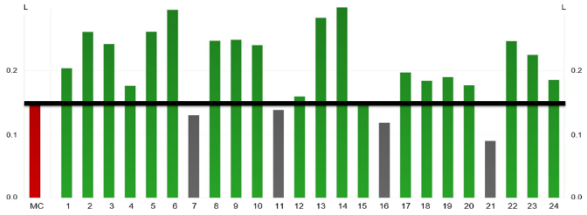
1% *A. sinensis in A. gigas*



1% *A. gigas in A. sinensis*

Kann sie Mischungen mit anderen Arten detektieren? → 3 asiatische Hauptarten von *Angelica*





Gegen einen Referenzstandard wird der Mindestgehalt überprüft

HPTLC - Fingerprints als Alternative zu (HPLC) Assays in TCM Drogen?

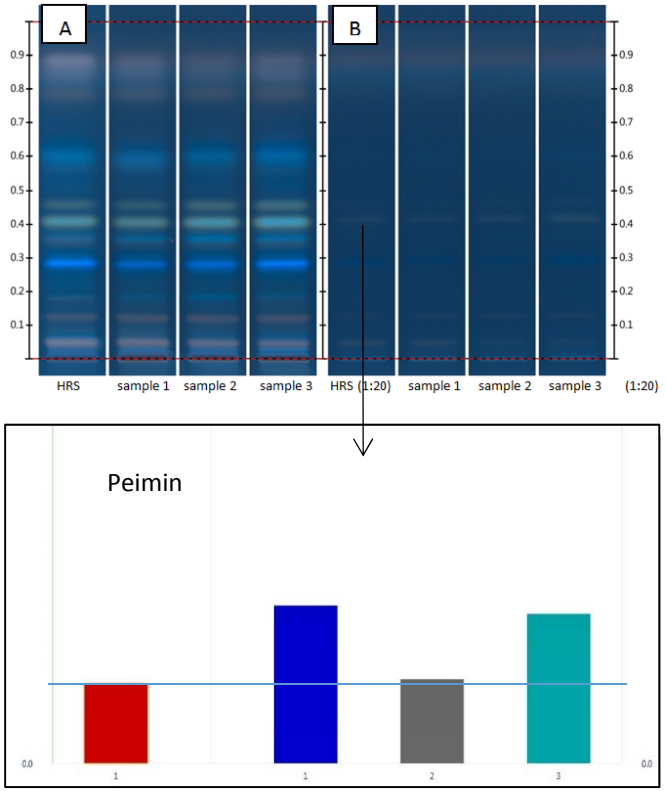
- Analytische Marker beschreiben nur einen kleinen Bruchteil der Droge
- Kann man Gehaltsbestimmung mit Identifizierung verbinden?
- Wie kann man (visuelle) HPTLC Chromatogramme quantitative auswerten?
- Welche Referenzpunkte gibt es?
- Sind die Ergebnisse mit bisherigen Daten vergleichbar?

→ Pilotprojekt in der WP TCM!

Fall 1: Referenzextrakt / Referenzdroge mit bekanntem Gehalt wird bis zur Sichtbarkeitsgrenze verdünnt, um einen Minimalgehalt zu definieren

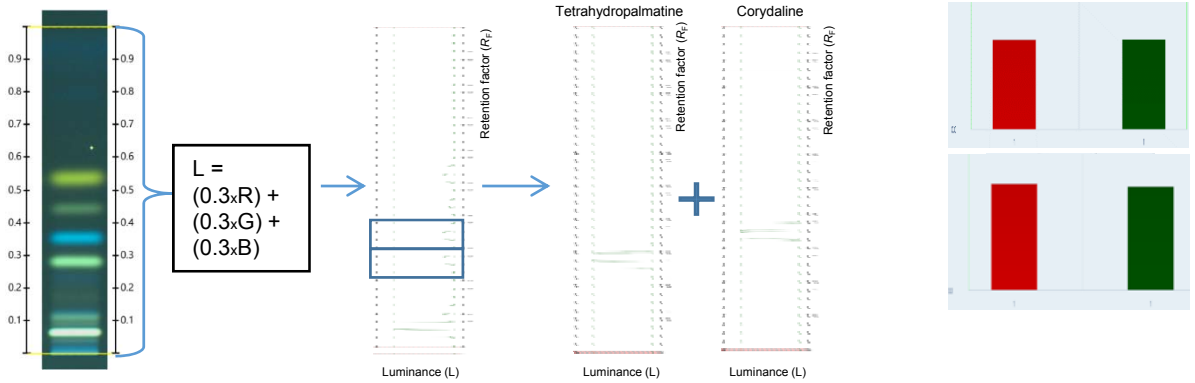
Fritillaria Zwiebeln

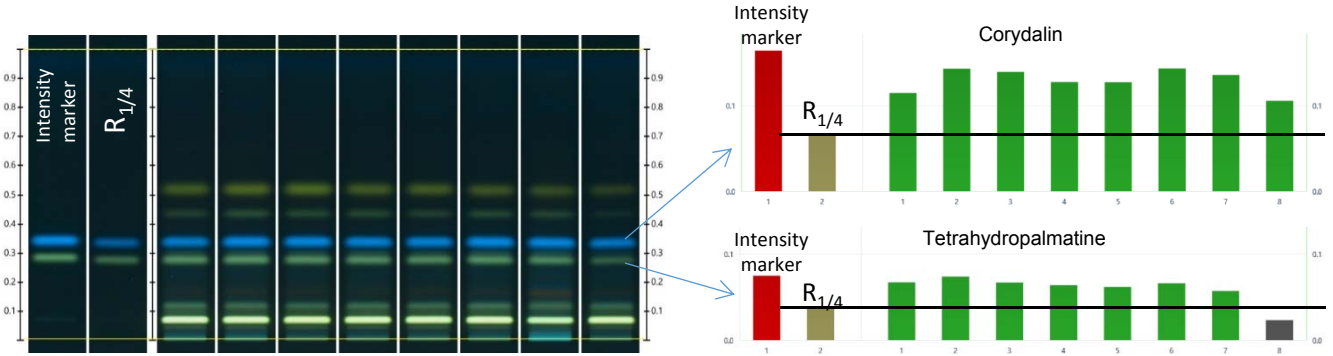
Top of the plate		
[a] 3 weak fluorescent zones --- [b] Peiminine: A blue fluorescent zone [c] A weak greenish fluorescent zone [d] Peimine: a greenish fluorescent zone [e] A weak bluish fluorescent zone --- [f] A bluish fluorescent zone [g] A weak brownish fluorescent zone [h] A weak brownish fluorescent zone	--- [a] A faint greenish zone (Peimine) ---	--- [a] A faint greenish zone (Peimine) ---
Reference solution	$R_{1:20}$	Test solution $T_{1:20}$



Fall 2: Minimalgehalt wird gegen einen Intensitätsmarker bestimmt

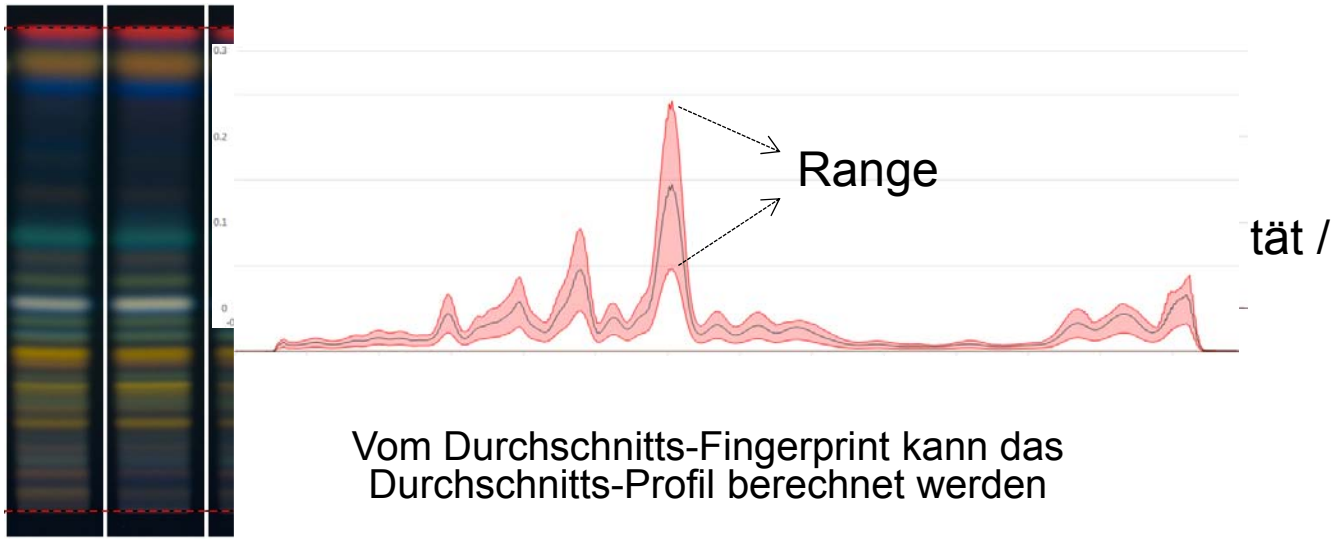
Corydalis





Fall 3: Fingerprint als “Limit Test”

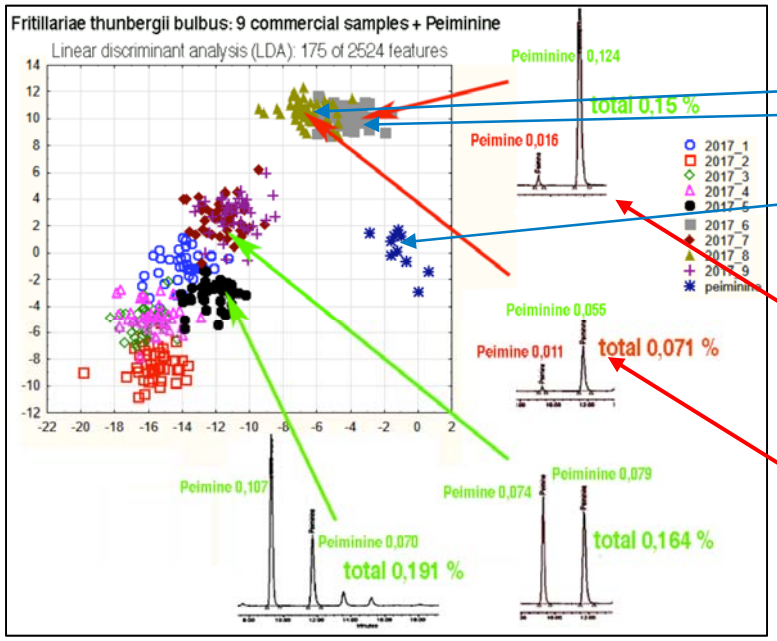
Durchschnitts-Fingerprint umfasst die natürliche Varabilität

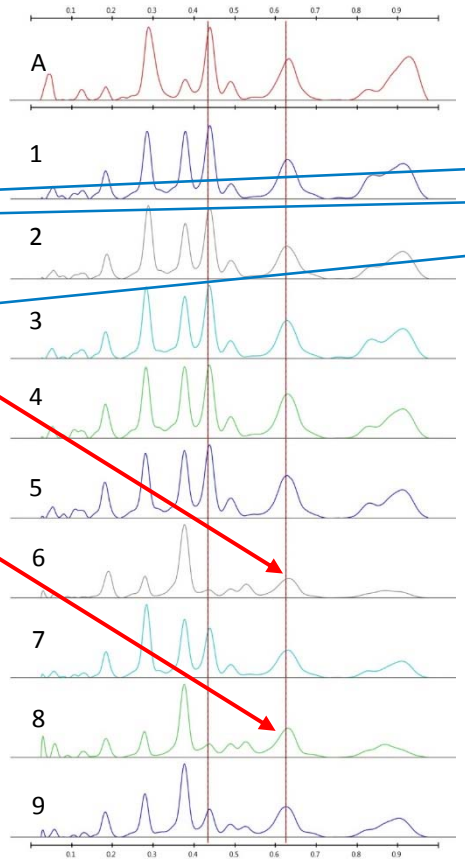


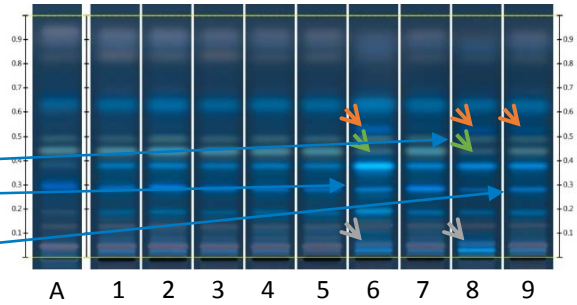
Vom Durchschnitts-Fingerprint kann das Durchschnitts-Profil berechnet werden

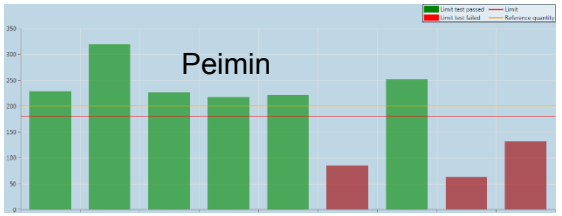
Fallstudie Fritillaria

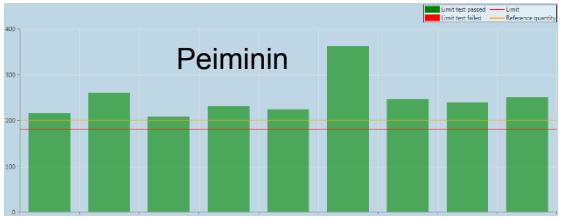
FTIR / HPLC





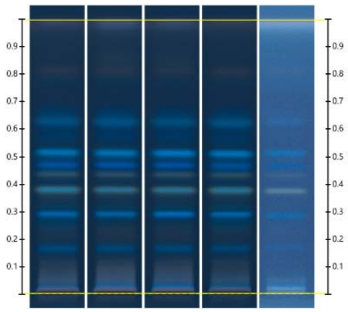




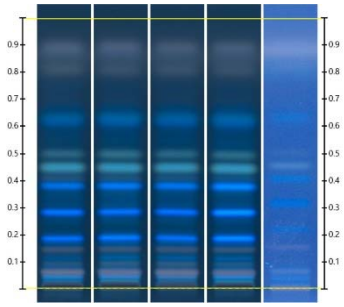


Fallstudie Fritillaria

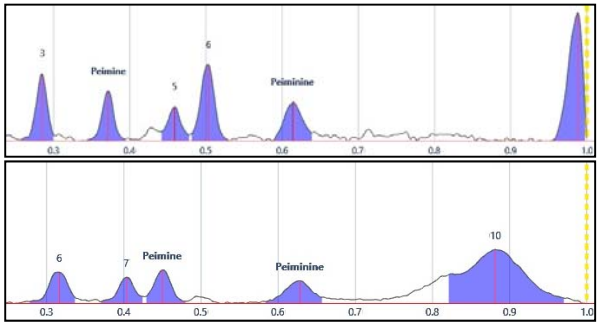
Optimierung der mobile Phase



Neu: Toluen, Aceton, Diethylamin (9:9:1.2); **ungesätigte** Kammer

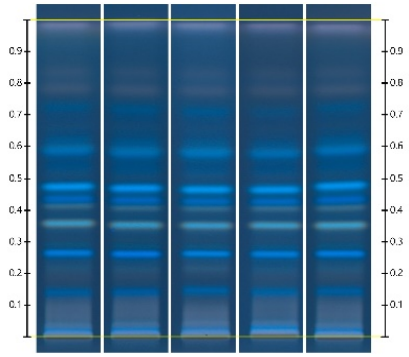


Bisher: Ethylacetat, Methanol, Ammoniak 25% (85:10:5); **gesättigte** Kammer



Repeatability

- Gleiche Instrumente
- 5 Analysen
  - ΔRF ≤ 0.02

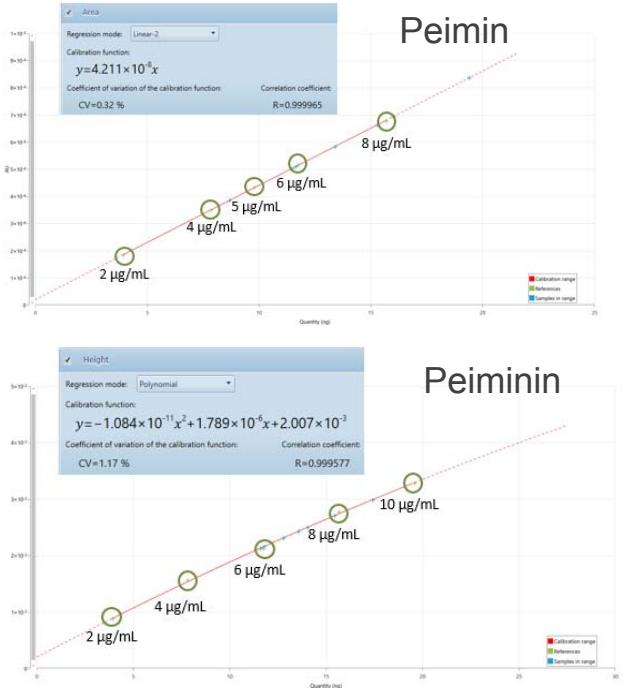


Extraktionsmethode

Peimine								
Method	1	2	3	4	5	6	7	8
Description	Son.10'	Son. 20'	Son. 30'	Shaking 10'	Shaking 20'	Shaking 30'	HPLC	Shaking 20' MeOH
Amount %	0.061	0.055	0.056	0.056	0.061	0.055	0.057	0.056
CV %	16.66	6.67	9.75	7.87	6.15	8.21	9.12	4.22
Peiminine								
Method	1	2	3	4	5	6	7	8
Description	Son.10'	Son.20'	Son.30'	Shaking 10'	Shaking 20'	Shaking 30'	HPLC	Shaking 20', MeOH
Amount %	0.090	0.082	0.078	0.087	0.094	0.093	0.083	0.114
CV %	18.60	11.30	14.80	8.95	7.40	7.65	7.04	6.86

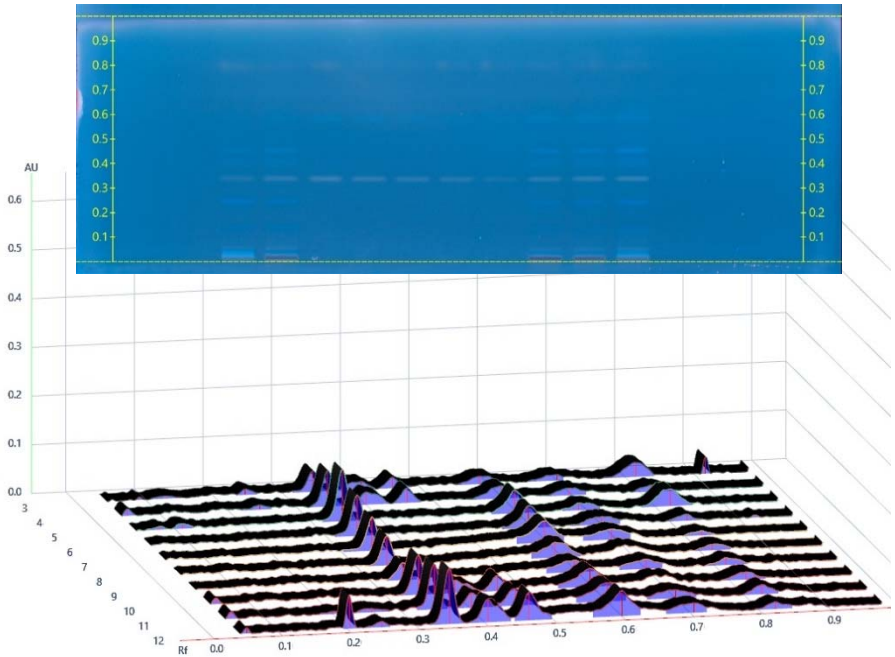
Fallstudie Fritillaria

Kalibrierkurven für 2 Marker: Peimin und Peiminin



Fingerprints (ID) und Referenstandards als Peakprofile

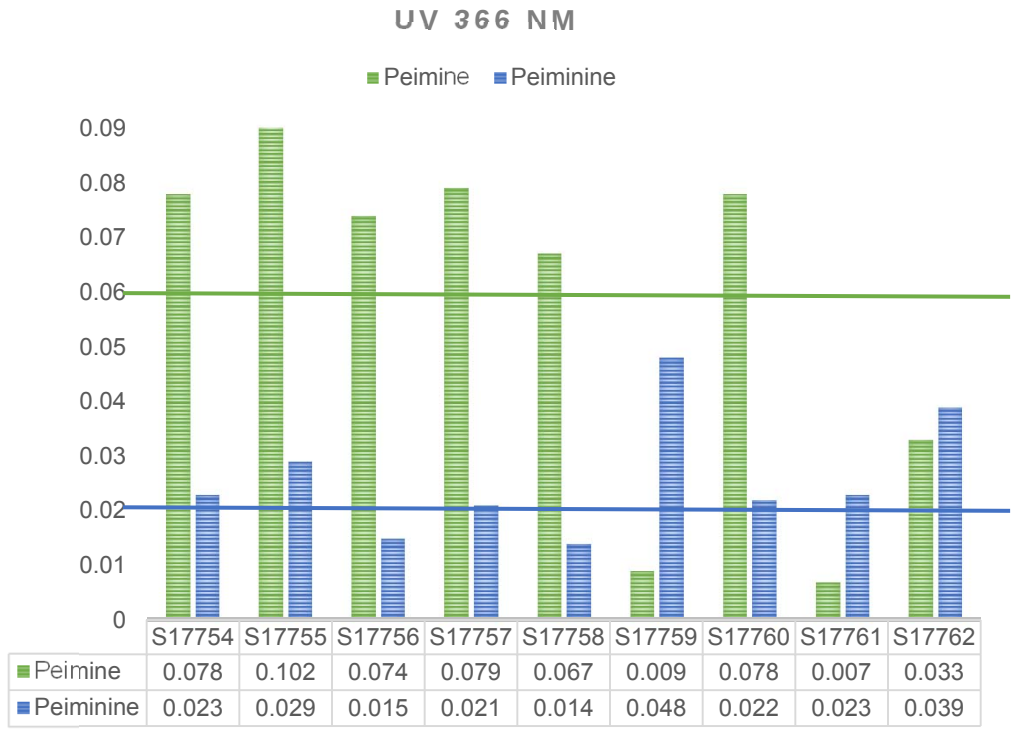
Verdünnung der Proben 1:25 → im Arbeitsbereich



Fallstudie Fritillaria

- Gehalt an Markern (Peimin und Peiminin) wurde für 9 Proben bestimmt

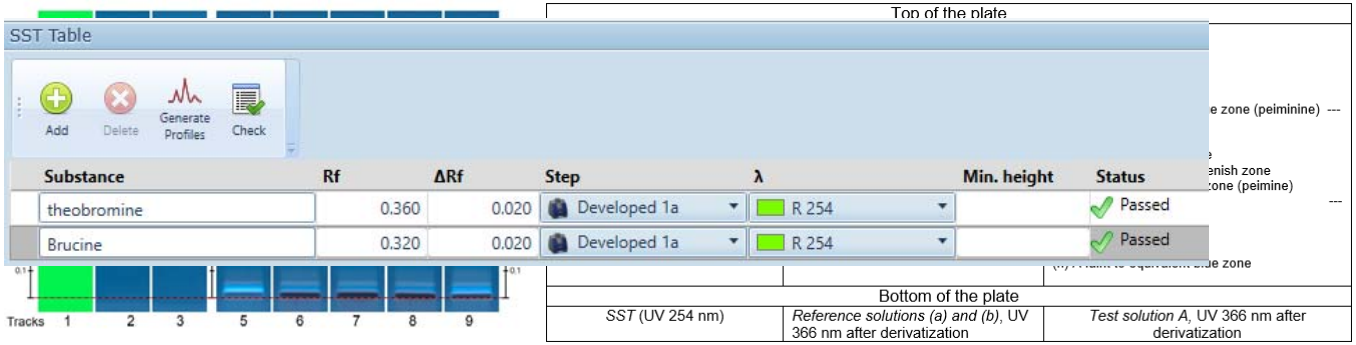
	Peimine %	Peiminine %
S17754	0.078	0.023
S17755	0.102	0.029
S17756	0.074	0.015
S17757	0.079	0.021
S17758	0.067	0.014
S17759	0.009	0.048
S17760	0.078	0.022
S17761	0.007	0.023
S17762	0.033	0.039
average	**0.058**	**0.026**



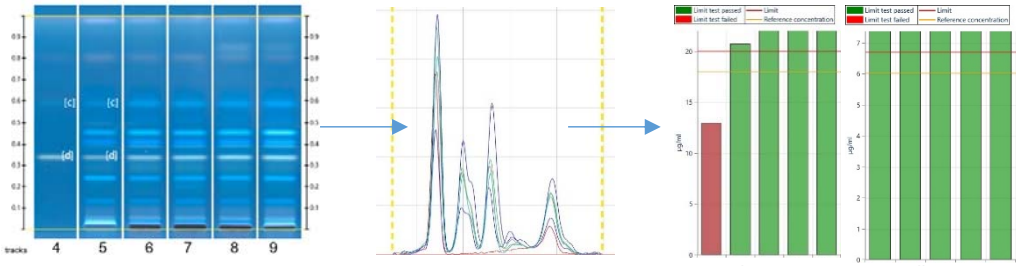
SOP für einen Ringversuch


SOP A for the collaborative trial:
HPTLC of Fritillaria bulbs

SOP → beschreibt Identifizierung der Droge und Test auf Mindestgehalt für 2 Marker



Fingerprints werden in Peakprofile umgewandelt



Zusammenfassung

- Die HPTLC hatte einen langen und steinigen Weg bis zum allgemeinen Kapitel 2.8.25.
- Neben deutschen und britischen Kollegen haben vor allem Schweizer Experten massgeblich zur Entwicklung des HPTLC Konzeptes beigetragen
- Heute ist HPTLC allgemein akzeptiert und es wird geprüft, ob sie quantitativ eingesetzt werden kann, um den Einsatz von Monographien für pflanzliche Drogen in der Praxis zu vereinfachen

Herzlichen Dank für Ihre Aufmerksamkeit

CAMAG 60 YEARS