Method Collection

HPTLC Association

HPTLC methods for identification of medicinal plants (method collection)

Introduction

The use of herbal medicines and herbal dietary supplement has a long standing tradition and is increasingly popular in all countries of the world. In addition herbal raw materials find their way into various food and cosmetic products. Quality and safety of all those items is of utmost concern not only to consumers, but also to the industry and regulatory bodies.
The quality of any product depends primarily on the quality of its raw material. But what is quality? In the most general approach quality is the agreement with specifications. The more detailed such specifications are the broader the meaning of quality gets. There are some common quality standards applicable to all herbal materials concerning for example the content of heavy metals, pesticides, toxic substances, microbes, filth, foreign matter, etc. If specifications of lower limits can be met by a given product it is usually regarded as of better quality than a product that meets its own specification based on higher limits. Quality is commonly linked to the content of certain markers or ingredients which may or may not have any established activity. Unfortunately a product meeting its specification of a higher content is easily viewed as of higher quality than a product meeting its own specification of a lower content, particularly if the latter is sold at a higher price.

From the perspective of current Good Manufacturing Practice (cGMP) a lot of attention is being given to proper identity of a material. In this respect quality can become very difficult to define, because there are many different aspects of “identity”. Fortunately there are quality monographs available for many plants (American Herbal Pharmacopoeia, PhEur, USP, PhPRC, Quality standards of Indian Medicines, etc.). In addition to a definition including scientific name, plant part, harvest /drying conditions etc. such monographs describe also macroscopic, microscopic, and organoleptic characters for identification purpose. The element that links the (biological) identity to the “quality” of a plant material is its chemical composition. Unlike a synthetic drug, a plant is a very complex and diverse mixture of chemical compounds, but the exact composition is known for only very few plants. On top of that the chemical composition of plants is affected by natural variability, growing/harvesting conditions and storage. That is why analysis of one or few markers cannot describe quality sufficiently. A chemical fingerprint is better suited for assessment of quality because it can provide qualitative information of identity and quantitative information of potency. A suitable fingerprint of a material that is in accordance with a quality monograph (or other suitable specification) can therefore be used as specification for a material.
There are many techniques for generating chemical fingerprints. High Performance Thin Layer Chromatography (HPTLC) is one of them. Developed from the classical Thin-Layer Chromatography (TLC), which is an integral part of most pharmacopoeia monographs on plants, it allows highly reproducible results and traceable records through a standardized methodology and the use of suitable instruments. In combination with validated methods HPTLC becomes the ideal tool for quality control, particularly for identification.

The International Association for the Advancement of High Performance Thin Layer Chromatography (HPTLC Association) promotes the use of HPTLC. It is dedicated to the

development and validation of common standards and methods for identification of plants and their adulterants. It is working to establish and maintain an “International Atlas of HPTLC Methods for Identification of Herbal Drugs” as a recognized reference tool for quality control. It wants to serve as the worldwide leading resource for information about and training in HPTLC to the scientific community.

This book is a selection of HPTLC methods for identification featured on the Association’s web site. It shall not only give an impression of the kind of results that can be obtained by HPTLC but also provide detailed instructions enabling the analyst who wants to use this technique to achieve the same.

Basel, December 2012

Eike Reich
Chair of the Method Review Committee
HPTLC Association

Definition

HPTLC is based on a standardized methodology and the use of suitable instruments (typically controlled by software) for all steps of the analysis. A system suitability test is used to qualify results.
The stationary phase is a HPTLC glass plate coated with a uniform thin layer (typically 200 micron) of porous particles (2 - 10 µm) with an average particle size of 5 µm. The layer typically consists of silica gel with a pore size of 60 Angstroms, a polymeric binder and a so called fluorescence indicator (F_{254}). The standard format of the plate is 20 cm x 10 cm.
The apparatus for HPTLC consist of

- A device suitable for application of samples as bands providing control of dimension and position of the application as well as applied sample volume
- A suitable chromatographic chamber (typically a twin trough chamber) providing control of saturation and developing distance
- A device suitable for controlling the activity of the stationary phase via relative humidity
- A device suitable for reproducible drying of the developed plate
- Suitable devices for reagent transfer and heating as part of the derivatization procedure
- A device suitable for electronic documentation of chromatograms under UV 254 nm, UV 366 nm, and white light
- For quantitative determinations a densitometer or image evaluation software

The general SOP provided on the following pages is the basis for all work published in this book.

General Methodology for HPTLC

I. PURPOSE

This SOP provides general guidance for analysis by HPTLC.

II. APPLICATION

This SOP applies to all work regarding HPTLC analysis unless the resulting chromatograms or the applied method require adjustments.

III. PROCEDURE

General

1. Prior to using an instrument, log-in the corresponding instrument log according to SOP 007.
2. Observe safety and housekeeping rules according to SOP 009 and SOP 010.
3. For use of instrument refer to SOP 007.
4. Record any performed work in appropriate worksheets as specified in SOP 008. Include temperature and humidity at least once a day in any active worksheets.

A) Plate material

Handle plates with extreme caution to avoid any damage to the layer. Store plates in the original package with the lid closed. Remove plate from storage only immediately prior to use.
Plates are generally handled only at the upper edge to avoid contamination.
Unless otherwise necessary Merck HPTLC plates silica gel 60 F254 in the format 20x10 cm are used. For most work plates are used without pre-treatment unless chromatography produces impurity fronts due to contamination of the plate. For reproducibility studies and quantitative analyses plates are pre-washed as follows.

1. Mark the direction of development with a pencil at the upper edge of the plate.
2. Develop plate with 20 mL methanol per trough in a 20 x 10 cm Twin Trough Chamber (TTC) to the upper edge. Up to two 20 x 10 cm plates can be developed back to back in each trough of the TTC.
3. Remove the plate and dry for 20 minutes in a clean drying oven at 120°C.
4. Equilibrate plate with lab atmosphere (temperature, relative humidity) in a suitable container providing protection from dust and fumes.

B) Sample application

Unless otherwise necessary apply samples using the spray-on technique with ATS 4 or Linomat 5.

Use the following application parameters:

Parameter	HPTLC
Distance from lower edge of plate for use in TTC	8 mm
x-position of first track	20 mm
Minimum space between bands	2 mm
Band length	8 mm
Maximum number of tracks on a 20 x 10 cm plate	15

C) Preparation and storage of developing solvents

Developing solvents consisting of more than 1 component are prepared by measuring the required volume (respectively weight) of each component separately and transferring them into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content.

Volumes smaller than 1 mL are measured with a suitable micropipette. Volumes up to 20 mL are measured with a graduated volumetric pipette of suitable size. Volumes larger than 20 mL are measured with a graduated cylinder of appropriate size.

To minimize volume errors developing solvents are prepared in a volume that is sufficient for one working day.

D) Development

Plates are generally developed in a saturated Twin Trough Chamber according to the following procedure:

1. Prepare the appropriate volume (30 mL for 20x10 cm TTC) of developing solvent.
2. Open chamber and place a correctly sized (20x10 cm) piece of filter paper in the rear trough.
3. Pour appropriate volume (20 mL for 20x10 cm TTC) of solvent into rear trough of the chamber so that the filter paper is thoroughly wetted and adheres to rear wall of TTC.
4. Pour appropriate volume (10 mL for 20x10 cm TTC) of solvent into front trough of the chamber.
5. Set chamber on bench, replace the lid and let chamber equilibrate for 20 minutes.
6. Mark the desired developing distance (70 mm from lower edge of plate) with a pencil on the right edge of the plate.
7. Slide off the lid to the side.
8. Insert the plate into the front trough. The layer should face the filter paper and the back of the plate is resting against front wall of TTC.
9. Replace lid.
10. Develop plate to the mark.
11. Open lid, remove plate.
12. Dry the plate (vertically in direction of chromatography) 5 minutes in a stream of cold air.
13. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber is dried and, if necessary, also cleaned.

Alternatively the automatic developing chamber ADC 2 can be used for development.

- To adjust the activity of the HPTLC plate the humidity control option of the ADC 2 is used. Consult the ADC2 manual for handling.
- If the instrument is operated in stand alone mode, and unless otherwise specified, the standard method S2 is applied using $MgCl_2$ for humidity control.
- If the instrument is operated under software control, and unless otherwise specified, developing distance is set to 70 mm from lower edge of plate, chamber saturation is set to 20 min, and the activity of the plate is adjusted to 33% relative humidity using saturated magnesium chloride solution for 10 min.
- The following manual steps are performed:

1. If a developing solvent is used for the first time the ADC2 is rinsed using 7 mL of developing solvent for each inlet.
2. Insert plate in ADC2.
3. Place an ADC2-filter paper in the rear trough of the twin-trough chamber.
4. Position the twin-trough chamber in the ADC2.

5. Pour 10 mL of solvent into the left inlet of the ADC2 (for development) and 25 mL into the right inlet (for saturation).
6. For humidity control, make sure the appropriate bottle is attached.
7. Start development using winCATS software, or in stand alone mode by pressing the “run” button.
8. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber is dried and, if necessary, also cleaned.

E) Derivatization

Transfer of reagent for derivatization of samples on a HPTLC plate may be accomplished by spraying or dipping. Dipping is the preferred method and should be used whenever possible. Spraying is done in the TLC spray cabinet. If derivatization includes heating the plate heater is used. Always refer to the HPTLC method for details of the derivatization procedure.

Dipping (Chromatogram Immersion Device)

1. Charge tank of immersion device with 200 mL of reagent.
2. Place plate in holder of immersion device, set parameters according to method and press start.
3. Let excess reagent drip off plate, wipe off back of the plate with paper towel.
4. Wait until no liquid film is seen on the plate surface.
5. Remove plate from plate holder.

Spraying (Glass sprayer)

1. Charge the bottle of the sprayer with up to 50 mL of reagent.
2. Place plate in spray cabinet against a filter paper or a paper towel.
3. Spray plate with horizontal and vertical motion until it is homogeneously covered with reagent avoiding any local over-saturation of layer.

Heating (Plate Heater)

1. Turn on plate heater and select temperature.
2. Wait until the temperature is stable.
3. Place plate onto plate heater.
4. After the time specified by the method remove hot plate from heater.

F) Documentation of plates (Visualizer / DigiStore 2)

Each developed plate is documented with the digital documentation system under UV 254 nm, UV 366 nm and white light.
If a type of light does not produce usable information that fact must be documented. If a plate is derivatized images are taken prior and after derivatization.
All images are labeled and listed in the project work sheet.

G) Labeling

Plates

Each plate is given an individual identification number (ID), which will be written in pencil in the top right corner. The ID includes project number, dash, year (YY), month (MM), day (DD), dash, and a consecutive number each day. Example: The first plate developed for Project P91 on January 10. 2002 is labeled P91-020110-01. The sixth plate developed for this project on the same day will have the ID P91-020110-06.

Images

Electronic images are saved as part of the winCats file corresponding to each plate. When exporting images as cpf-file or in other formats they are labeled individually with a file name

corresponding to the plate ID followed by the description of light used for capturing. Example: The image of the first plate of January 10. 2002 for Project P91 taken under UV 254 nm is saved as P91-020110-01-254. The image of a derivatized plate will additionally be labeled with a letter abbreviating the reagent name. Example: P91-020110-01-A366.cpf is the image of the first plate on January 10. 2002 for Project P91 after derivatization with anisaldehyde under UV 366 nm.

The following abbreviations are used:

254	short-wave UV light, reflection
366	long-waver UV light, reflection
W	white light (reflectance)
WT	white light (transmittance)
WRT	white light (reflectance / transmittance)
A	anisaldehyde reagent
S	Sulfuric acid reagent
NP	Natural products reagent
NPP	Natural products reagent followed by polyethylene glycol

Other letters to indicate other specific reagents as specified on the work sheet

Image Comparison Viewer
Image comparison Viewer files (.cx6) are saved with the project number and if necessary with a descriptor (e.g. P194 different solvents.cx6) in the electronic folder of the corresponding project. The content of the .cx6 file is briefly described in the worksheet.

H) Quantitative Evaluation
Quantitative evaluation is performed either with the TLC Scanner 3 using winCATS software or based on digital images with VideoScan.
The winCATS analysis files are labeled to reflect the plate ID and any additional descriptive information if multiple evaluations under different conditions is performed.
Example: P91-020110-01-A-520.cna for the analysis file containing scan data at 520 nm obtained after derivatization with anisaldehyde reagent as opposed to P91-020110-01-210.cna containing scan data at 210 nm obtained prior to derivatization.
The Video scan file is saved with the name of the corresponding image file and the addition “scan”. Example P91-020110-01-A366-scan.cpf

I) Documentation of work
All work performed is documented in a project worksheet.

Alphabetical Table of Content (Latin)

Agrimonia eupatoria (Agrimony herb) 10
Alchemilla vulgare (Lady's Mantle herb) 4
Aloysia citriodora (Lemon verbena leaf) 6
Amomum krervanh, Amomum compactum (Round cardamom fruit) 9
Amomum villosum, Amomum longiligulare (Villous amomum fruit) 12
Angelica archangelica (Angelica root) 14
Angelica dahurica (Dahurian Angelica root) 17
Angelica pubescens (Doubleteeth Pubescent Angelica root, Du huo) 20
Angelica sinensis (Chinese Angelica root, Dang gui) 23
Arctostaphylos uva ursi (Uva ursi leaf) 26
Arnebia euchroma or Arnebia guttata (Arnebia root, zi ca) 28
Arnica Montana (Arnica flower) 31
Artemisia annu (Sweet wormwood leaf, qing hao) 33
Astragalus membranaceus (Astragalus root, huang qi) 35
Avena sativa (Oat herb) 37
Calendula officinalis (Calendula flower) 39
Camellia sinensis (Green Tea leaf) 41
Capsicum annum / Capsicum frutescens (Cayenne pepper) 43
Carum carvi (Caraway fruit (flavonoids)) 45
Carum carvi (Caraway fruit) 47
Carum carvi (Caraway oil) 50
Cetraria islandica (Iceland moss) 53
Chamaemelum nobile (Roman chamomile flower) 56
Clematis armandii (Armand's clematis root, xiao mu tong) 59
Coix lacryma-jobi var. ma-yuen (Job's tears seed (syn. Coix seed), yi yi ren) 61
Curcuma longa (Turmeric) 63
Curcuma longa (Turmeric) Test for adulteration with nimesulide 65
Drynaria fortune Drynaria rhizome, gu sui bu 68
Eclipta prostrate (False daisy herb, mo han lian) 70
Equisetum palustre (Marsh horsetail herb) 72
Foeniculum vulgare ssp. vulgare var. dulce (Sweet Fennel fruit) 74
Foeniculum vulgare ssp. vulgare var. vulgare (Bitter Fennel fruit) 76
Fraxinus excelsior or Fraxinus oxyphylla (Ash leaf) 78
Harpagophytum procumbens and/or Harpagophytum zeyheri (Devil's claw root) 80
Hibiscus sabdariffa (Roselle flower) 82
Hordeum vulgare (Barley grass) 84

Isatis tinctoria (Isatis root, ban lan gen) 86
Lavandula angustifolia (Lavender flower) 88
Lavandula angustifolia (Lavender oil) 90
Lavandula latifolia (Spike lavender oil) 92
Levisticum officinale (Lovage root) 94
Ligusticum sinense, Ligusticum jeholense (Chinese lovage root, gao ben)....97
Malva sylvestris (Mallow flower) 100
Matricaria recutita (Chamomile flower) 102
Melaleuca quinquenervia, Melaleuca viridiflora, Melaleuca leucadendra (Niaouli oil)....105
Melissa officinalis (Melissa leaf dry extract) 107
Monarda didyma (Oswego tea) 109
Paeonia lactiflora (White paeony root, bai shao)....112
Paeonia lactiflora or Paeonia veitchii (Red paeony root, chi shao) 115
Paeonia suffruticosa (Tree paeony root bark, mu dan pi) 118
Passiflora incarnate (Passion flower)....121
Peumus boldus (Boldo leaf)....123
Phyllanthus emblica (Amla fruit, yu gan zi) 126
Phyllanthus emblica, Terminalia bellerica, Terminalia chebula (Triphala powder)....129
Pimpinella anisum (Anise fruit (flavonoids)) 132
Pimpinella anisum (Anise oil)....134
Pimpinella anisum (Aniseed) 136
Piper methysticum (Kava rhizome) 139
Plantago lanceolata (Ribwort plantain leaf)....142
Polygonum multiflorum (Fleeceflower, he shou wu)....145
Primula veris (Cowslip Primrose Flower) 147
Pueraria montana var. chinensis (Thomson's kudzu root, gen ge)....149
Pueraria montana var. lobata (Kudzu root, ge gen) 151
Rosmarinus officinalis (Rosemary leaf) 153
Rosmarinus officinalis (Rosemary oil)....155
Salvia fruticosa, syn. S. triloba (Three-lobed sage leaf) 157
Salvia lavandulifolia (Spanish sage oil)....159
Salvia miltiorrhiza (Chinese Sage, dan shen) 161
Salvia miltiorrhiza (Chinese Sage root, dan shen) Identification 164
Salvia officinalis (Sage leaf)....167
Salvia officinalis (Sage oil)....169
Saposhnikovia divaricate (Saposhnikovia root, Fang feng)....171
Silybum marianum (Milk thistle fruit) 173

Solidago gigantean (Giant goldenrod herb) 175
Solidago virgaurea (European goldenrod herb) 177
Tanacetum parthenium (Feverfew flower) 179
Terminalia belleric (Belleric myrobalan fruit, mao he zi) 182
Terminalia chebula (Chebulic myrobalan fruit, he zi) 184
Tilia cordata, Tilia platyphyllos, Tilia × vulgaris, or mixtures (Lime flower) 186
Triticum aestivum (Wheat grass) 189
Malva sylvestris (Mallow flower) 191
Verbascum thapsus L., V. densiflorum Bertol. syn. V. thapsiforme Schrad, V. phlomoides L. (Mullein flower) 193
Verbena officinalis (European vervain herb) 195
Viola arvensis, V. tricolor (Wild pansy flowering aerial parts) 198
Ziziphus jujuba var. spinosa (Spine date seed, Suan zao ren) 200

Agrimonia eupatoria (Agrimony herb)

1. Scope

This method identifies dried flowering tops of Agrimony herb (Agrimonia eupatoria L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph. Eur. 6.7 (change in sample preparation, change of mobile phase)

3. Procedure

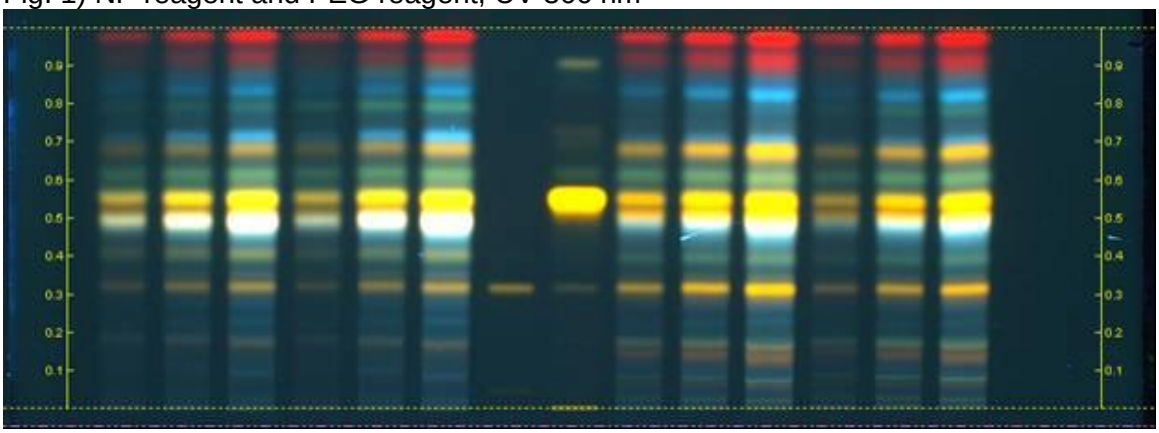
Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 mg of rutin and 3 mg of isoquercitrin individually in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid 98%, water, ethyl methyl ketone 50:10:10:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%

Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2.5 µL	Agrimony 1 (Ph. Eur. extraction)	9	4 µL	Agrimony 2 (Ph. Eur. extraction)
2	5 µL	Agrimony 1 (Ph. Eur. extraction)	10	8 µL	Agrimony 2 (Ph. Eur. extraction)
3	10 µL	Agrimony 1 (Ph. Eur. extraction)	11	16 µL	Agrimony 2 (Ph. Eur. extraction)
4	1 µL	Agrimony 1	12	1 µL	Agrimony 2
5	**3 µL**	**Agrimony 1**	**13**	**3 µL**	**Agrimony 2**
6	6 µL	Agrimony 1	14	6 µL	Agrimony 2
7	5 µL	Rutin	15		Blank
8	2 µL	Isoquercitrin			

System suitability test
Rutin: yellow fluorescent zone at Rf ~ 0.32
Isoquercitrin: yellow fluorescent zone at Rf ~ 0.55

Identification
Compare result under UV 366 nm with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.

The chromatogram of the test solution shows a yellow zone at the position of rutin. At the position of isoquercitrin a yellow zone is seen together with a white zone just below as well as a green fluorescent zone and another yellow fluorescent zone just above. Close to the solvent front two red zones above a blue zone are seen. (NOTE: The Ph. Eur. 6.7 monograph lists 4 orange yellow zones).

Alchemilla vulgare
(Lady's Mantle herb)

1. Scope

This method identifies Lemon verbena leaf (Aloysia citriodora Palau (syn. Aloysia triphylla (L'Hér.) Kuntze; Verbena triphylla L'Hér.; Lippia citriodora Kunth.) by HPTLC fingerprint and detects the adulterant European vervain herb (Verbena officinalis L.).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references)

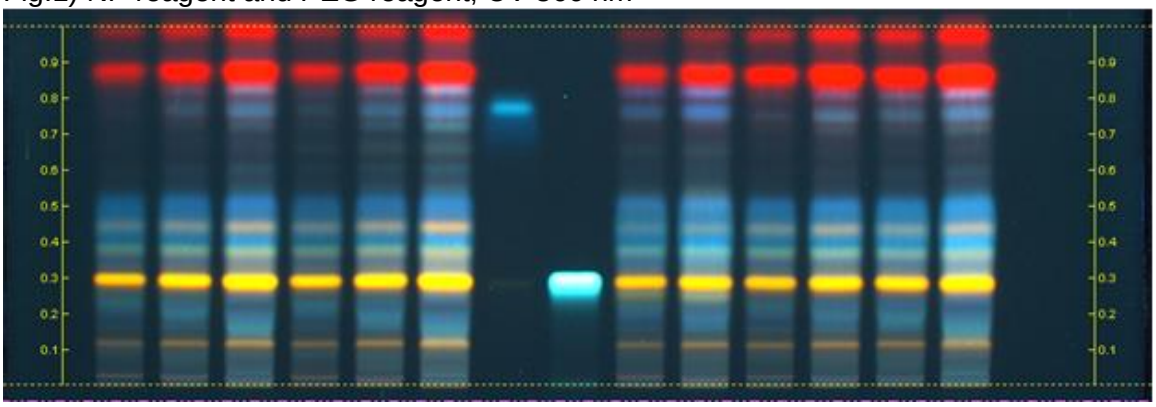
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3 mg of caffeic acid and 1.5 mg of chlorogenic acid individually in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid 98%, water 84:8:8 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 ml ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate 3 min at 100 °C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP reagent and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Lady's Mantle herb 1	9	3 µL	Lady's Mantle herb 2
2	**4 µL**	**Lady's Mantle herb 1**	**10**	**6 µL**	**Lady's Mantle herb 2**
3	8 µL	Lady's Mantle herb 1	11	3 µL	Lady's Mantle herb 3
4	1 µL	Lady's Mantle herb 1 (Ph. Eur. extraction)	12	6 µL	Lady's Mantle herb 3
5	2 µL	Lady's Mantle herb 1 (Ph. Eur. extraction)	13	3 µL	Lady's Mantle herb 4
6	4 µL	Lady's Mantle herb 1 (Ph. Eur. extraction)	14	6 µL	Lady's Mantle herb 4
7	2 µL	Caffeic acid	15		Blank
8	2 µL	Chlorogenic acid			

System suitability test
Caffeic acid: blue fluorescent zone at Rf ~ 0.77
Chlorogenic acid: blue white fluorescent zone at ~ Rf 0.29

Identification
Compare result under UV 366 nm with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a yellow zone at the position of chlorogenic acid. A bluish zone is seen at the position of caffeic acid. Between the positions of chlorogenic acid and caffeic acid there are several blue, green or yellow zones (yellow arrow). Two red fluorescent zones corresponding to chlorophyll are detected close to the solvent front.

Aloysia citriodora
(Lemon verbena leaf)

1. Scope

This method identifies the whole or cut, dried flowering aerial parts of Lady's Mantle herb (Alchemilla vulgare L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references)

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of rutin in 5 mL of methanol. Dissolve 1 mg of arbutin in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Formic acid, acetic acid, water, ethyl acetate 11:11:27:100 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT and UV 254 nm 2.) UV 254 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

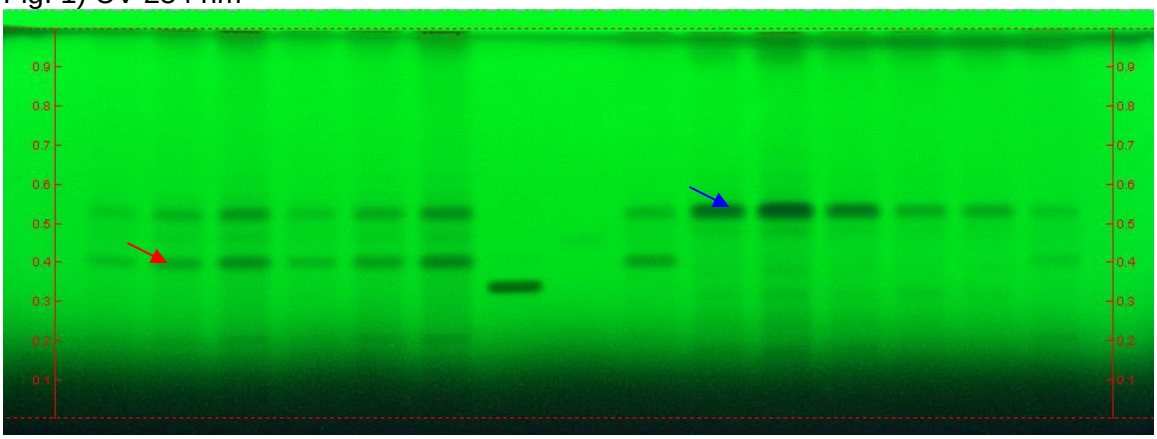
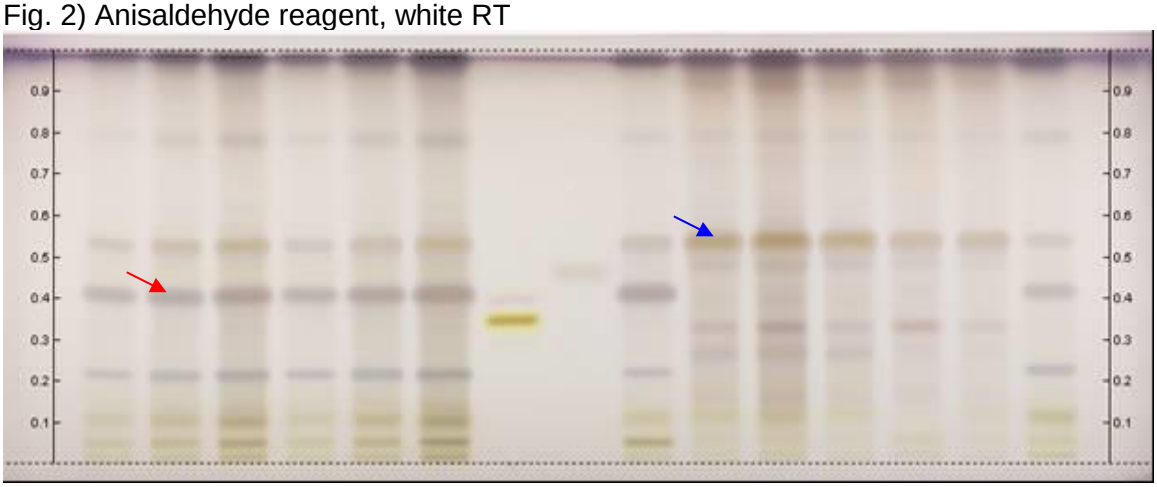


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	European vervain herb 1	9	3 µL	European vervain herb 2
2	5 µL	European vervain herb 1	**10**	**5 µL**	**Lemon verbena leaf 1**
3	7 µL	European vervain herb 1	11	7 µL	Lemon verbena leaf 1
4	3 µL	European vervain herb 1 (Ph.Eur. extraction)	12	5 µL	Lemon verbena leaf 2
5	5 µL	European vervain herb 1 (Ph.Eur. extraction)	13	5 µL	Lemon verbena leaf 3
6	7 µL	European vervain herb 1 (Ph.Eur. extraction)	14	5 µL	Lemon verbena leaf 4
7	5 µL	Rutin	15	5 µL	European vervain herb 3
8	5 µL	Arbutin			

System suitability test
Rutin: light yellow zone at Rf ~ 0.34 (white RT).
Arbutin: light brown zone at Rf ~ 0.46 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows one quenching zone at Rf ~ 0.53. Under white RT there is a brownish grey zone at Rf ~ 0.53 above the position of reference arbutin (blue arrow). A greyish violet double zone is seen around Rf ~ 0.30 just below reference rutin.

Test for adulteration
No zone is seen at Rf ~ 0.40 (red arrow, European vervain herb).

Amomum krervanh, Amomum compactum (Round cardamom fruit)

1. Scope

This method identifies dried Round cardamom fruit (Amomum krervanh Pierre ex Gagnep, Amomum compactum Soland ex Maton), by HPTLC fingerprint and discriminates Villous amomum fruit (Amomum villosum Lour., Amomum longiligulare T.I. Wu), dried Black cardamom fruit (Amomum tsao-ko Crevost & Lamerié), dried Light galangal fruit (Alpinia zerumbet (Pers.) Burtt & Sm.), dried Sharp-leaf galangal fruit (Alpinia oxyphylla) and dried Amomum aurantium fruit.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of toluene, xylene 1:1 (v/v) and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 10 µL of cineole in 1 mL of methanol. Dissolve 10 µL of linalool in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 5 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 93:7 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice cooled methanol is mixed with 20 mL acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Clean plate, white RT 2.) UV 366 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 366 nm

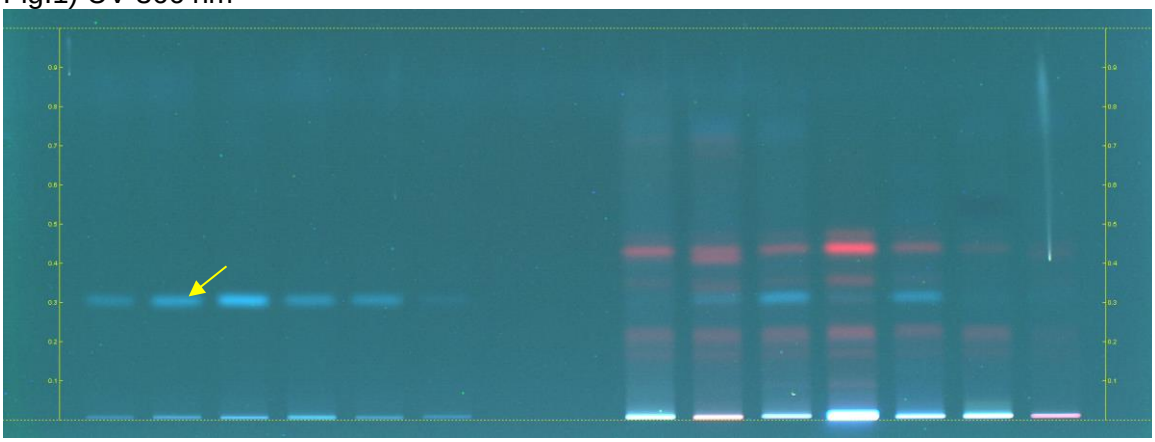
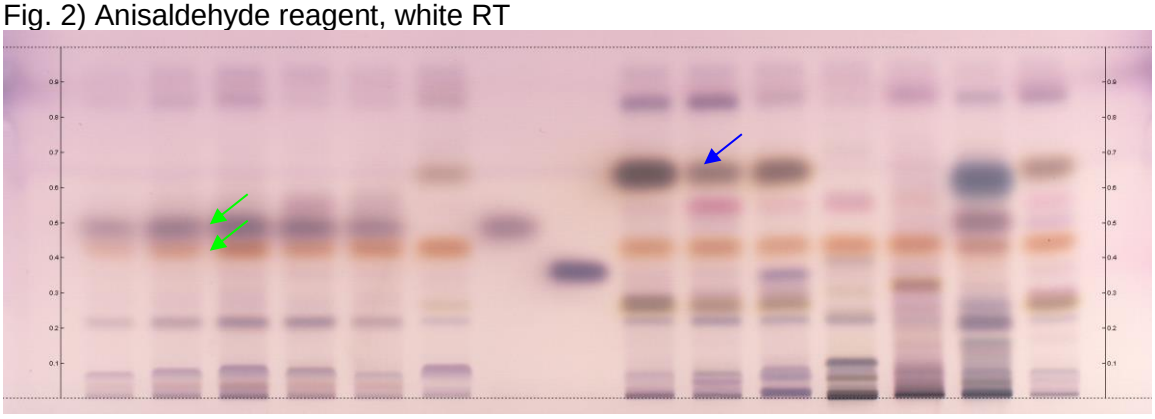


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Round cardamom fruit 1 (A. compactum)	9	5 µL	Villous amomum fruit 1 (Amomum villosum)
2	**5 µL**	**Round cardamom fruit 1** (A. compactum)	10	5 µL	Villous amomum fruit 2 (Amomum longiligulare)
3	7 µL	Round cardamom fruit 1 (A. compactum)	11	5 µL	Villous amomum fruit 3 (Amomum villosum)
4	5 µL	Round cardamom fruit 2 (A. compactum)	12	5 µL	Light galangal fruit
5	5 µL	Round cardamom fruit 3 (A. compactum)	13	5 µL	Sharp-leaf galangal fruit
6	5 µL	Round cardamom fruit 4 (A. compactum, adulterated)	14	5 µL	Black cardamom fruit
7	1 µL	Cineole	15	5 µL	Amomum aurantium fruit
8	1 µL	Linalool			

System suitability test
Cineole: violet zone at Rf ~ 0.47 (white RT).
Linalool: violet zone at Rf ~ 0.35 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Prior to derivatization under UV 366 nm the chromatogram of the test solution shows one distinct fluorescent zone at Rf ~ 0.30 (yellow arrow).
After derivatization under white RT the chromatogram of the test solution shows a violet zone at Rf ~ 0.47 corresponding to reference substance cineole, and an orange zone just below it (green arrows). There is a violet zone at Rf ~0.21 and another violet zone right above the application position.

Test for other species
No zone is seen at Rf ~ 0.60 (Round cardamom fruit, blue arrow).). The other species show distinctively different fingerprints (see chromatogram).

Amomum villosum, Amomum longiligulare (Villous amomum fruit)

1. Scope

This method identifies dried Villous amomum fruit (Amomum villosum Lour., Amomum longiligulare T.I. Wu) by HPTLC fingerprint and discriminates dried Round cardamom fruit (Amomum krervanh Pierre ex Gagnep, Amomum compactum Soland ex Maton), dried Black cardamom fruit (Amomum tsao-ko Crevost & Lamerié), dried Light galangal fruit (Alpinia zerumbet (Pers.) Burtt & Sm.), dried Sharp-leaf galangal fruit (Alpinia oxyphylla) and dried Amomum aurantium fruit.

2. Source of method

CAMAG

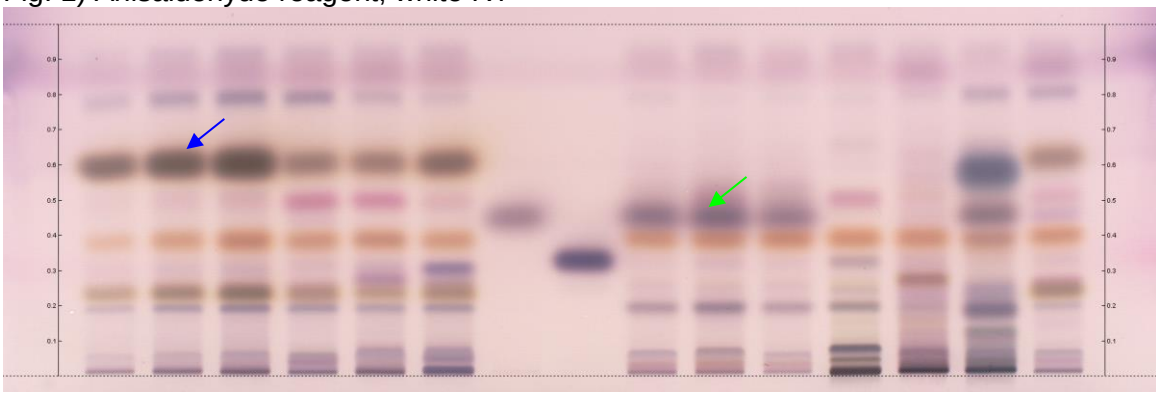
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of toluene, xylene 1:1 (v/v) and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 10 µL of cineole in 1 mL of methanol. Dissolve 10 µL of linalool in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 5 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 93:7 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice cooled methanol is mixed with 20 mL acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Villous amomum fruit 1 (Amomum villosum)	9	5 µL	Round cardamom fruit 1 (Amomum compactum)
2	**5 µL**	**Villous amomum fruit 1** (Amomum villosum)	10	5 µL	Round cardamom fruit 2 (Amomum compactum)
3	7 µL	Villous amomum fruit 1 (Amomum villosum)	11	5 µL	Round cardamom fruit 3 (Amomum compactum)
4	5 µL	Villous amomum fruit 2 (Amomum longiligulare)	12	5 µL	Light galangal fruit
5	5 µL	Villous amomum fruit 3 (Amomum villosum)	13	5 µL	Sharp-leaf galangal fruit
6	5 µL	Villous amomum fruit 4 (Amomum villosum)	14	5 µL	Black cardamom fruit
7	1 µL	Cineole	15	5 µL	Amomum aurantium fruit
8	1 µL	Linalool			

System suitability test

Cineole: violet zone at Rf ~ 0.47.
Linalool: violet zone at Rf ~ 0.35.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a violet zone below the solvent front. Below it there is an intense brown zone at Rf ~ 0.60 (blue arrow). Right above the position of reference cineole there may be a pink zone. Below cineole there is an orange zone at Rf ~ 0.38. There is a brown zone at Rf ~ 0.24 and just below it a blue zone. A violet zone is seen right above the application position.

Test for other species

No zone is seen at the position of cineole (Round cardamom fruit, green arrow). The other species show distinctively different fingerprints (see chromatogram).

Angelica archangelica
(Angelica root)

1. Scope

This method identifies Angelica root (Angelica archangelica L.) by HPTLC fingerprint and detects the adulterants Chinese Angelica root (Angelica sinensis (Oliv.) Diels), Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.), Lovage root (Levisticum officinale W. Koch), and Chinese lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.).

2. Source of method

CAMAG, under evaluation by Ph.Eur

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions **NOTE**: apply reference standards separately with method Reference standards ATS4.cme or Reference standards Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes

Fig. 1) UV 254 nm

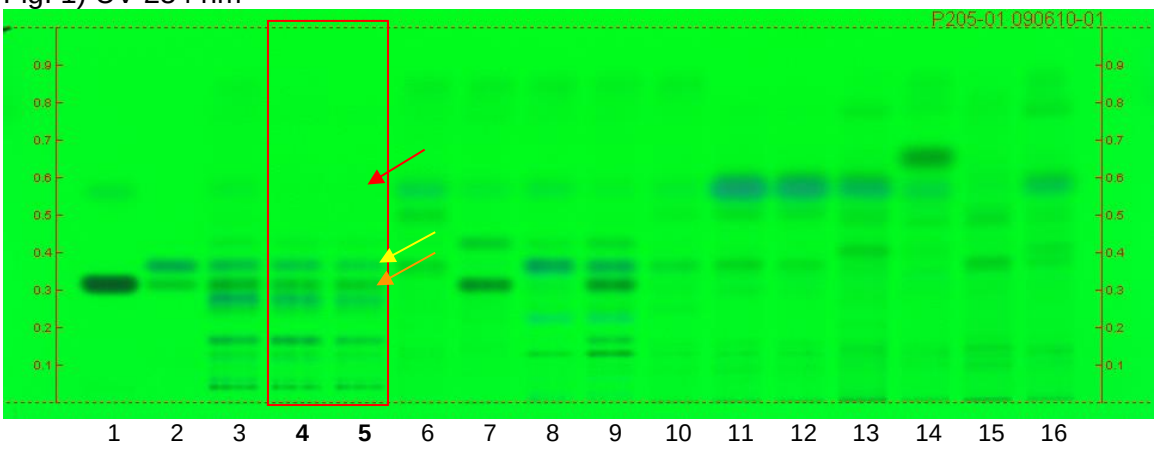
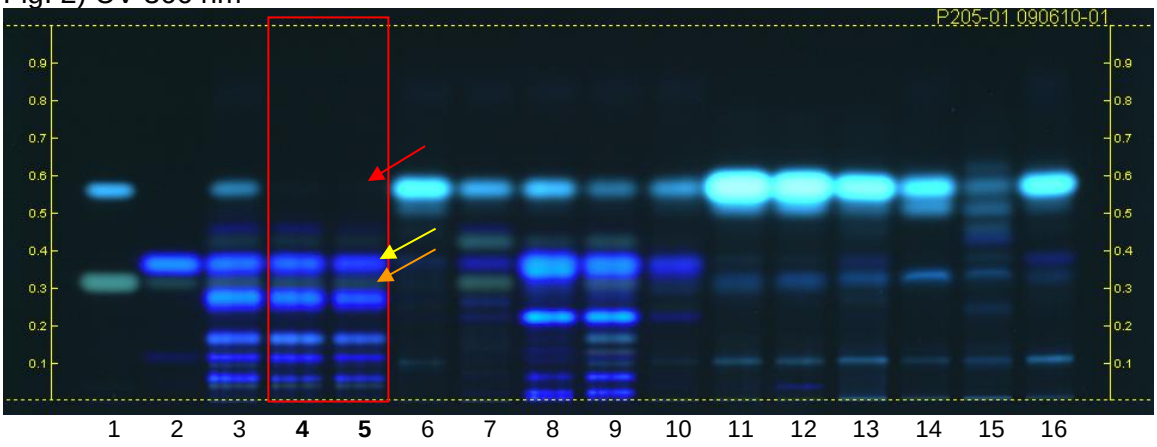


Fig. 2) UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	9	4 µL	Doubleteeth pubescent Angelica root 2
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	11	4 µL	Lovage root 2
4	**4 µL**	**Angelica root 2**	12	4 µL	Lovage root 3
5	**4 µL**	**Angelica root 3**	13	4 µL	Chinese lovage root *(Ligusticum sinensis)*
6	4 µL	Chinese Angelica root	14	4 µL	Chinese lovage root 1 *(Ligusticum jeholense)*
7	4 µL	Dahurian Angelica root	15	4 µL	Chinese lovage root 2 *(Ligusticum jeholense)*
8	4 µL	Doubleteeth pubescent Angelica root 1	16	4 µL	Chinese lovage root *(Ligusticum chuanxiong)*

*these samples do not comply with Ph.Eur specifications

System suitability test
Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. Under UV 254 nm the chromatogram of the test solution shows quenching zones corresponding to references imperatorin and osthole (Rf ~ 0.31 and Rf ~ 0.36, orange and yellow arrow). Below these zones several quenching zones are detected.
Under UV 366 nm the chromatogram of the test solution shows a dark blue fluorescent zone corresponding to the reference osthole. Right below a greenish fluorescent zone corresponding to imperatorin is detected. Below this zone several blue fluorescent zones are detected.

Test for adulteration
No zone is seen at or directly below Rf ~ 0.57 (red arrow) (Chinese Angelica root, Dahurian Angelica root, Doubleteeth pubescent root, Lovage root, Chinese lovage root).

Angelica dahurica
(Dahurian Angelica root)

1. Scope

This method identifies Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), by HPTLC fingerprint and detects the adulterants Angelica root (Angelica archangelica L.), Chinese Angelica root (Angelica sinensis (Oliv.) Diels), Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.), Lovage root (Levisticum officinale W. Koch) and Chinese lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.).

2. Source of method

CAMAG, under evaluation by Ph.Eur

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions **NOTE**: apply reference standards separately with method Reference standards ATS4.cme or Reference standards Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid with 180 mL methanol. Use: Dip for 1 s, heat at 100°C for 5 min
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) UV 366 nm 4.) Sulfuric acid, WRT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

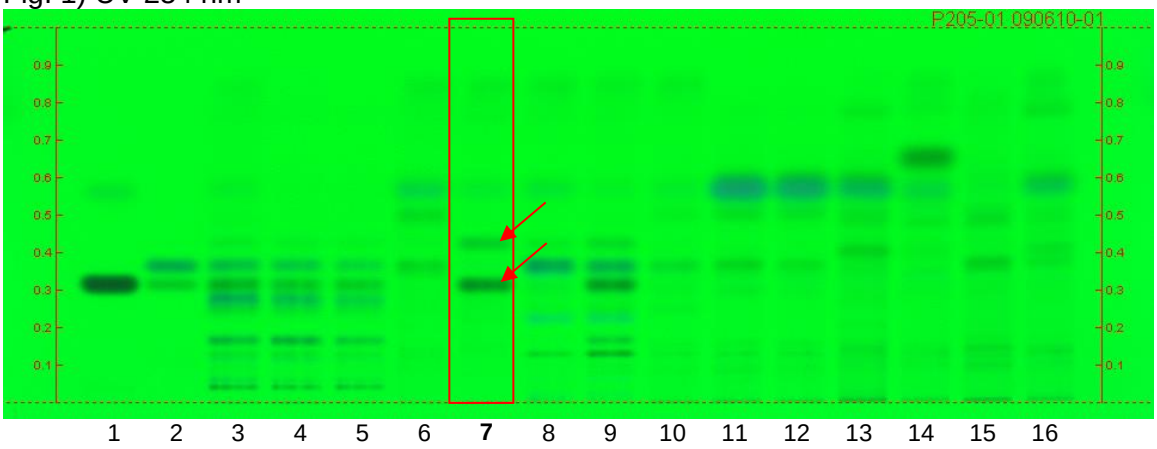


Fig. 2) UV 366 nm

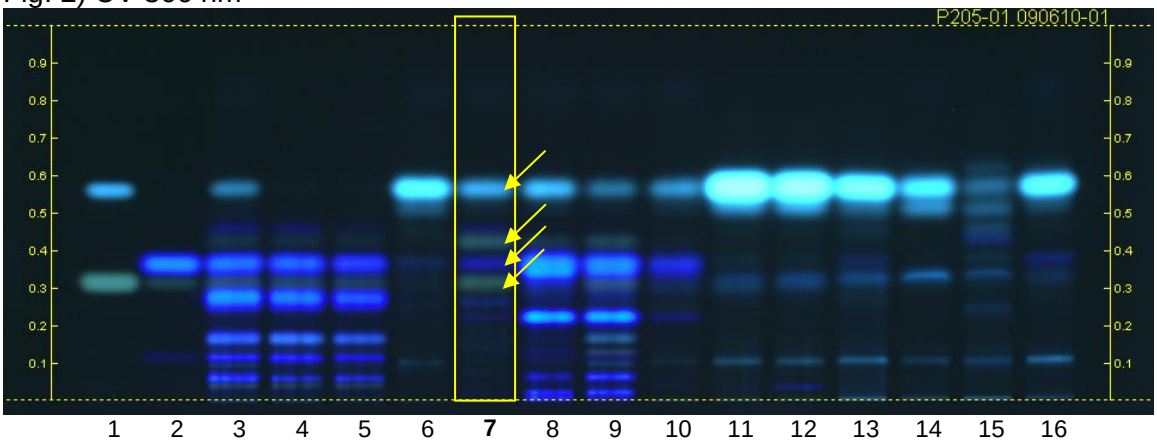
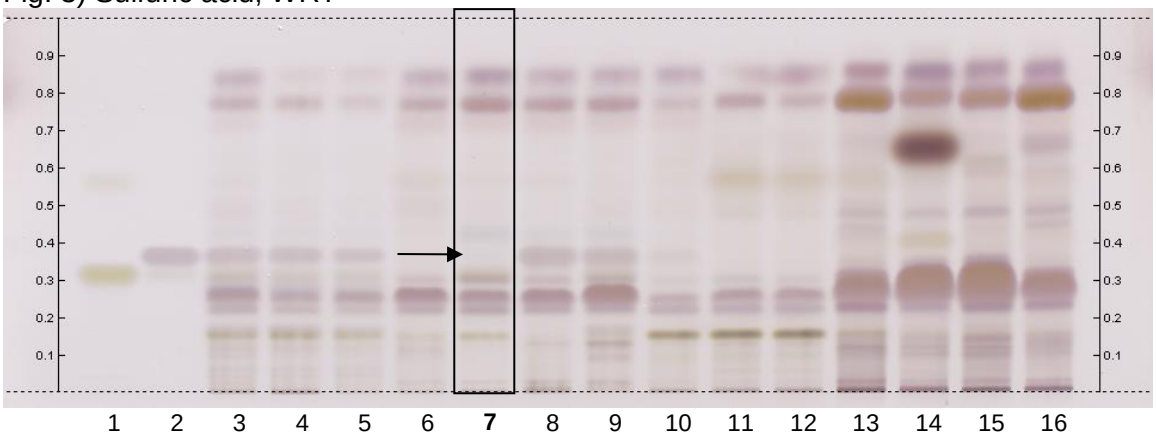


Fig. 3) Sulfuric acid, WRT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	9	4 µL	Doubleteeth pubescent Angelica root 2
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	11	4 µL	Lovage root 2
4	4 µL	Angelica root 2	12	4 µL	Lovage root 3
5	4 µL	Angelica root 3	13	4 µL	Chinese lovage root (*Ligusticum sinensis*)
6	4 µL	Chinese Angelica root	14	4 µL	Chinese lovage root 1 (*Ligusticum jeholense*)
7	**4 µL**	**Dahurian Angelica root**	15	4 µL	Chinese lovage root 2 (*Ligusticum jeholense*)
8	4 µL	Doubleteeth pubescent Angelica root 1	16	4 µL	Chinese lovage root (*Ligusticum chuanxiong*)

System suitability test
Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone corresponding to reference imperatorin and a quenching zone right above the position of reference osthole. There may be a faint quenching zone at the position of z-ligustilide (but no fluorescent zone).
Under UV 366 nm the chromatogram of the test solution shows a greenish zone at the position of reference imperatorin, a dark blue zone at the position of reference osthole, and above it another greenish zone. A white blue fluorescent zone is seen at the position of reference Z-ligustilide at Rf ~ 0.57.
After derivatization under white light there are two prominent purple zones below the solvent front. Below this zone there is a weak blue zone. At the position of imperatorin there is a greenish yellow zone and just below it a violet double zone.

Identification
Under UV 254 nm no blue fluorescent zone is seen at Rf ~ 0.57 and no quenching zone is seen at the position of osthole or below the position of imperatorin. Under UV 366 nm there is no intense blue fluorescent zone below the position of imperatorin. After derivatization under white light there is no purple zone at the position of osthole (black arrow) (Angelica root, Chinese Angelica root, Doubleteeth pubescent root, Lovage root, Chinese lovage root).

Angelica pubescens
(Doubleteeth Pubescent Angelica root, Du huo)

1. Scope

This method identifies Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.) by HPTLC fingerprint and detects the adulterants Angelica root (Angelica archangelica L.), Chinese Angelica root (Angelica sinensis (Oliv.) Diels), Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), Lovage root (Levisticum officinale W. Koch), and Chinese Lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.).

2. Source of method

CAMAG, under evaluation by Ph.Eur

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions **NOTE**: apply reference standards separately with method Reference standards ATS4/Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid with 180 mL methanol. Use: Dip for 1 s, heat at 100°C for 5 min2.) PEG reagent
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) UV 366 nm 4.) Sulfuric acid, WRT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

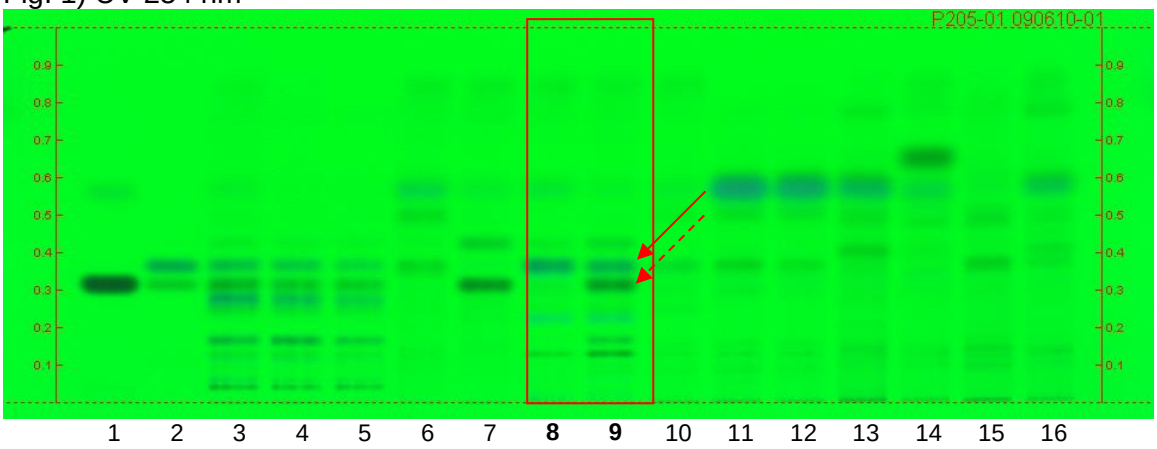


Fig. 2) UV 366 nm

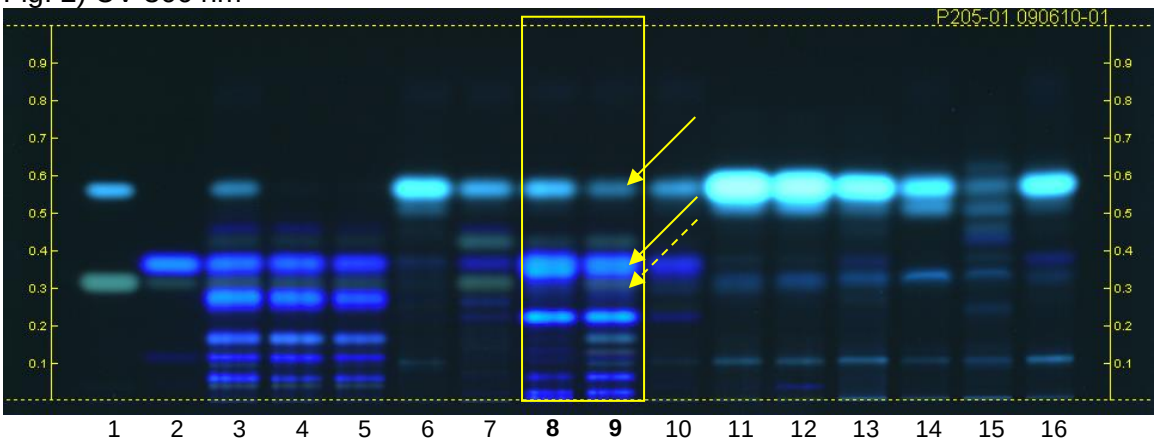
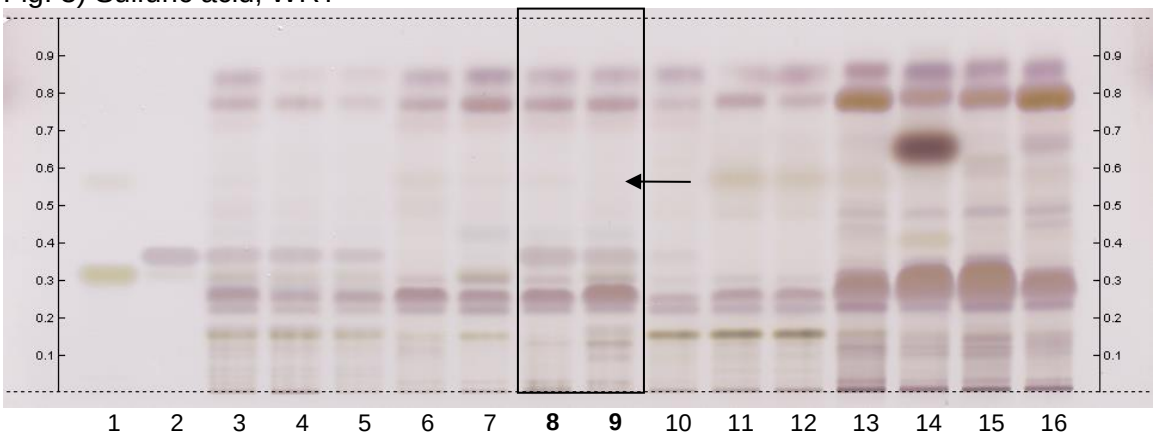


Fig. 3) Sulfuric acid, WRT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	**9**	**4 µL**	**Doubleteeth pubescent Angelica root 2**
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	11	4 µL	Lovage root 2
4	4 µL	Angelica root 2	12	4 µL	Lovage root 3
5	4 µL	Angelica root 3	13	4 µL	Chinese lovage root (*Ligusticum sinensis*)
6	4 µL	Chinese Angelica root	14	4 µL	Chinese lovage root 1 (*Ligusticum jeholense*)
7	4 µL	Dahurian Angelica root	15	4 µL	Chinese lovage root 2 (*Ligusticum jeholense*)
8	**4 µL**	**Doubleteeth pubescent Angelica root 1**	16	4 µL	Chinese lovage root (*Ligusticum chuanxiong*)

*these samples do not comply with Ph.Eur specifications

System suitability test
Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone corresponding to reference osthole (Rf ~ 0.36) (red arrow). There may be a quenching zone corresponding to imperatorin (dashed red arrow) and there may be a quenching zone at the position of z-ligustilide (but no fluorescent zone). There are several weak quenching zones below these zones.
Under UV 366 nm the chromatogram of the test solution shows a white blue fluorescent zone at Rf ~ 0.57 and a prominent blue fluorescent zone corresponding to osthole (yellow arrows). Above this zone there may be a very faint green whitish zone. There may be a green whitish fluorescent zone corresponding to imperatorin (dashed yellow arrow). Below this zone there is a prominent blue fluorescent zone and several weak bluish and greenish zones.
After derivatization under white light there are two prominent purple zones below the solvent front. A purple zone corresponding to osthole is detected and there may be a purple zone corresponding to imperatorin. Below this zone there are several yellow and purple zones.
NOTE: the two samples of Doubleteeth pubescent Angelica root show variation.

Test for adulteration
Under UV 254 nm no blue fluorescent zone is seen at Rf ~ 0.57. Under UV 366 nm no blue fluorescent zone just below the position of imperatorin is seen. After derivatization under white light no zone is seen at Rf ~ 0.57 (black arrow) (Angelica root, Chinese Angelica root, Dahurian Angelica root, Lovage root, Chinese lovage root).

Angelica sinensis
(Chinese Angelica root, Dang gui)

1. Scope

This method identifies dried Chinese Angelica root (Angelica sinensis (Oliv.) Diels) by HPTLC fingerprint and detects the adulterants dried Angelica root (Angelica archangelica L.), Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.), Lovage root (Levisticum officinale W. Koch), and Chinese Lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions NOTE: apply reference standards separately with method Reference standards ATS4.cme or Reference standards Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

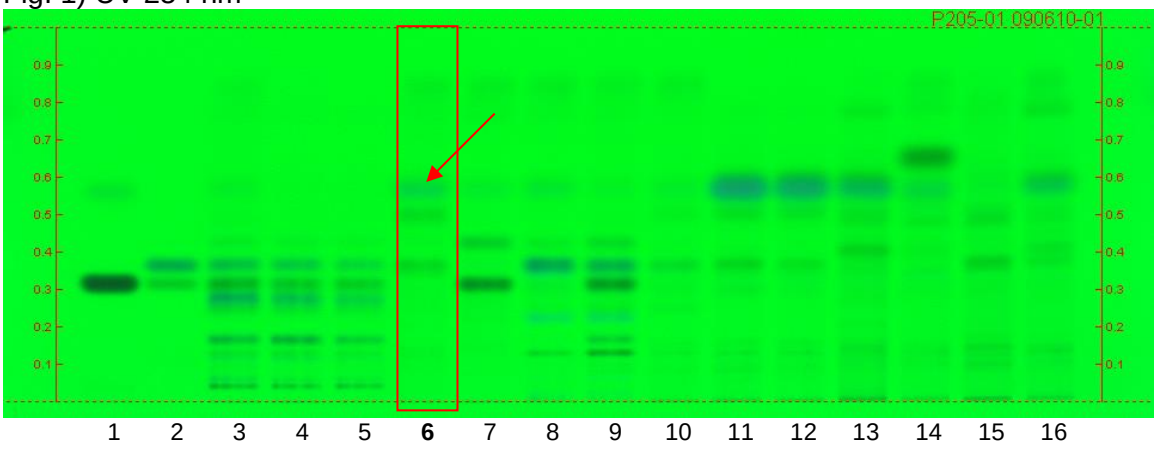
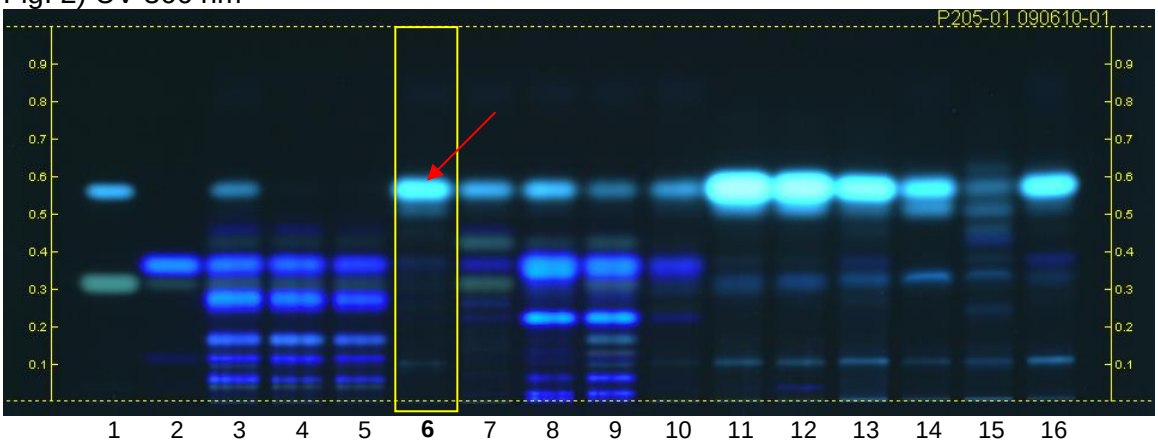


Fig. 2) UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	9	4 µL	Doubleteeth pubescent Angelica root 2
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	11	4 µL	Lovage root 2
4	4 µL	Angelica root 2	12	4 µL	Lovage root 3
5	4 µL	Angelica root 3	13	4 µL	Chinese lovage root (*Ligusticum sinensis*)
6	**4 µL**	**Chinese Angelica root**	14	4 µL	Chinese lovage root 1 (*Ligusticum jeholense*)
7	4 µL	Dahurian Angelica root	15	4 µL	Chinese lovage root 2 (*Ligusticum jeholense*)
8	4 µL	Doubleteeth pubescent Angelica root 1	16	4 µL	Chinese lovage root (*Ligusticum chuanxiong*)

*these samples do not comply with Ph.Eur specifications

System suitability test

Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a blue fluorescent zone corresponding to reference Z-ligustilide (red arrow). Below this zone there are two weak quenching zones, one of them at the position corresponding to osthole.

Under UV 366 nm the chromatogram of the test solution shows an intense blue white fluorescent zone corresponding to reference Z-ligustilide.

Test for adulteration
Under UV 254 nm no zone is seen at or below the position of imperatorin. Under UV 366 nm no zone is seen at or below the position of osthole (Angelica root, Dahurian Angelica root, Doubleteeth pubescent root, Lovage root, Chinese lovage root.

Arctostaphylos uva ursi
(Uva ursi leaf)

1. Scope

This method identifies dried Uva ursi leaf (Bearberry leaf) (Arctostaphylos uva-ursi L. Spreng) by HPTLC fingerprint.

2. Source of method

dified from Ph. Eur 6.7 (change in sample preparation and derivatization reagent)

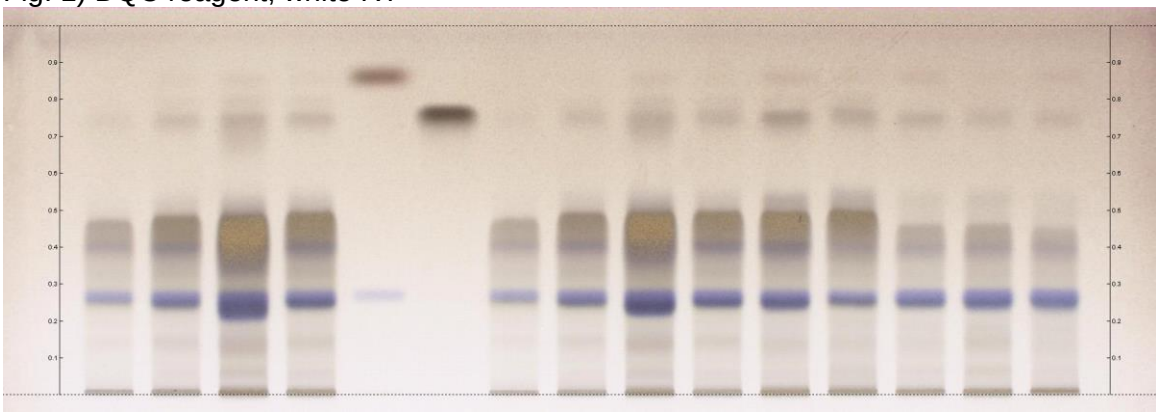
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol-water 1:1 and sonicate for 10 minutes at 60°C, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2.5 mg of arbutin in 1 mL of methanol. Dissolve 2.5 mg of hydroquinone in 1 mL of methanol. Optional: Dissolve 2.5 mg of gallic acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 88:6:6 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	2, 6-Dichloroquinone-4-chloroimide (DQC) reagent Preparation: 250 mg of 2, 6-Dichloroquinone-4-chloroimide are dissolved in 50 mL of methanol. Use: spray, dry in a stream of cold air for 3 min, expose to ammonia vapor (hold plate in a tank saturated with 32% ammonia) for 2 s or until blue zones are visible
Documentation:	1.) Clean plate, white RT 2.) DQC reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) DQC reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Uva ursi leaf 1	9	4 µL	Uva ursi leaf 3
2	**2 µL**	**Uva ursi leaf 1**	10	2 µL	Uva ursi leaf 4
3	4 µL	Uva ursi leaf 1	11	2 µL	Uva ursi leaf 5 (old)
4	2 µL	Uva ursi leaf 2	12	2 µL	Uva ursi leaf 6 (old)
5	2 µL	Arbutin, hydroquinone (with increasing Rf)	13	2 µL	Uva ursi leaf 7 (old)
6	2 µL	Gallic acid	14	2 µL	Uva ursi leaf 8 (old)
7	1 µL	Uva ursi leaf 3	15	2 µL	Uva ursi leaf powdered (old)
8	2 µL	Uva ursi leaf 3			

System suitability test
Arbutin: blue zone at Rf ~ 0.27.
Hydroquinone: brown zone at Rf ~ 0.87.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an intense blue zone at Rf ~ 0.27 corresponding to reference substance arbutin. Above it there is another blue zone Rf ~ 0.40 and overlapping a very broad and diffuse brown zone. A faint brown zone is seen at Rf ~ 0.75 (gallic acid). Some samples show a faint brown zone at Rf ~ 0.87 (hydroquinone).

Arnebia euchroma or Arnebia guttata (Arnebia root, zi ca)

1. Scope

This method identifies dried Arnebia root (Arnebia euchroma (Royle) Johnst. or Arnebia guttata Bunge) by HPTLC fingerprint and discriminates dried Yunnan onosma root (Onosma paniculatum Bureau & Franch.) and Tibetan onosma root (Onosma hookeri C. B. Clarke var. longiflorum (Duthie) Duthie ex Stapf).

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dilute CAMAG test dye mixture III 1:10 with toluene .
Stationary phase:	HPTLC Si 60 RP-18
Application:	3 µL of references, 7 µL of test solutions
Mobile phase:	acetone, 5% aqueous formic acid 8:2 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate, white RT 2.) UV 366 nm 3.) White RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 366 nm

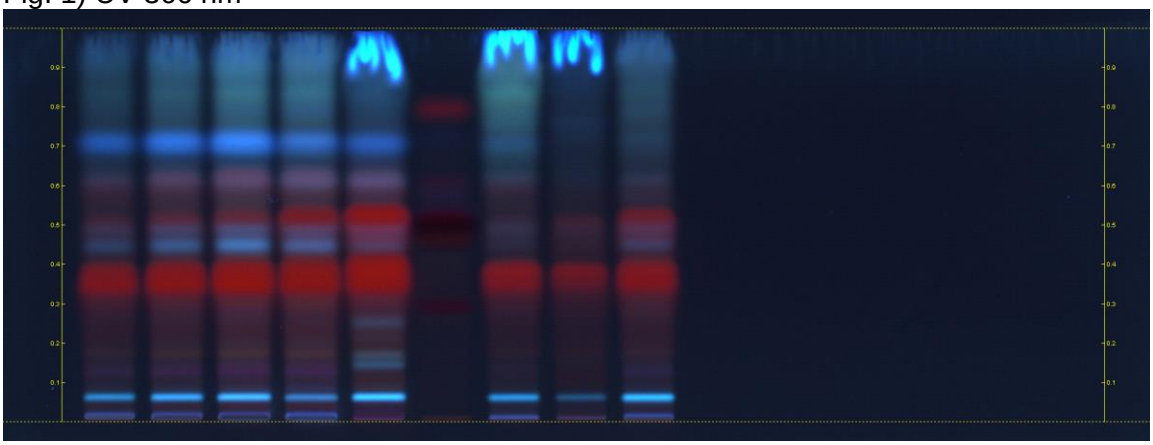
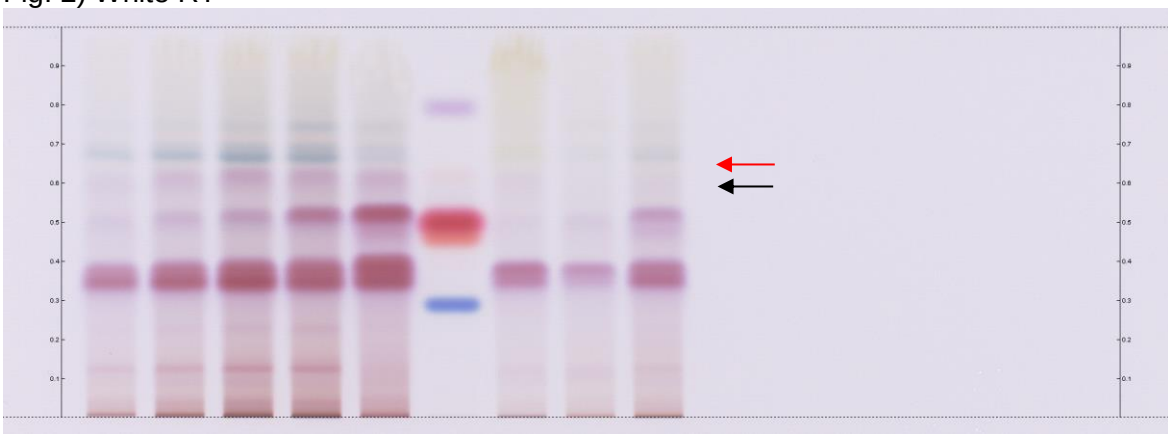


Fig. 2) White RT



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Arnebia root 1	9	7 µL	Tibetan onosma root
2	5 µL	Arnebia root 1	10		Blank
3	**7 µL**	**Arnebia root 1**	11		Blank
4	7 µL	Arnebia root 2	12		Blank
5	7 µL	Arnebia root 3	13		Blank
6	3 µL	CAMAG test dye mixture III	14		Blank
7	7 µL	Arnebia root 4 (old sample)	15		Blank
8	7 µL	Yunnan onosma root			

System suitability test (under white RT)
CAMAG test dye mixture III: A blue zone: at Rf ~ 0.28 and an orange red double zone at Rf ~ 0.48.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 366 nm the chromatogram of the test solution shows a blue zone right above the application position, a diffuse red zone at Rf ~ 0.35, above it another blue, a red and a purple zone and a characteristic blue zone at Rf ~ 0.71.
Under white RT there is an intense and diffuse purple zone at Rf ~ 0.35. Above it there is a purple zone at Rf ~ 0.50 and at Rf ~ 0.60. A blue zone is visible at Rf ~ 0.67.

Test for adulteration
Under white RT no zones are seen at Rf ~ 0.60 (black arrow) and Rf ~ 0.67 (red arrow) (Yunnan onosma root, Tibetan onosma root, or low quality/old Arnebia root).
.

Arnica Montana
(Arnica flower)

1. Scope

This method identifies the whole or partially broken, dried flower-heads of Arnica (Arnica montana L.) by HPTLC fingerprint and discriminates the adulterants dried Chamisso arnica flower (Arnica chamissonis Less.), False arnica flower (Heterotheca inuloides Cass.), and Calendula flower (Calendula officinalis L.).

2. Source of method

Modified from Ph. Eur 6.7 (change in sample preparation)

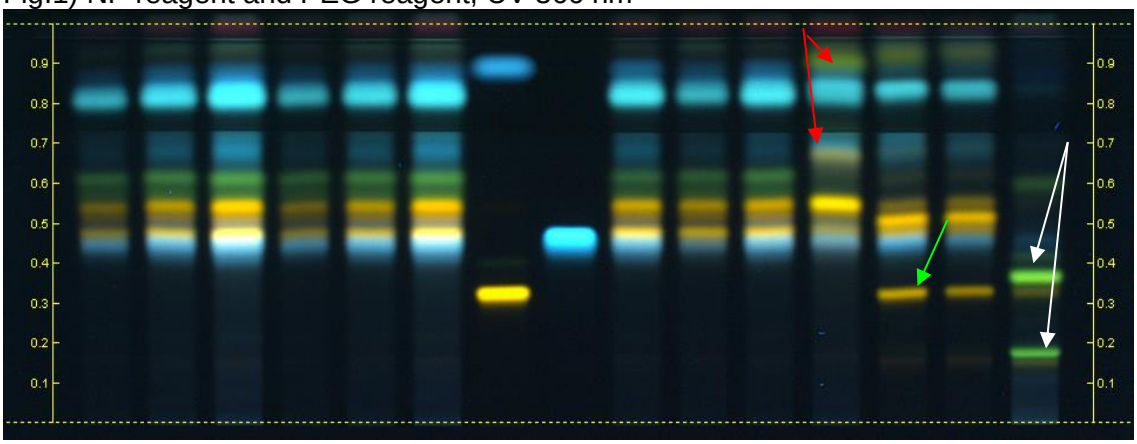
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2 mg of caffeic acid, 3.5 mg of rutin and 2.5 mg of chlorogenic acid individually in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid 98%, water, ethyl methyl ketone 50:10:10:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL methylene chloride Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent while still hot, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP reagent and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Arnica flower 1	**9**	**4 µL**	**Arnica flower 2**
2	**4 µL**	**Arnica flower 1**	**10**	**4 µL**	**Arnica flower 3**
3	6 µL	Arnica flower 1	**11**	**4 µL**	**Arnica flower 4**
4	2 µL	Arnica flower 1	12	4 µL	Chamisso arnica flower
5	**4 µL**	**Arnica flower 1**	13	4 µL	False arnica flower 1
6	6 µL	Arnica flower 1	14	4 µL	False arnica flower 2
7	2 µL	Rutin, caffeic acid (increasing Rf)	15	4 µL	Calendula flower
8	2 µL	Chlorogenic acid			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.32
Chlorogenic acid: bluish fluorescent zone at Rf ~ 0.46
Caffeic acid: bluish fluorescent zone at Rf ~ 0.89

Identification

Compare result under UV 366 nm with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a blue-white zone corresponding to chlorogenic acid. There are two yellow zones and a green zone above the reference material chlorogenic acid. At the upper part of the chromatogram there are one to two blue-white zones. Below the NOTE: No prominent zone is seen at or below the position of rutin.

Test for adulteration

No greenish fluorescent zone is seen at or just above the position of caffeic acid and no yellow fluorescent zone is seen at Rf ~ 0.65 (red arrows; Chamisso arnica flower). No yellow zone is seen at the position of rutin (green arrow; False arnica flower). No green zone is seen below or just above the position of rutin (white arrows; Calendula flower).

Artemisia annu
(Sweet wormwood leaf, qing hao)

1. Scope

This method identifies dried (and fresh) Sweet wormwood leaf (Artemisia annua L.) by HPTLC fingerprint and detects the adulterant Chinese wormwood leaf (Artemisia apiacea Hance).

2. Source of method

CAMAG

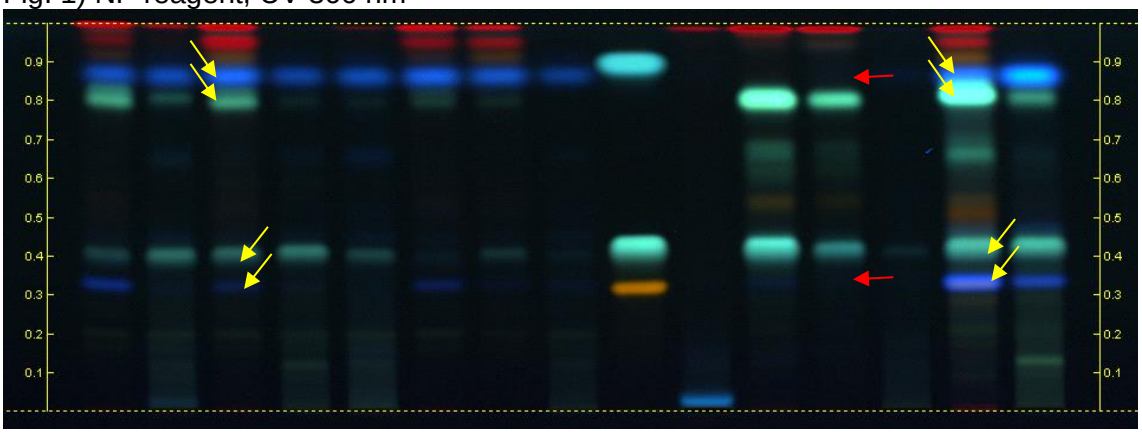
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chlorogenic acid in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol. Optional: Dissolve 1 mg of rutin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Ethyl acetate, water, acetic acid, formic acid 100:26:11:11 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5)
Documentation:	1.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Sweet wormwood fresh leaf 1 (extracted with water)	9	5 µL	Rutin, chlorogenic acid, caffeic acid (with increasing Rf)
2	5 µL	Sweet wormwood fresh leaf 1 (extracted with ethanol)	10	5 µL	Chinese wormwood fresh leaf (extracted with water)
3	5 µL	Sweet wormwood fresh leaf 1 (extracted with methanol)	11	5 µL	Chinese wormwood fresh leaf (extracted with ethanol)
4	5 µL	Sweet wormwood fresh leaf 1 (extracted with hot water)	12	5 µL	Chinese wormwood fresh leaf (extracted with methanol)
5	5 µL	Sweet wormwood fresh leaf 2 (extracted with water)	13	5 µL	Chinese wormwood fresh leaf (extracted with hot water)
6	5 µL	Sweet wormwood fresh leaf 2 (extracted with ethanol)	**14**	**5 µL**	**Sweet wormwood dried leaf 3 (extracted with methanol)**
7	5 µL	Sweet wormwood fresh leaf 2 (extracted with methanol)	15	5 µL	Sweet wormwood dried leaf 3 (extracted with hot water)
8	5 µL	Sweet wormwood fresh leaf 2 (extracted with hot water)			

Sweet wormwood fresh leaf 1: source Switzerland; Sweet wormwood fresh leaf 2: source Korea

System suitability test

Chlorogenic acid: green fluorescent zone at Rf ~ 0.40.
Caffeic acid: green fluorescent zone at Rf ~ 0.90.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a green fluorescent zone at Rf ~ 0.40 corresponding to reference chlorogenic acid and below it a blue zone at Rf ~ 0.32 (yellow arrows). In the upper part of the chromatogram there is an intense green zone at Rf ~ 0.81 and a blue one just above it at Rf ~ 0.86. Below the solvent front there are two red zones. Similar but fainter zones are seen in the fresh sample (track 3).

Test for adulteration

The blue zones at Rf ~ 0.32 and Rf ~ 0.86 are missing (red arrows, Chinese wormwood leaf).

Astragalus membranaceus (Astragalus root, huang qi)

1. Scope

This method identifies dried Astragalus root (Astragalus membranaceus (Fisch.ex Link) Bunge) by HPTLC fingerprint and discriminates the adulterant dried Hedysarum root (Hedysarum polybotris Hand.-Mazz.).

2. Source of method

CAMAG, under evaluation by Ph.Eur

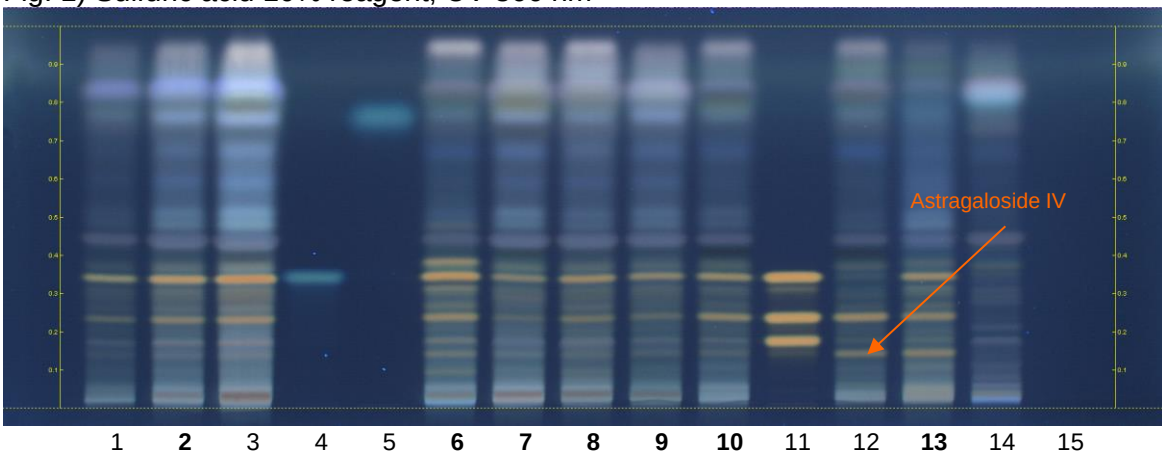
3. Procedure

Sample preparation:	Heat 3 g of powdered sample with 50 mL of methanol for 50 min under reflux, filter. Evaporate the filtrate under reduced pressure to dryness, take up the residue in 1 mL of water. Transfer this solution onto a 6 mL C18 SPE column (Bakerbond SPE C18 is suitable), that was conditioned with 3 mL of methanol and 3 mL of water. Wash the SEP column with 15 mL of water followed by 15 mL of 30% methanol. Discard both washings. Elute the sample with 20 mL of methanol and collect the eluate. Evaporate the eluate under reduced pressure to dryness, take up the residue with 2 mL of methanol.
Reference substances:	Dissolve 1 mg of daidzin in 1 mL of methanol. Dissolve 1 mg of daidzein in 1 mL of methanol. Optional: dissolve 1 mg each of astragalosides I to IV individually in 1 mL of methanol
Stationary phase:	HPTLC Si 60 F_{254}
Application:	3 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, methanol, water 100:13.5:10 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid 10% reagent Preparation: Solve 20 mL of sulfuric acid careful in 200 mL of methanol. Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Sulfuric acid 10% reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Sulfuric acid 10% reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	1µL	Astragalus root 1	**9**	**3µL**	**Astragalus root 5**
2	3µL	**Astragalus root 1**	**10**	**3µL**	**Astragalus root 6**
3	6µL	Astragalus root 1	11	3µL	Astragaloside 3 to 1 (with incr. Rf)
4	3µL	Daidzin	12	3µL	Astragalus root 7
5	3µL	Daidzein	**13**	**3µL**	**Astragalus root 8**
6	**3µL**	**Astragalus root 2**	14	3µL	Hedysarum root
7	**3µL**	**Astragalus root 3**	15		Blank
8	**3µL**	**Astragalus root 4**			

System suitability test

Daidzin: dark blue zone at Rf ~ 0.34
Daidzein: dark blue zone at Rf ~ 0.76

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
After derivatization the chromatogram of the test solution shows four orange zones corresponding to astragalosides I-IV between Rf ~ 0.15 and 0.35. The reference material daidzin and the reference material astragaloside I show more or less the same Rf value. In the upper part of the chromatogram several diffuse blue zones are present. One corresponds in color and position to daidzein at Rf ~ 0.76.

Test for adulteration

Note: The chromatogram of Hedysarum root does not show orange zones at Rf ~ 0.23 and Rf ~ 0.33.

Avena sativa
(Oat herb)

1. Scope

This method identifies dried Oat herb (Avena sativa L.) by HPTLC fingerprint and detects dried Barley grass (Hordeum vulgare L.) and dried Wheat grass (Triticum aestivum L.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

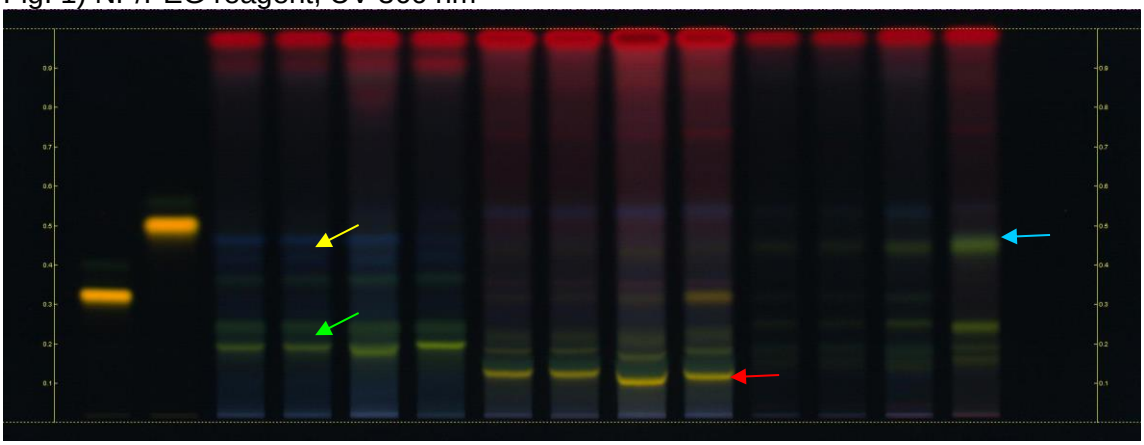
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of rutin in 1 mL of methanol. Dissolve 1 mg of hyperoside in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references, 10 µL of test solutions
Mobile phase:	Formic acid, water, methyl ethyl ketone, ethyl acetate 10:10:30:50 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat plate 3 min at 100 °C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP/PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Rutin	9	15 µL	Barley grass #1
2	4 µL	Hyperoside	10	10 µL	Barley grass #2
3	7 µL	Oat herb #1	11	7 µL	Wheat grass #1
4	**10 µL**	**Oat herb #1**	12	10 µL	Wheat grass #1
5	15 µL	Oat herb #1	13	15 µL	Wheat grass #1
6	10 µL	Oat herb #2	14	10 µL	Wheat grass #2
7	7 µL	Barley grass #1	15		Blank
8	10 µL	Barley grass #1			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.32.
Hyperoside: orange fluorescent zone at Rf ~ 0.50.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Between the orange zones corresponding to references rutin and hyperoside the chromatogram of the test solution shows two faint blue zones at Rf ~ 0.42 and Rf ~ 0.46 (yellow arrow) and below them a faint greenish zone. Below reference rutin there are two green zones at Rf ~ 0.25 and Rf ~ 0.18 (green arrow). A prominent red zone is located close to the solvent front.

Test for other species

No zone is seen between the application position and the green zone at Rf ~ 0.18 (Barley grass, red arrow). No green zone is seen at Rf ~ 0.44 (Wheat grass, blue arrow).

Calendula officinalis
(Calendula flower)

1. Scope

This method identifies dried Calendula flower (Calendula officinalis L.) by HPTLC fingerprint and discriminates dried Arnica flower (Arnica montana L.).

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation)

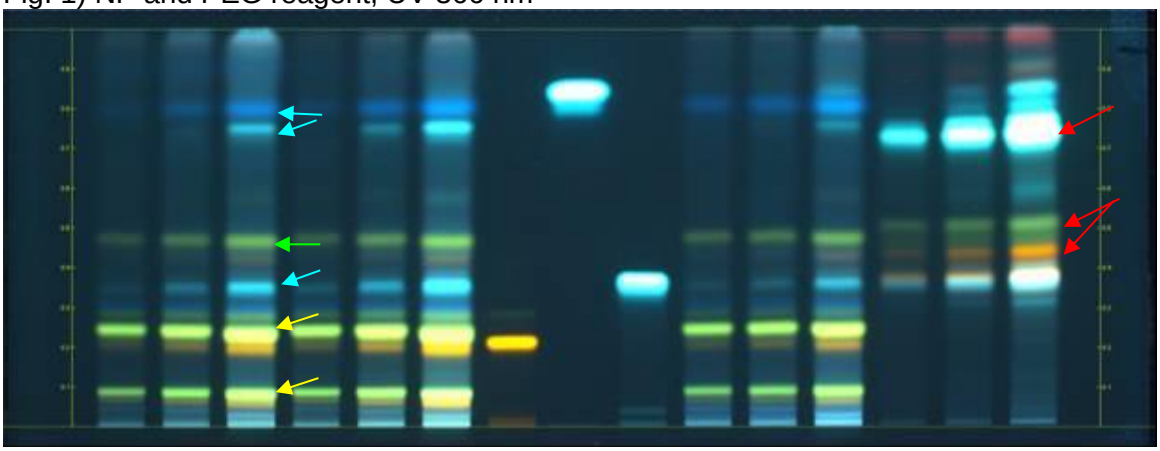
3. Procedure

Sample preparation:	Mix 0.5 g of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2.5 mg of rutin in 5 mL of methanol. Dissolve 3 mg of caffeic acid in 5 mL of methanol. Optional: dissolve 1.5 mg of chlorogenic acid in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 6 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid 98%, water 84:8:8 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent while still hot, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	NP reagent and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Calendula flower	9	2 µL	Chlorogenic acid
2	**6 µL**	**Calendula flower**	10	4 µL	Calendula flower (old)
3	12 µL	Calendula flower	11	8 µL	Calendula flower (old)
4	1 µL	Calendula flower (Ph. Eur. extraction)	12	16 µL	Calendula flower (old)
5	2 µL	Calendula flower (Ph. Eur. extraction)	13	2 µL	Arnica flower
6	4 µL	Calendula flower (Ph. Eur. extraction)	14	4 µL	Arnica flower
7	2 µL	Rutin	15	8 µL	Arnica flower
8	2 µL	Caffeic acid			

System suitability test
Rutin: a yellow fluorescent zone at Rf ~ 0.21
Caffeic acid: a white-blue fluorescent zone at Rf ~ 0.85

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a yellow zone corresponding to the reference material rutin. Right above and below rutin there are a green zone each at Rf ~ 0.23 and 0.09 (yellow arrows). A green fluorescent zone in the middle of the chromatogram is detected at Rf ~ 0.46 (green arrow). Three blue-white zones are present at Rf ~ 0.35, 0.75 and 0.80, the two last zones are right below caffeic acid (blue arrows).

Test for adulteration
No orange and green zones are seen at Rf ~ 0.45 and 0.51 and no blue-white zone is seen at Rf ~ 0.72 (red arrows, Arnica flower).

Camellia sinensis (Green Tea leaf)

1. Scope

This method identifies dried Green Tea leaf (Camellia sinensis (L.) Kuntze) by HPTLC fingerprint.

2. Source of method

CAMAG Method of Analysis MOA001

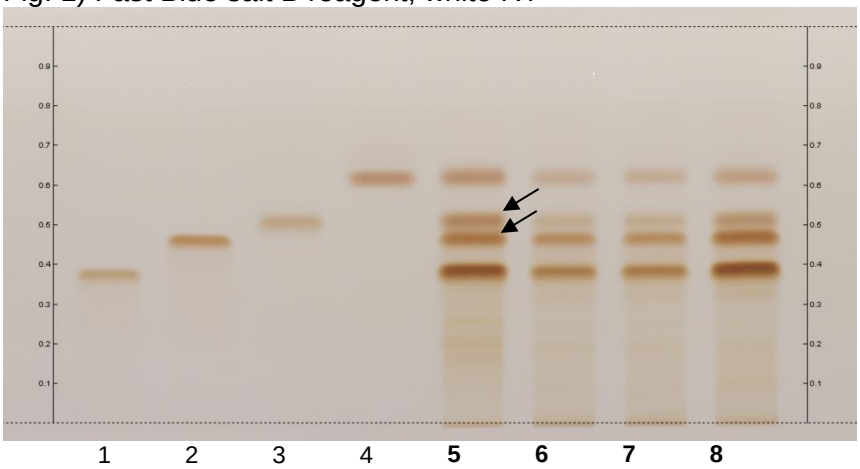
3. Procedure

Sample preparation:	Mix 100 mg of powdered sample with 10 mL of methanol, water 4:1 and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Individually dissolve 1 mg of (-)-epigallocatechin and 1 mg of (-)-epicatechin gallate each in 20 mL of methanol. Optional: Individually dissolve 1 mg of (-)-epigallo-catechin-3-O-gallate and 1 mg of (-)-epicatechin each in 20 mL of methanol. Store all solutions at -20°C..
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 1 µL of test solutions
Mobile phase:	Toluene, acetone, formic acid 9:9:2 (v/v/v)
Development:	**Unsaturated chamber** Developing distance **60 mm** from lower edge Relative humidity 33%
Derivatization reagent:	Fast Blue salt B reagent Preparation: dissolve 140 mg of Fast Blue salt B in 10 mL of water and add 140 mL of methanol and 50 mL of dichloromethane. Store reagent in the dark at 4°C. Use: preheat the plate to 100°C for 2 min, then dip (time 0, speed 5), dry for 5 min in the fume hood
Documentation:	1.) Clean plate, white RT 2.) Fast Blue salt B reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Fast Blue salt B reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	(-)-Epigallocatechin-3-O-gallate	9		Blank
2	5 µL	(-)-Epigallocatechin	10		Blank
3	5 µL	(-)-Epicatechin gallate	11		Blank
4	5 µL	(-)-Epicatechin	12		Blank
5	**1 µL**	**Green Tea leaf 1**	13		Blank
6	**1 µL**	**Green Tea leaf 2**	14		Blank
7	**1 µL**	**Green Tea leaf 3**	15		Blank
8	**1 µL**	**Green Tea leaf 4**			

System suitability test
(-)-Epigallocatechin: brown zone at Rf ~ 0.46
(-)-Epicatechin gallate: brown zone at Rf ~ 0.52

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows four brownish-orange zones corresponding to reference substance epigallocatechin-3-O-gallate (Rf ~ 0.37), (-)-epigallocatechin (Rf ~ 0.46), (-)-epicatechin gallate (Rf ~ 0.52), and (-)-epicatechin (Rf ~ 0.62). The lowest zone is the most intense and the upper zone is the faintest. The two zones in between are clearly separated (black arrows).

Capsicum annum / Capsicum frutescens (Cayenne pepper)

1. Scope

This method identifies dried Cayenne pepper (Capsicum annum L. var. minimum (Miller) Heiser) and (Capsicum frutescens L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation)

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2 mg of capsaicin in 5 mL of methanol. Dissolve 2 mg of dihydrocapsaicin in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 RP-18 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Water, methanol 20:80 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Dichloroquinone chlorimide reagent Preparation: 50 mg dichloroquinone chlorimide in 200 mL of ethyl acetate Use: Dip (time 0, speed 5), dry with cold air for 5 min, then expose to ammonia vapor
Documentation:	1.) Clean plate, white RT 2.) Dichloroquinone chlorimide reagent and ammonia vapor, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Dichloroquinone chlorimide reagent and ammonia vapour, white RT

Track	Volume	Sample	Track	Volume	Sample
1	3 µL	*Capsicum annum* (Ph.Eur.)	9	2 µL	*Capsicum frutescens* 1
2	4 µL	*Capsicum annum* (Ph.Eur.)	10	2 µL	*Capsicum frutescens* 2
3	5 µL	*Capsicum annum* (Ph.Eur.)	11	8 µL	Cayenne pepper 1
4	3 µL	*Capsicum annum*	12	10 µL	Cayenne pepper 1
5	**4 µL**	***Capsicum annum***	13	6 µL	Cayenne pepper 2
6	5 µL	*Capsicum annum*	14	8 µL	Cayenne pepper 2
7	2 µL	Capsaicin	15		Blank
8	2 µL	Dihydrocapsaicin			

System suitability test
Capsaicin: blue zone at Rf ~ 0.47
Dihydrocapsaicin: blue zone at Rf ~ 0.40

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows two blue zones at Rf ~ 0.40 and Rf ~ 0.47.

Carum carvi
(Caraway fruit (flavonoids))

1. Scope

This method identifies dried Caraway fruit (Carum carvi L.) by HPTLC fingerprint and discriminates dried Bitter Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. vulgare), Sweet Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. dulce) and Anise fruit (Pimpinella anisum L.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

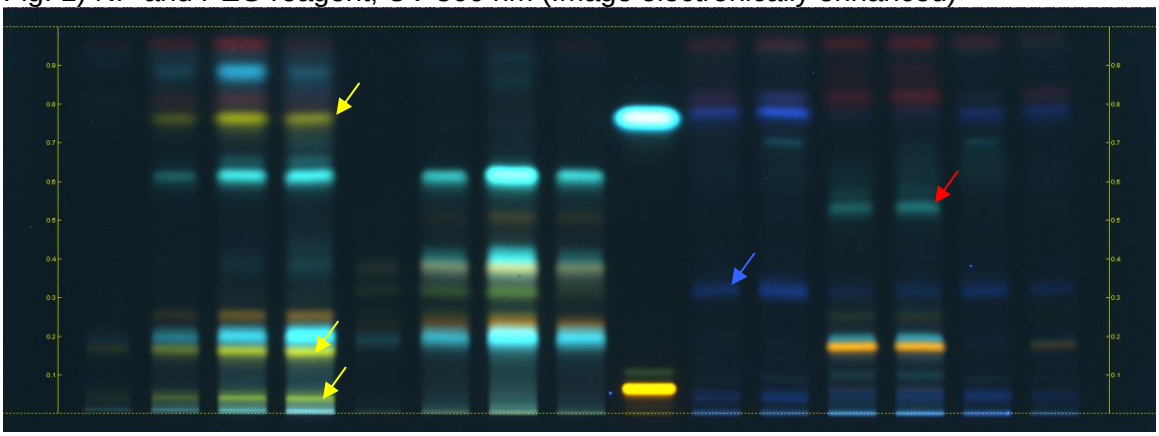
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3 mg of rutin in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent while still hot, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm (Image electronically enhanced)



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Anise fruit 1	9	3 / 1µL	Rutin, Caffeic acid (with incr. Rf)
2	3 µL	Anise fruit 1	10	3 µL	Bitter Fennel fruit 1
3	6 µL	Anise fruit 1	11	3 µL	Bitter Fennel fruit 2
4	3 µL	Anise fruit 2	12	3 µL	Sweet Fennel fruit 1
5	1 µL	Caraway fruit 1	13	3 µL	Sweet Fennel fruit 2
6	**3 µL**	**Caraway fruit 1**	14	3 µL	Wild Fennel fruit
7	6 µL	Caraway fruit 1	15	3 µL	Fennel tea
8	**3 µL**	**Caraway fruit 2**			

System suitability test
Rutin: orange zone at Rf ~ 0.07
Caffeic acid: light bluish zone at Rf ~ 0.77

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a brown zone at Rf ~ 0.23 and a light blue zone right above it at Rf ~ 0.20. Between Rf ~ 0.30 and 0.41 there are three zones (yellowish, brownish and light blue). Above these zones a weak brown zone at Rf ~ 0.51 is present and a light blue zone at Rf ~ 0.61 is seen.

Test for other species
No yellow zone is seen at Rf ~ 0.04, 0.16 and 0.76 (yellow arrows, Anise fruit). No greenish blue zone is seen at Rf ~ 0.53 (red arrow, Sweet Fennel fruit) and no blue zone is seen at Rf ~ 0.32 (blue arrow, Bitter Fennel fruit).

Carum carvi
(Caraway fruit)

1. Scope

This method identifies dried Caraway fruit (Carum carvi L.) by HPTLC fingerprint and discriminates the essential oil of Caraway.

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation)

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. (Caraway oil: Dissolve 40 µL of sample in 1 mL of toluene.)
Reference substances:	Dissolve 2 µL of carvone in 1 mL of ethyl acetate. Dissolve 5 µL of olive oil in 1 mL of ethyl acetate.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, toluene 5:95 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 4 min
Documentation:	1.) Clean plate, white RT and UV 254 nm 2.) UV 254 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

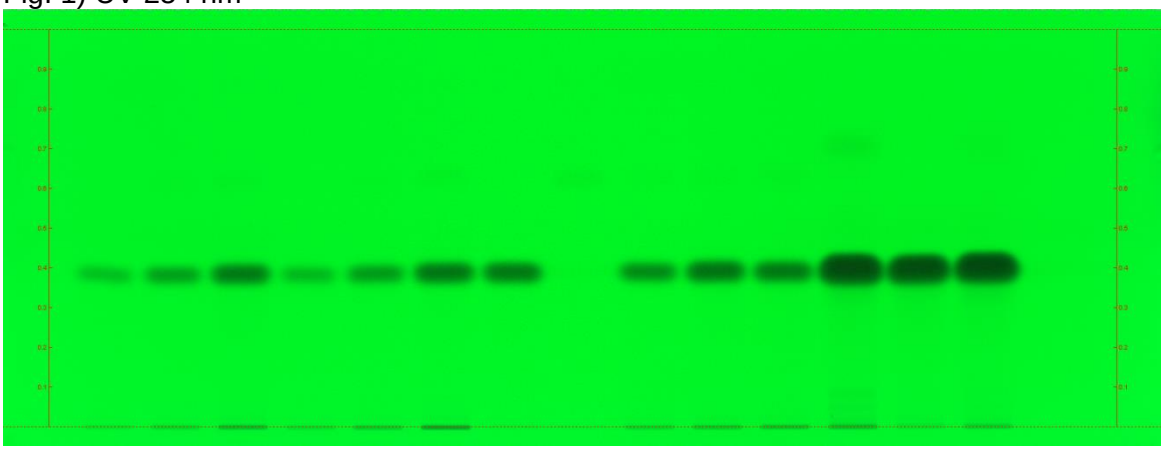
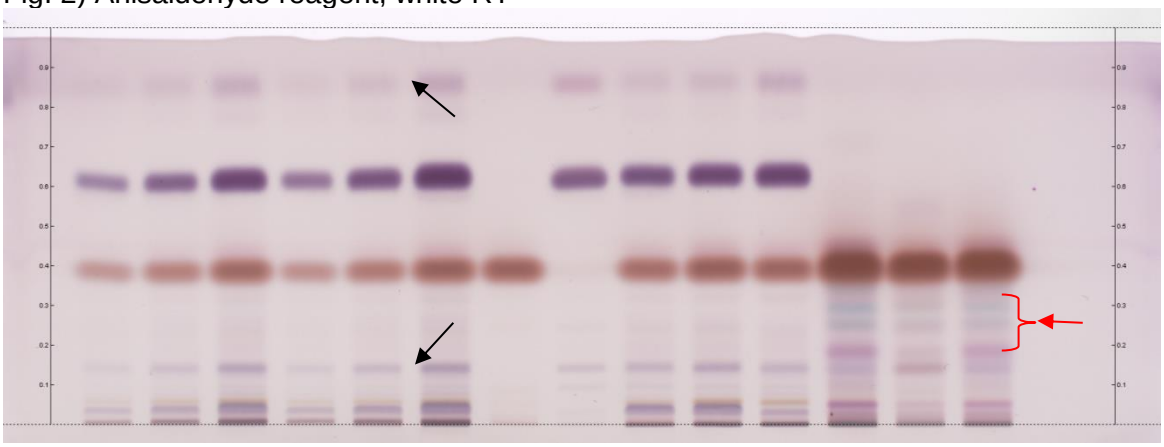


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Caraway fruit 1 (Ph. Eur. extraction)	**9**	**2 µL**	**Caraway fruit 2**
2	2 µL	Caraway fruit 1 (Ph. Eur. extraction)	**10**	**2 µL**	**Caraway fruit 3**
3	4 µL	Caraway fruit 1 (Ph. Eur. extraction)	**11**	**2 µL**	**Caraway fruit 4**
4	1 µL	Caraway fruit 1	12	2 µL	Caraway oil 1
5	**2 µL**	**Caraway fruit 1**	13	2 µL	Caraway oil 2
6	4 µL	Caraway fruit 1	14	2 µL	Caraway oil 3
7	2 µL	Carvone	15		Blank
8	2 µL	Olive oil			

System suitability test
Carvone: a brown zone at Rf ~ 0.39 (WRT)
Olive oil: a violet zone at Rf ~ 0.62 (WRT)

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone at the position of carvone reference substance at Rf ~ 0.39. After derivatization there is a brown zone at the position of carvone at Rf ~ 0.39 and a violet zone at the position of the olive oil reference substance at Rf ~ 0.62. Furthermore there is a weak violet zone close to the solvent and another violet zone at Rf ~ 0.14 (black arrows).

Test for Caraway essential oil
After derivatization no blue violet zones are present below the position of carvone (red arrow).

Carum carvi
(Caraway oil)

1. Scope

This method identifies the essential oil of Caraway (Carum carvi L.) by HPTLC fingerprint and discriminates the dried fruits of Caraway.

2. Source of method

Ph.Eur. 6.7

3. Procedure

Sample preparation:	Dissolve 40 µL of sample in 1 mL toluene. (Caraway fruit: Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.).
Reference substances:	Dissolve 2 µL of carvone in 1 mL of ethyl acetate. Dissolve 5 µL of olive oil in 1 mL of ethyl acetate.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, toluene 5:95 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 4 min
Documentation:	1.) Clean plate, white RT and UV 254 nm 2.) UV 254 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

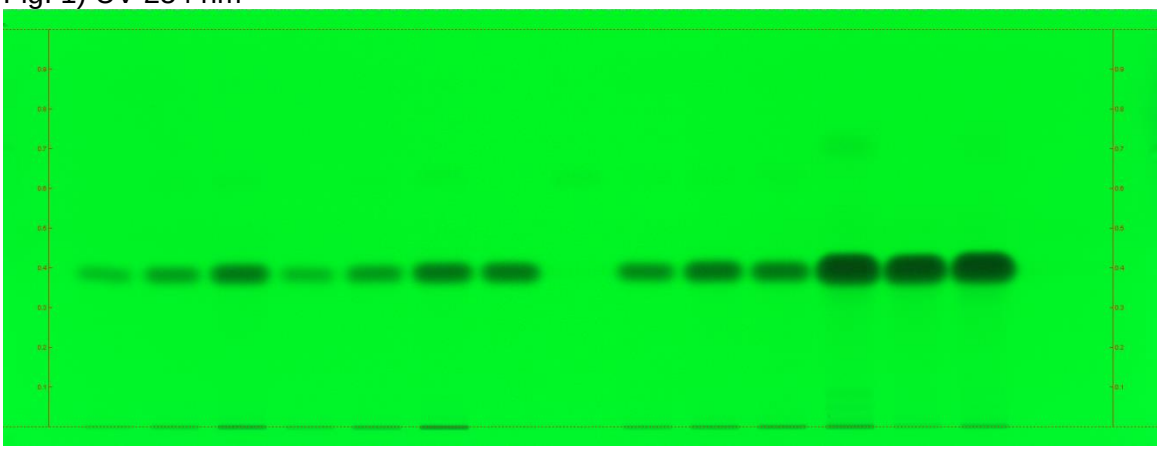
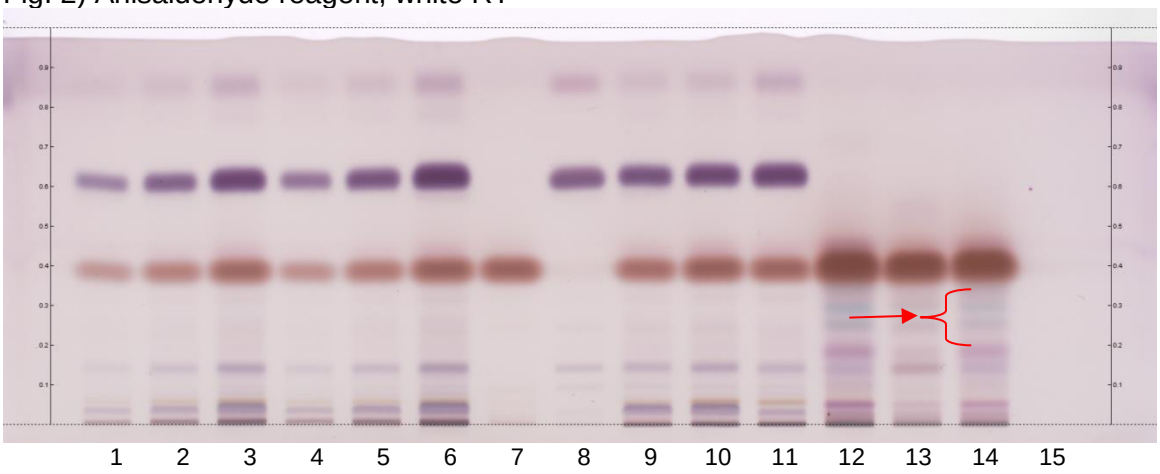


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Caraway fruit 1 (Ph. Eur. extraction)	9	2 µL	Caraway fruit 2
2	2 µL	Caraway fruit 1 (Ph. Eur. extraction)	10	2 µL	Caraway fruit 3
3	4 µL	Caraway fruit 1 (Ph. Eur. extraction)	11	2 µL	Caraway fruit 4
4	1 µL	Caraway fruit 1	**12**	**2 µL**	**Caraway oil 1**
5	2 µL	Caraway fruit 1	**13**	**2 µL**	**Caraway oil 2**
6	4 µL	Caraway fruit 1	**14**	**2 µL**	**Caraway oil 3**
7	2 µL	Carvone	15		Blank
8	2 µL	Olive oil			

System suitability test
Carvone: a brown zone at Rf ~ 0.39
Olive oil: a violet zone at Rf ~ 0.62

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone corresponding to the reference material carvone at Rf ~ 0.39. After derivatization there is a brown zone at the position of carvone at Rf ~ 0.39. Above this zone several weak blue-violet zones are present (red arrow). (The reddish-violet zone at Rf ~ 0.19 is carveol).

Test for adulteration
After derivatization there is no violet zone at the position of the olive oil reference substance at Rf ~ 0.62.

Cetraria islandica (Iceland moss)

1. Scope

This method identifies dried Iceland moss (Cetraria islandica L. Ach.) by HPTLC fingerprint and detects the other species dried Usnea moss (Usnea barbata L. F.H. Wigg.) and dried Oak moss (Evernia prunastri L. Ach.).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples/references and in detection)

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 5 mL of ethyl acetate and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of caffeic acid in 10 mL of methanol. Dissolve 2 mg of anethole in 10 mL of toluene. Optional: Dissolve 1 mg of usnic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 10 µL of test solutions
Mobile phase:	Acetone, methanol, formic acid, toluene 5:5:10:80 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT, UV 254 nm 2.) UV 254 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

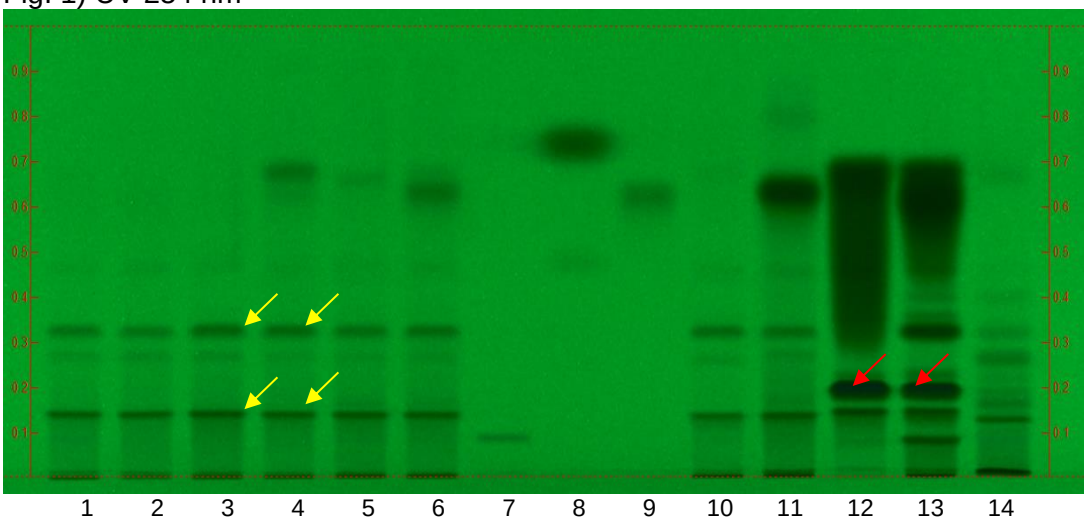
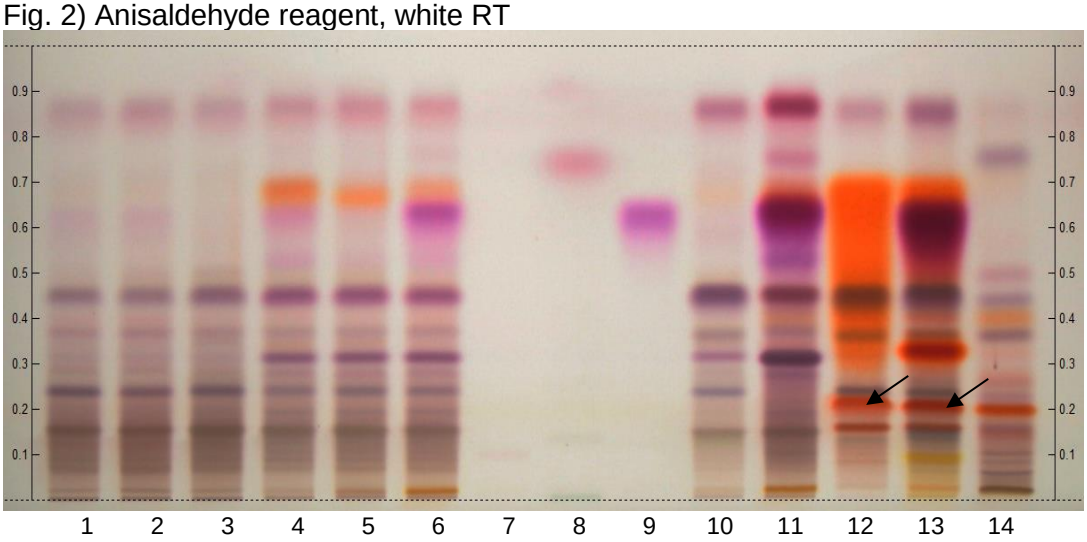


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	10 µL	Iceland moss 1	9	5 µL	Usnic acid
2	10 µL	Iceland moss 2	10	10 µL	Iceland moss 7
3	**10 µL**	**Iceland moss 3**	11	10 µL	Iceland moss 8
4	**10 µL**	**Iceland moss 4**	12	10 µL	Oak moss
5	10 µL	Iceland moss 5	13	10 µL	Usnea moss
6	10 µL	Iceland moss 6	14	10 µL	Usnea tincture
7	5 µL	Caffeic acid	15		blank
8	5 µL	Anethole			

System suitability test
Caffeic acid: quenching zone at Rf ~ 0.10 (UV 254 nm).
Anethole: quenching zone at Rf ~ 0.75 (UV 254 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone at Rf ~ 0.32 and 0.14 (yellow arrows). Under white RT there are several grayish and pink zones in the lower part of the plate with an intense violet zone at Rf ~ 0.28 and an intense pink zone at Rf ~ 0.49. Above it there is a faint green zone.

Test for other species
Under UV 254 nm no quenching zone is seen at Rf ~ 0.20 (red arrows); this zone appears orange under white RT (black arrows) (Usnea moss, Oak moss).

Chamaemelum nobile
(Roman chamomile flower)

1. Scope

This method identifies dried Roman chamomile flower (Chamaemelum nobile (L.) All.) by HPTLC fingerprint and discriminates the adulterant dried Feverfew flower (Tanacetum parthenium (L.) Sch. Bip.), Feverfew flower from Mexico (Tanacetum parthenium), Matricaria flower (Matricaria recutita L.) and Matricaria flower oil (Matricaria recutita L.).

2. Source of method

CAMAG Method of Analysis MOA009

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Dissolve 10 µL of the essential oil in 1 mL of toluene.
Reference substances:	Dissolve 1.5 mg of apigenin in 5 mL of methanol. Dissolve 1 mg of parthenolide in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, cyclohexane 1:1 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 4 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, UV 366 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, UV 366 nm

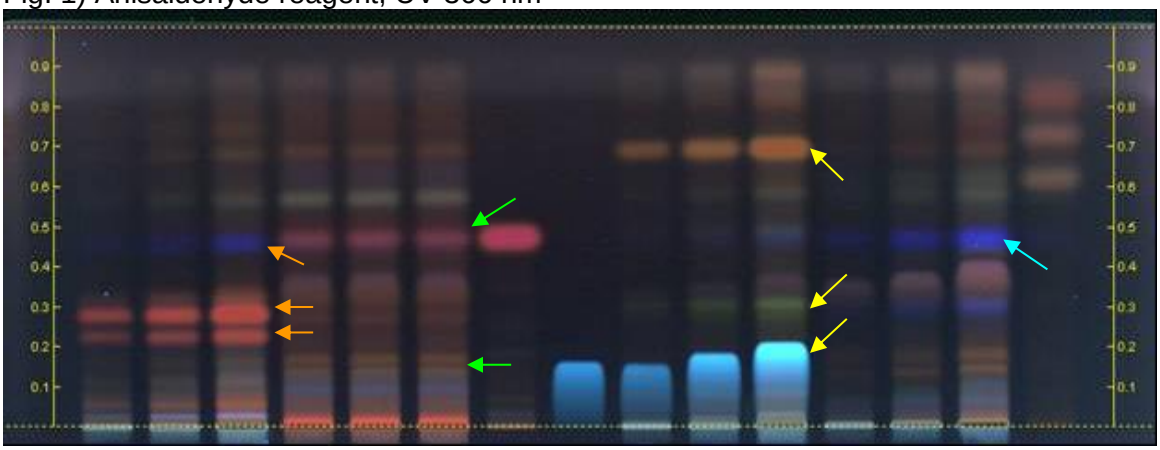
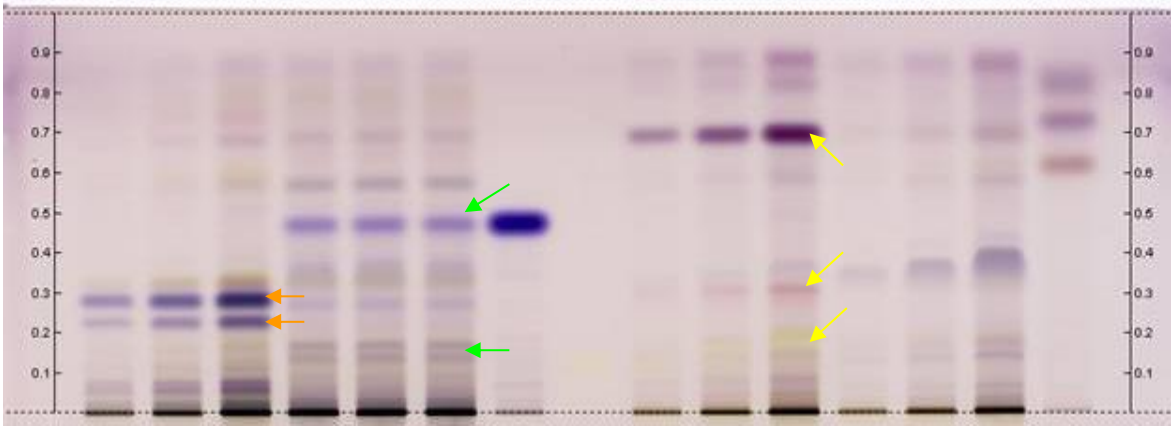


Fig. 2) Anisaldeyhde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Feverfew flower from Mexico	9	1 µL	Roman Chamomile flower
2	2 µL	Feverfew flower from Mexico	**10**	**2 µL**	**Roman Chamomile flower**
3	4 µL	Feverfew flower from Mexico	11	4 µL	Roman Chamomile flower
4	3 µL	Feverfew flower	12	1 µL	Matricaria flower
5	3.5 µL	Feverfew flower	13	2 µL	Matricaria flower
6	4 µL	Feverfew flower	14	4 µL	Matricaria flower
7	2 µL	Parthenolide	15	1 µL	Matricaria flower oil
8	2 µL	Apigenin			

System suitability test (UV 366 nm)
Apigenin: blue zone at Rf ~ 0.20
Parthenolide: pink zone at Rf ~ 0.48

Identification
Compare result under UV 366 nm with reference images. The fingerprint of the test Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 366 nm the chromatogram of the test solution shows a strongly tailing blue zone at the position of reference substance apigenin at Rf ~ 0.20. There is a characteristic greenish zone (red zone under white light) at Rf ~ 0.30 and an intense orange zone (violet zone under white light) at Rf ~ 0.7 (yellow arrows).

Test for adulteration
Under UV 366 nm there are no intense red zones (violet zone under white light) between Rf ~ 0.20 and 0.30. No blue zone is seen at the position of parthenolide (orange arrows; Feverfew flower from Mexico).
Under UV 366 nm no pink zone (violet zone under white light) at Rf ~ 0.48 corresponding to reference substance parthenolide is seen and there are no brown zones at the position of apigenin at Rf ~ 0.20 (green arrows, Feverfew flower).
Under UV 366 nm no blue zone is seen at the position of parthenolide (blue arrow; Chamomile flower).
Chamomile flower oil does not show any zones below Rf ~ 0.60.

Clematis armandii
(Armand's clematis root, xiao mu tong)

1. Scope

This method identifies Armand's clematis root (Clematis armandii Franch.) by HPTLC fingerprint and detects the adulterants Chinese clematis root (Clematis chinensis Retz.), Clematis manshurica Rupr. root, Clematis montana Buch.-Ham. ex. DC. root, and Clematis hexapetala Pall. root.

2. Source of method

CAMAG, under evaluation by Ph.Eur.

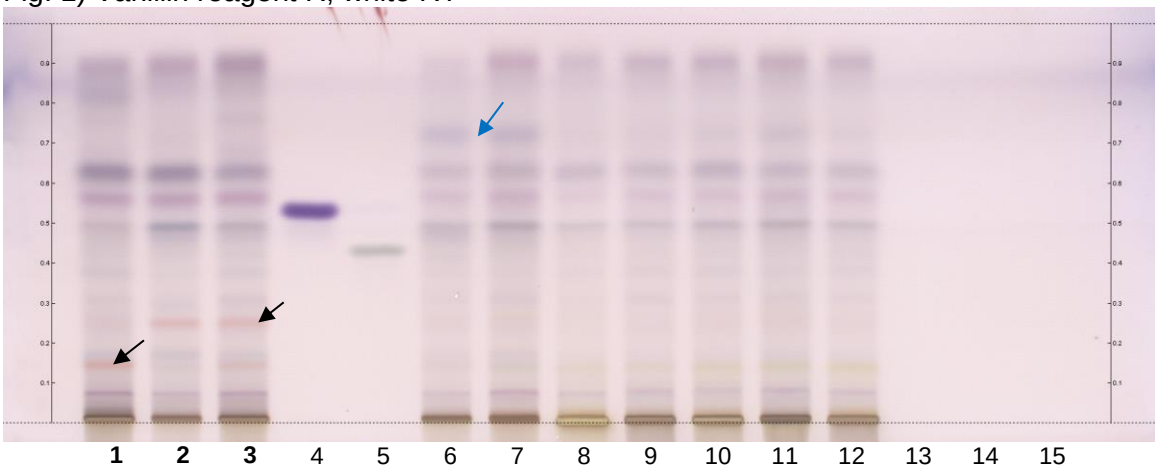
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 4.5 mg of oleanolic acid in 10 mL of methanol. Dissolve 4.5 mg of hederagenin in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	10 µL of references, 10 µL of test solutions
Mobile phase:	Toluene, acetone, acetic acid 32:8:2 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Vanillin reagent R Preparation: Dissolve 2 g of vanillin in 200 mL of ethanol and add 2 mL of sulfuric acid (only fresh preparation) Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Vanillin reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Vanillin reagent R, white RT



Track	Volume	Sample	Track	Volume	Sample
1	**10 µL**	**Armand's clematis root (from Hong Kong)**	9	10 µL	*Clematis manshurica* root
2	**10 µL**	**Armand's clematis root (from Germany)**	10	10 µL	*Clematis manshurica* root
3	**10 µL**	**Armand's clematis root (from Germany)**	11	10 µL	*Clematis hexapetala* root
4	10 µL	Oleanolic acid	12	10 µL	*Clematis manshurica* root
5	10 µL	Hederagenin	13		Blank
6	10 µL	*Clematis montana* root	14		Blank
7	10 µL	*Clematis chinensis* root; Chinese clematis root	15		Blank
8	10 µL	Armand's clematis root			

System suitability test
Oleanolic acid: violet zone at Rf ~ 0.53
Hederagenin: weak grey zone at Rf ~ 0.43

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows one blue grey zone between the positions of reference substances oleanolic acid and hederagenin. Above the position of oleanolic acid there is a weak pink zone at Rf ~ 0.57 and a blue grey zone at Rf ~ 0.62. In the lower part of the chromatogram there is a violet zone at Rf ~ 0.07. Orange zones are located at Rf ~ 0.15 and 0.25 (black arrows).

Test for adulteration and other species
Clematis chinensis, C. montana, C. manshurica and C. hexapetala root do not show orange zones in the upper part of the chromatogram.

Coix lacryma-jobi var. ma-yuen (Job's tears seed (syn. Coix seed), yi yi ren)

1. Scope

This method identifies dried Job's tears seed (syn. Coix seed) (Coix lacryma-jobi L. var. ma-yuen (Roman.) Stapf.) by HPTLC fingerprint.

2. Source of method

CAMAG, under evaluation by Ph.Eur (method for the identification of fatty oils Ph.Eur)

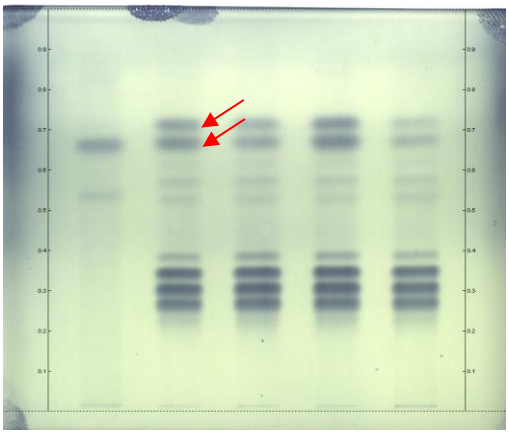
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of petroleum benzene (40-60°C) and sonicate for 10 minutes. Concentrate solution to 1 mL under vacuum. Dilute 1:10 with petroleum benzene (40-60°C).
Reference substances:	Dissolve 2 mg of oleic acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 RP-18 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Dichloromethane, acetic acid, acetone 20:40:50 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Phosphomolybdic acid reagent Preparation: 5 g of phosphomolybdic acid are dissolved in 200 mL of ethanol Use: Dip (time 0, speed 5), heat at 120°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Phosphomolybdic acid reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Phosphomolybdic acid reagent, white RT



1 2 3 4 5

Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Oleic acid	9		Blank
2	**2 µL**	**Job's tears seed 1**	10		Blank
3	2 µL	Job's tears seed 2	11		Blank
4	2 µL	Job's tears seed 3	12		Blank
5	2 µL	Job's tears seed 4	13		Blank
6		Blank	14		Blank
7		Blank	15		Blank
8		Blank			

System suitability test
Oleic acid: blue zone at Rf ~ 0.66.
Blue zone at Rf ~ 0.72.

Identification
Compare result with reference images in. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a blue zone at Rf ~ 0.66 corresponding to reference oleic acid and right above it another blue zone (red arrows). Two weak blue zones are seen between Rf ~ 0.50 and 0.60. A cluster of four blue zones is detected between Rf ~ 0.25 and 0.40.

Curcuma longa (Turmeric)

1. Scope

This method identifies Turmeric, the dried rhizome of Curcuma longa L. (syn. Curcuma domestica Val.) by HPTLC fingerprint and discriminates the dried rhizome of Curcuma xanthorrhiza Roxb.

2. Source of method

CAMAG, under evaluation by USP

3. Procedure

Sample preparation:	Mix 0.2 g of powdered sample with 3 mL of methanol, sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant/filtrate as test solution.
Reference substances:	Dissolve 2 mg of USP Curcuminoids RS in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references, 2 µL of test solutions
Mobile phase:	Toluene, acetic acid (4:1) (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate, white RT 2.) White RT 3.) UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) White RT

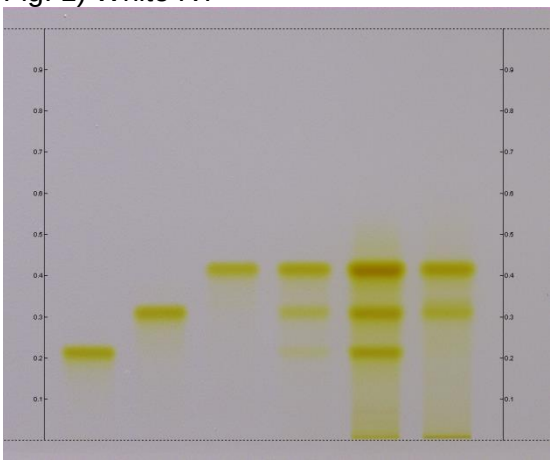
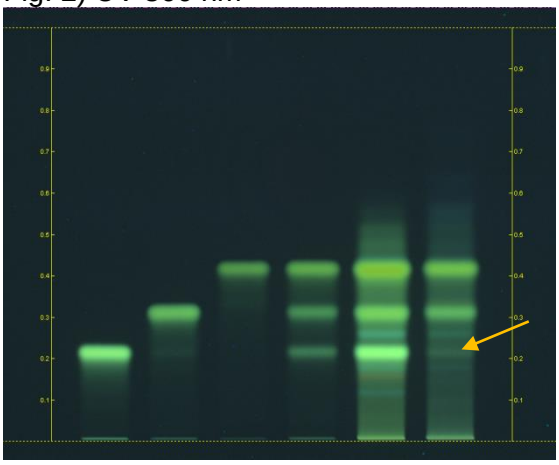


Fig. 2) UV 366 nm



Track	Volume	Sample
1	2 µL	Bisdesmethoxycurcumin
2	2 µL	Desmethoxycurcumin
3	2 µL	Curcumin
4	4 µL	Curcuminoids
5	**2 µL**	**Turmeric *(Curcuma longa)***
6	2 µL	*Curcuma xanthorrhiza*

System suitability test (UV 366 nm)
Curcuminoids: three yellowish-green zones at Rf ~ 0.21 (bisdesmethoxycurcumin), Rf ~ 0.32 (desmethoxycurcumin), and Rf ~ 0.42 (curcumin).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows three yellowish-green zones at Rf ~ 0.21, Rf ~ 0.32, and Rf ~ 0.42 corresponding to the three zones of the curcuminoids reference.

Test for other species
The chromatogram of Curcuma xanthorriza does not show an intense fluorescent zone at Rf ~ 0.21 (orange arrow).

Curcuma longa (Turmeric)
Test for adulteration with nimesulide

1. Scope

This method detects 1% adulteration of Turmeric supplements (Curcuma longa L.) with the synthetic anti-inflammatory drug nimesulide by HPTLC fingerprint.

2. Source of method

CAMAG, under evaluation by USP

3. Procedure

Sample preparation:	Mix 0.2 g of powdered sample with 3 mL of methanol, sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant/filtrate as test solution.
Reference substances:	Dissolve 1.0 mg of nimesulide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	10 µL of reference, 2 µL of test solution
Mobile phase:	Toluene, acetic acid (4:1) (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	2,5-Dichloro-1,4-benzoquinone reagent Preparation: 0.50 g of 2,5-Dichloro-1,4-benzoquinone is dissolved in 80 mL of dimethyl sulfoxide and then diluted with 160 mL of tetrahydrofuran. Use: Dip (time 0, speed 5), dry in a stream of cold air for 3 min.
Documentation:	1.) Clean plate, UV 254 nm 2.) Clean plate, white RT 3.) UV 254 nm 4.) 2,5-Dichloro-1,4-benzoquinone reagent, UV 366 nm 5.) 2,5-Dichloro-1,4-benzoquinone reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm

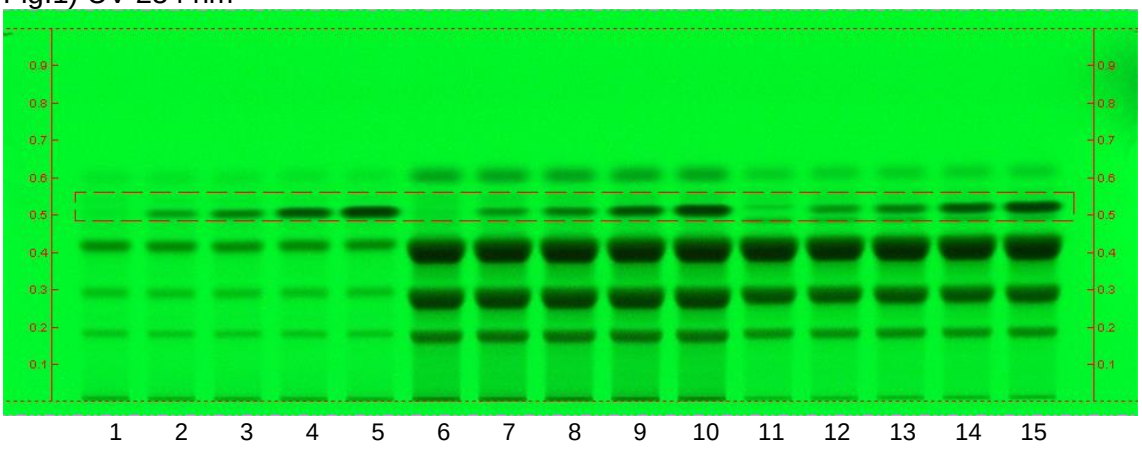


Fig.2) 2,5-Dichloro-1,4-benzoquinone reagent, UV 366 nm

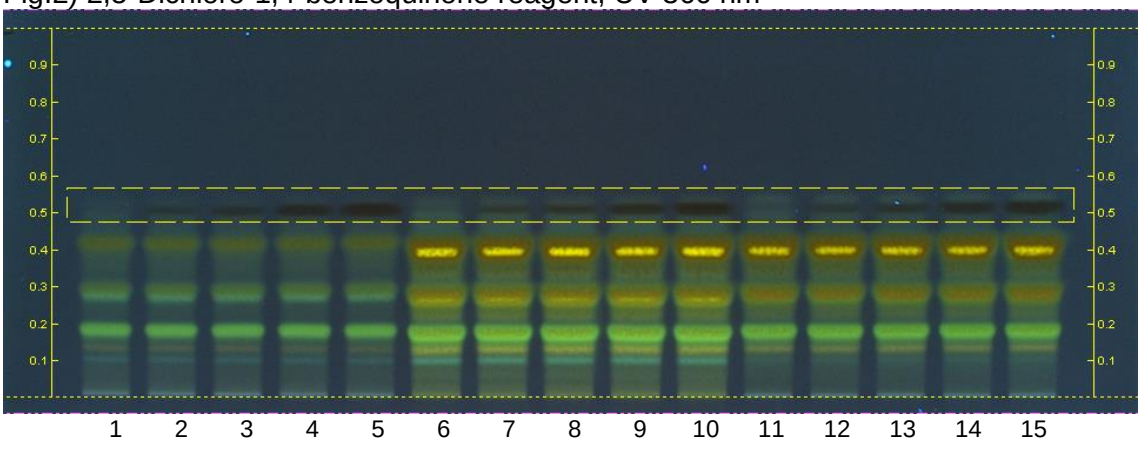
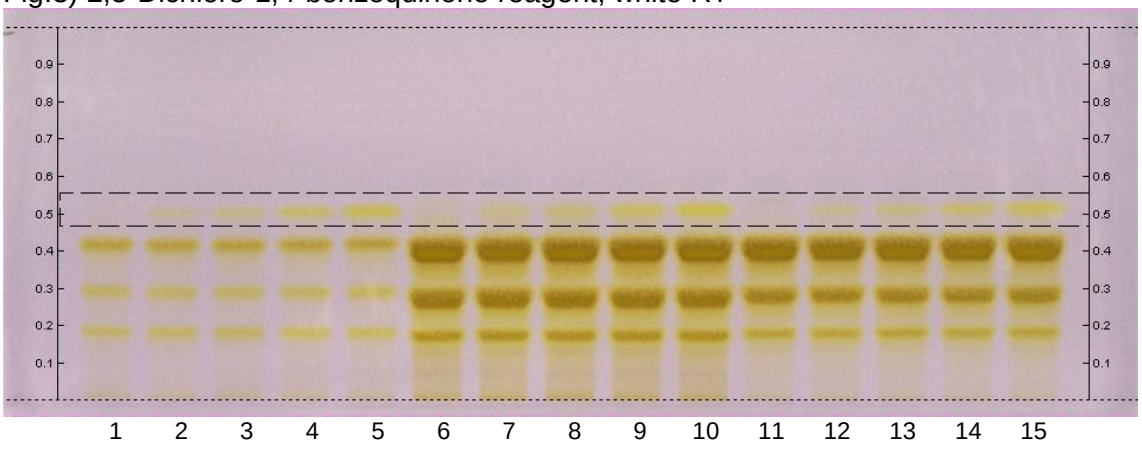


Fig.3) 2,5-Dichloro-1,4-benzoquinone reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Turmeric 1 (not spiked)	9	2 µL	Turmeric 2 (w/ 5% nimesulide)
2	2 µL	Turmeric 1 (spiked w/ 1% nimesulide)	10	2 µL	Turmeric 2 (w/ 10% nimesulide)
3	2 µL	Turmeric 1 (w/ 2% nimesulide)	11	2 µL	Turmeric 3 (not spiked)
4	2 µL	Turmeric 1 (w/ 5% nimesulide)	12	2 µL	Turmeric 3 (w/ 1% nimesulide)
5	2 µL	Turmeric 1 (w/ 10% nimesulide)	13	2 µL	Turmeric 3 (w/ 2% nimesulide)
6	2 µL	Turmeric 2 (not spiked)	14	2 µL	Turmeric 3 (w/ 5% nimesulide)
7	2 µL	Turmeric 2 (w/ 1% nimesulide)	15	2 µL	Turmeric 3 (w/ 10% nimesulide)
8	2 µL	Turmeric 2 (w/ 2% nimesulide)			

System suitability test
Nimesulide: Rf ~ 0.51

Test for adulteration
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows no zone at Rf ~ 0.51 corresponding in color and position to that of the nimesulide reference standard.

Drynaria fortune
Drynaria rhizome, gu sui bu

1. Scope

This method identifies dried Drynaria rhizome (*Drynaria fortunei* (Kunze) J.Sm.) by HPTLC fingerprint and discriminates the dried rhizomes of the other species *Humata tyermanni* Moore, *Drynaria bonii*, *Drynaria rigidula*, and *Drynaria sparsisora.*

2. Source of method

CAMAG, under evaluation by Ph.Eur

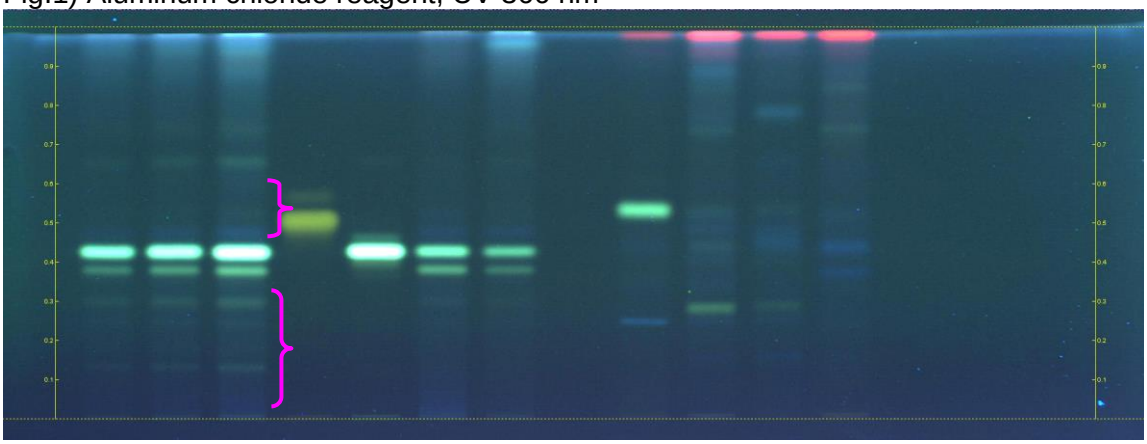
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of hyperoside in 1 mL of methanol. Dissolve 1 mg of naringin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 10 µL of test solutions
Mobile phase:	Ethyl acetate, acetic acid, formic acid, water 100:11:11:26 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Aluminum chloride reagent Preparation: Dissolve 4 g of aluminum chloride in 200 mL of a 5% solution (v/v) of glacial acetic acid in methanol. Use: dip (time 0, speed 5)
Documentation:	1.) Aluminum chloride reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Aluminum chloride reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Drynaria rhizome 1	9	10 µL	*Humata tyermanni* Moore rhizome
2	**10 µL**	**Drynaria rhizome 1**	10	10 µL	*Drynaria bonii* rhizome
3	15 µL	Drynaria rhizome 1	11	10 µL	*Drynaria rigidula* rhizome
4	5 µL	Hyperoside	12	10 µL	*Drynaria sparsisora* rhizome
5	5 µL	Naringin	13		Blank
6	10 µL	Drynaria rhizome 2	14		Blank
7	10 µL	Drynaria rhizome 3	15		Blank
8		Blank			

System suitability test

Hyperoside: yellow fluorescent zone at Rf ~ 0.50.
Naringin: blue white fluorescent zone at Rf ~ 0.42.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an intense greenish blue white zone at Rf ~ 0.42 corresponding to reference substance naringin. Right below this zone there is a less intense greenish blue white zone at Rf ~ 0.38.

Test for other species

No intense zone is seen between the application position and Rf ~ 0.30 (*Humata tyermanni* Moore, *Drynaria bonii, Drynaria rigidula, Drynaria sparsisora*) and no zone is seen at the position of reference substance hyperoside (*Humata tyermanni* Moore) (pink arrows).

Eclipta prostrate
(False daisy herb, mo han lian)

1. Scope

This method identifies dried False daisy herb (Eclipta prostrata L.) by HPTLC fingerprint and discriminates dried Great St. John's wort herb (Hypericum ascyron).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

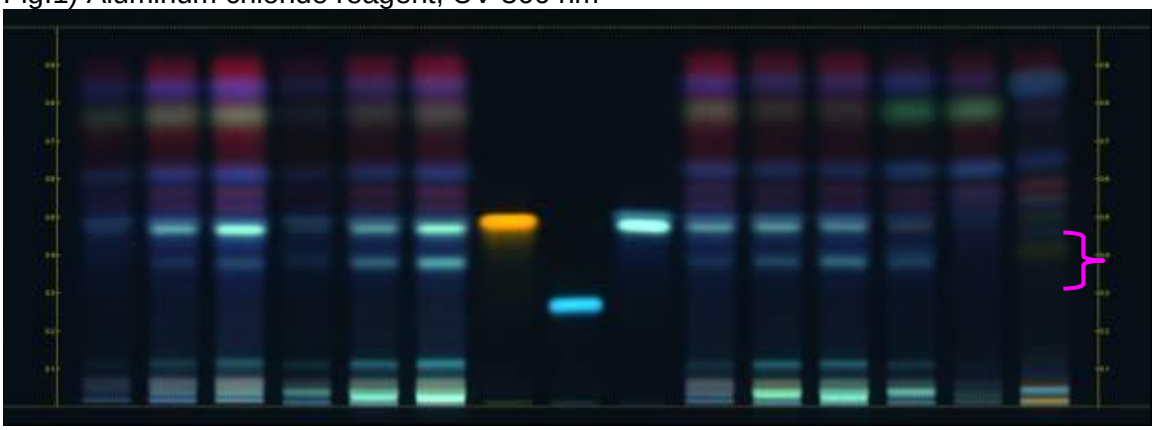
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes at 60°C, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of quercetin and 1 mg of rosmarinic acid in 1 mL of methanol. Optional: dissolve 1 mg of wedelolactone in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	3 µL of references, 5 µL of test solutions
Mobile phase:	Toluene, acetone, formic acid 11:6:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Aluminum chloride reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	False daisy herb 1	9	3 µL	Wedelolactone
2	**5 µL**	**False daisy herb 1**	**10**	**5 µL**	**False daisy herb 3**
3	7 µL	False daisy herb 1	**11**	**5 µL**	**False daisy herb 4**
4	2 µL	False daisy herb 2	**12**	**5 µL**	**False daisy herb 5**
5	**5 µL**	**False daisy herb 2**	**13**	**5 µL**	**False daisy herb 6**
6	7 µL	False daisy herb 2	14	5 µL	False daisy herb (questionable)
7	3 µL	Quercetin	15	5 µL	Great St. John's wort herb
8	3 µL	Rosmarinic acid			

System suitability test

Quercetin: orange fluorescent zone at Rf ~ 0.48.
Rosmarinic acid: blue fluorescent zone at Rf ~ 0.27.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows several blue white or greenish white zones just above the start position. There is a faint blue white zone below the position of reference quercetin and a blue white zone (wedelolactone) at the position of quercetin. Above it there is a pale blue zone. Above this zone, just below the solvent front, there is a pattern of three diffuse zones, a greenish, a blue violet and a red zone.

Test for adulteration

No blue white zones are seen at the position of quercetin and just below it (between Rf ~ 0.37-0.47) (purple arrow, Great John's wort herb).

Equisetum palustre
(Marsh horsetail herb)

1. Scope

This method identifies dried Marsh horsetail herb (Equisetum palustre L.) by HPTLC fingerprint and discriminates dried Common horsetail herb (Equisetum arvense L.).

2. Source of method

CAMAG

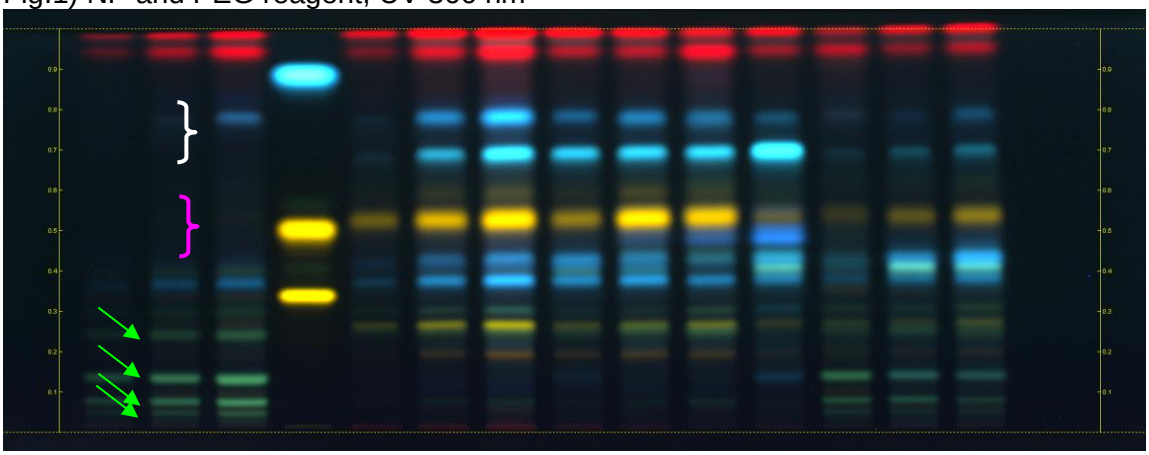
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Individually dissolve 1 mg of caffeic acid, 1 mg of rutin, and 1 mg of hyperoside each in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 5 µL of test solutions
Mobile phase:	Ethyl acetate, water, acetic acid, formic acid 134:36:15:15 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Marsh horsetail herb 1	9	5 µL	Common horsetail herb 3
2	**5 µL**	**Marsh horsetail herb 1**	10	5 µL	Common horsetail herb 4
3	8 µL	Marsh horsetail herb 1	11	5 µL	Common horsetail herb 5
4	1 µL	Rutin, hyperoside, caffeic acid (with increasing Rf)	12	5 µL	Common horsetail herb 6 (adulterated with Marsh horsetail herb)
5	2 µL	Common horsetail herb 1	13	5 µL	Common horsetail herb 2 (adulterated with Marsh horsetail herb)
6	5 µL	Common horsetail herb 1	14	5 µL	Common horsetail herb 3 (adulterated with Marsh horsetail herb)
7	8 µL	Common horsetail herb 1	15		Blank
8	5 µL	Common horsetail herb 2			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.34.
Hyperoside: orange fluorescent zone at Rf ~ 0.50.
Caffeic acid: light blue fluorescent zone at Rf ~ 0.88.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solutions shows four green zones between the application position and the zone due to reference substance rutin (green arrows). Right above the zone due to rutin there is a faint blue zone. Just below the solvent front there are two red zones due to chlorophylls.

Test for adulteration

There is no yellow zone at or right above the position of hyperoside (pink arrow); between hyperoside and caffeic acid there are not two intense blue zones (white arrow, Common horsetail herb).

Foeniculum vulgare ssp. vulgare var. dulce (Sweet Fennel fruit)

1. Scope

This method identifies dried Sweet Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. dulce) by HPTLC fingerprint and discriminates dried Bitter Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. vulgare), Anise fruit (Pimpinella anisum L.) and Caraway fruit (Carum carvi L.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

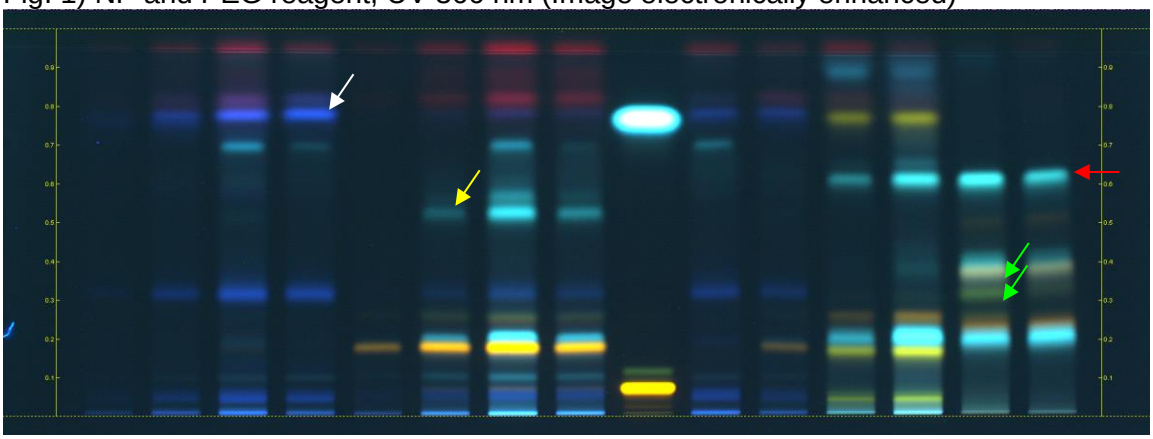
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3 mg of rutin in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 ml ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat the plate at 100°C for 5 min, dip (time 0, speed 5) while still hot, dry in air.
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm (Image electronically enhanced)



1 2 3 4 5 **6** 7 **8** 9 10 11 12 13 14 15

Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Bitter Fennel fruit 1	9	3 / 1µL	Rutin, Caffeic acid (with incr. Rf)
2	3 µL	Bitter Fennel fruit 1	10	3 µL	Wild Fennel fruit
3	6 µL	Bitter Fennel fruit 1	11	4 µL	Fennel tea
4	3 µL	Bitter Fennel fruit 2	12	3 µL	Anise fruit 1
5	1 µL	Sweet Fennel fruit 1	13	3 µL	Anise fruit 2
6	**3 µL**	**Sweet Fennel fruit 1**	14	3 µL	Caraway fruit 1
7	6 µL	Sweet Fennel fruit 1	15	3 µL	Caraway fruit 2
8	**3 µL**	**Sweet Fennel fruit 2**			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.07
Caffeic acid: light bluish fluorescent zone at Rf ~ 0.77.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a yellow zone at Rf ~ 0.17, a light blue zone at Rf ~ 0.20, a faint yellow zone at Rf ~ 0.26 and a faint blue zone at Rf ~ 0.32. A light blue zone is seen in the center part of the chromatogram at Rf ~ 0.53 (yellow arrow).

Test for adulteration

No intense dark blue zone is seen at Rf ~ 0.78 (white arrow, Bitter Fennel fruit). No light blue zone is seen at Rf ~ 0.62 (red arrow, Anise fruit or Caraway fruit). No brownish zones are seen at Rf ~ 0.32 and 0.38 (green arrows, Caraway fruit).

Foeniculum vulgare ssp. vulgare var. vulgare (Bitter Fennel fruit)

1. Scope

This method identifies dried Bitter Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. vulgare) by HPTLC fingerprint and discriminates dried Sweet Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. dulce), Anise fruit (Pimpinella anisum L.) and Caraway fruit (Carum carvi L.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

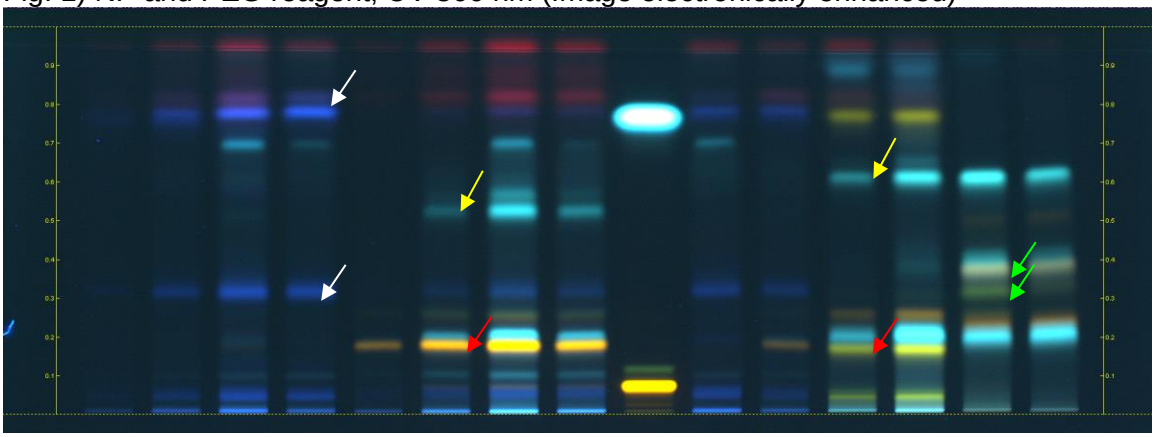
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3 mg of rutin in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 ml ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat the plate at 100°C for 5 min, dip (time 0, speed 5) in NP reagent while still hot, dry in air, dip in PEG reagent.
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm (Image electronically enhanced)



1 **2** 3 **4** 5 6 7 8 9 10 11 12 13 14 15

Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Bitter Fennel fruit 1	9	3 / 1µL	Rutin, caffeic acid (with incr. Rf)
2	**3 µL**	**Bitter Fennel fruit 1**	10	3 µL	Wild Fennel fruit
3	6 µL	Bitter Fennel fruit 1	11	4 µL	Fennel tea
4	**3 µL**	**Bitter Fennel fruit 2**	12	3 µL	Anise fruit 1
5	1 µL	Sweet Fennel fruit 1	13	3 µL	Anise fruit 2
6	3 µL	Sweet Fennel fruit 1	14	3 µL	Caraway fruit 1
7	6 µL	Sweet Fennel fruit 1	15	3 µL	Caraway fruit 2
8	3 µL	Sweet Fennel fruit 2			

System suitability test

Rutin: orange zone at Rf ~ 0.07
Caffeic acid: light bluish zone at Rf ~ 0.77

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a dark blue zone at Rf ~ 0.78 slightly above the position of reference caffeic acid and a dark blue zone at Rf ~ 0.31 (white arrows).

Test for other species

No yellow zone is seen at Rf ~ 0.17 (red arrows, Anise fruit, Sweet Fennel fruit). A light blue zone is neither seen at Rf ~ 0.53 (yellow arrow, Sweet Fennel fruit) nor at Rf ~ 0.62 (yellow arrow, Anise fruit, Caraway fruit). No brownish zones are seen at Rf ~ 0.32 and 0.38 (green arrows, Caraway fruit).

Fraxinus excelsior or Fraxinus oxyphylla (Ash leaf)

1. Scope

This method identifies dried Ash leaf (Fraxinus excelsior L. or Fraxinus oxyphylla M. Blieb) by HPTLC fingerprint.

2. Source of method

Modified from Ph. Eur. 6.7 (change in preparation of sample and reference substances)

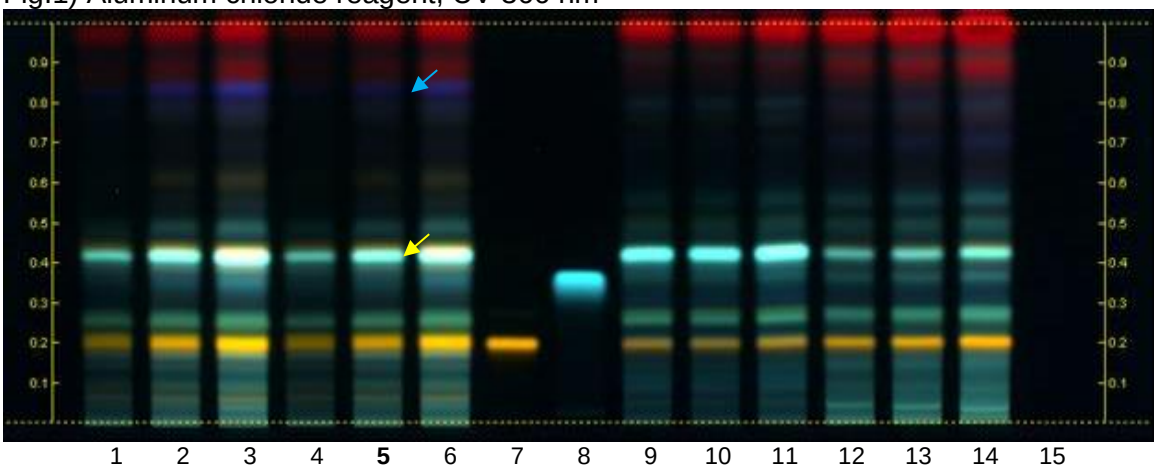
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3.5 mg of rutin in 5 mL of methanol. Dissolve 2.5 mg of chlorogenic acid in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 80:10:10 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat plate 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Aluminum chloride reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Ash leaf 1 (Ph. Eur. extraction)	9	8 µL	Ash leaf 2
2	4 µL	Ash leaf 1 (Ph. Eur. extraction)	10	10 µL	Ash leaf 2
3	6 µL	Ash leaf 1 (Ph. Eur. extraction)	11	12 µL	Ash leaf 2
4	2 µL	Ash leaf 1	12	6 µL	Ash leaf 3
5	**4 µL**	**Ash leaf 1**	13	8 µL	Ash leaf 3
6	6 µL	Ash leaf 1	14	10 µL	Ash leaf 3
7	2 µL	Rutin	15		Blank
8	2 µL	Chlorogenic acid			

System suitability test

Rutin: yellow fluorescent zone at Rf ~ 0.20
Chlorogenic acid: blue fluorescent zone at Rf ~ 0.36.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a yellow zone corresponding to reference rutin and a faint blue zone corresponding to chlorogenic acid. There is an intense bluish zone at Rf ~ 0.42 just above the zone corresponding to chlorogenic acid (yellow arrows). There is a faint dark blue zone at Rf ~ 0.83 (blue arrow).

Harpagophytum procumbens and/or Harpagophytum zeyheri (Devil's claw root)

1. Scope

This method identifies dried Devil's claw root (Harpagophytum procumbens DC. and/or Harpagophytum zeyheri Decne.) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 6.7 (change in detection)

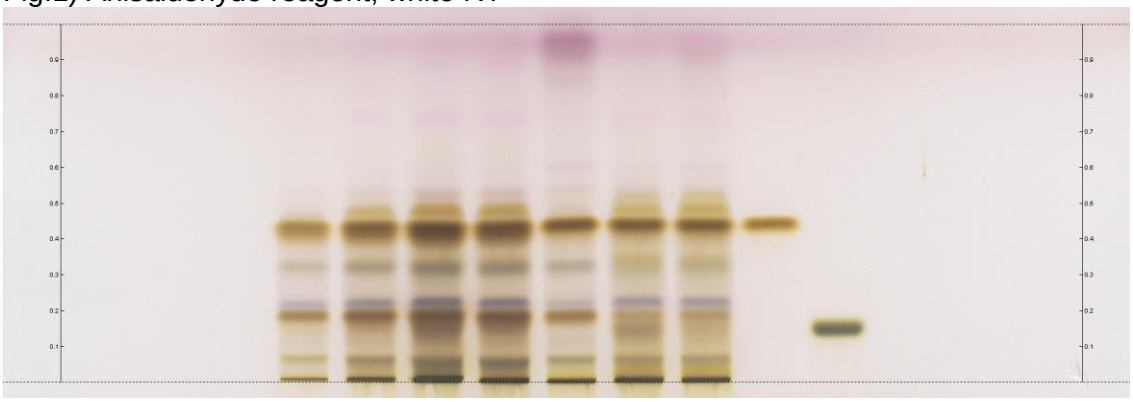
3. Procedure

Sample preparation:	Heat 1.0 g of the powdered drug with 10 mL of methanol, sonicate on a water-bath at 60°C for 10 min. Centrifuge and reduce the filtered solution to 2 mL under reduced pressure at a temperature not exceeding 40°C.
Reference substances:	Dissolve 1 mg of harpagoside in 1 mL of methanol. Dissolve 1 mg of fructose in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, methanol, water 77:15:8 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of glacial acetic acid, 10 mL of concentrated sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, white RT



1 2 3 4 **5** 6 7 8 9 10 11 12 13 14 15

Track	Volume	Sample	Track	Volume	Sample
1		Blank	9	2 µL	Devil's claw root 4 (old sample)
2		Blank	10	2 µL	Devil's claw root 5 (old sample)
3		Blank	11	5 µL	Harpagoside
4	1 µL	Devil's claw root 1	12	5 µL	Fructose
5	**2 µL**	**Devil's claw root 1**	13		Blank
6	4 µL	Devil's claw root 1	14		Blank
7	2 µL	Devil's claw root 2	15		Blank
8	2 µL	Devil's claw root 3 (old sample)			

System suitability test

Harpagoside: brown zone at Rf ~ 0.44.
Fructose: greenish zone at Rf ~ 0.14.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a brown zone (Rf ~ 0.06) below the zone due to reference substance fructose. At the position of fructose there may be a greenish zone (see track 9). Above fructose there is an intense brown zone at Rf ~ 0.18, just above it a blue zone at Rf ~ 0.22 and at Rf ~ 0.32 a weak brown zone. An intense brown zone corresponding to harpagoside is seen at Rf ~ 0.44.

Hibiscus sabdariffa
(Roselle flower)

1. Scope

This method identifies the whole or cut dried calyces and epicalyces of Roselle (Hibiscus sabdariffa L.) by HPTLC fingerprint.

2. Source of method

Ph.Eur. 6.7 (change in sample preparation).

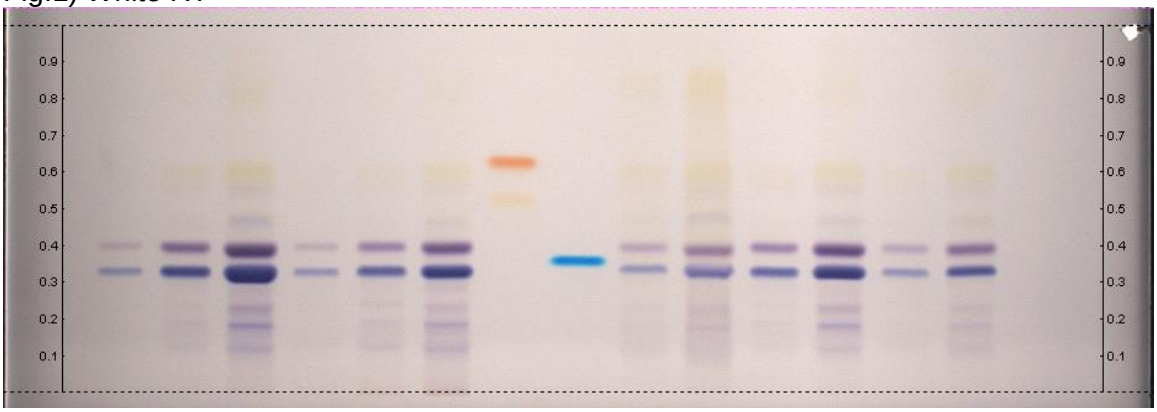
3. Procedure

Sample preparation:	Mix 1 g of powered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants /filtrates as test solutions.
Reference substances:	Dissolve 2.6 mg of patent blue V in 10 mL of ethanol. Dissolve 2.8 mg of quinaldine red in 10 mL of ethanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 6 µL of test solutions
Mobile phase:	1-Butanol, formic acid, water 40:10:12: (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	no derivatization
Documentation:	1.) Clean plate, white RT 2.) Developed plate, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) White RT



Track	Volume	Sample	Track	Volume	Sample
1	1.0 µL	Roselle flower 1	9	8.0 µL	Roselle flower 2
2	3.0 µL	Roselle flower 1	10	16.0 µL	Roselle flower 2
3	6.0 µL	Roselle flower 1	11	3.0 µL	Roselle flower 3
4	0.5 µL	Roselle flower 1 (Ph. Eur. extract.)	12	6.0 µL	Roselle flower 3
5	1.0 µL	Roselle flower 1 (Ph. Eur. extract.)	13	3.0 µL	Roselle flower 4
6	2.0 µL	Roselle flower 1 (Ph. Eur. extract.)	14	6.0 µL	Roselle flower 4
7	2.0 µL	Quinaldine red	15		Blank
8	2.0 µL	Patent blue V			

System suitability test

Patent blue V: blue zone at Rf ~ 0.36.
Quinaldine red: orange zone at Rf ~ 0.63.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The test solution shows two violet-blue zones at Rf ~ 0.33 and Rf ~ 0.40.

Hordeum vulgare
(Barley grass)

1. Scope

This method identifies dried Barley grass (Hordeum vulgare L.) by HPTLC fingerprint and detects dried Oat herb (Avena sativa L.) and dried Wheat grass (Triticum aestivum L.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

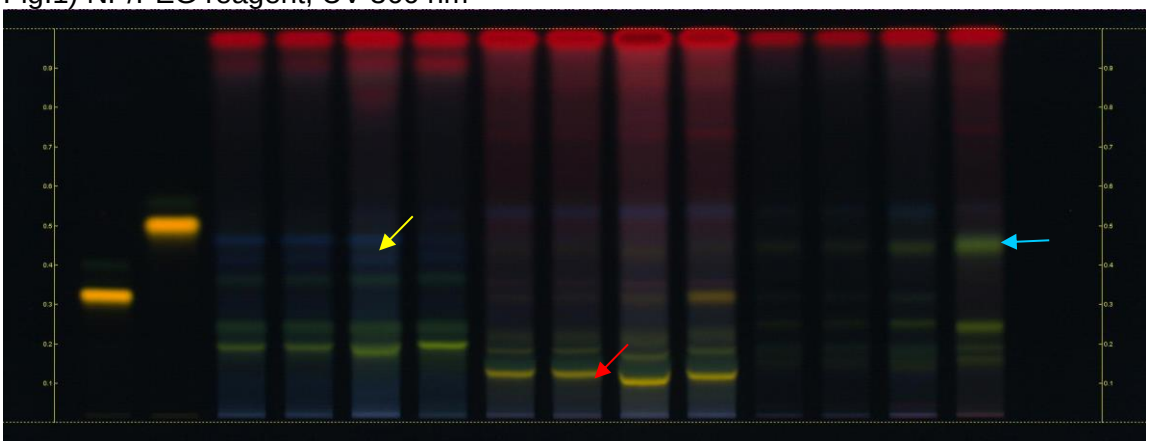
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of rutin in 1 mL of methanol. Dissolve 1 mg of hyperoside in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references, 10 µL of test solutions
Mobile phase:	Formic acid, water, methyl ethyl ketone, ethyl acetate 10:10:30:50 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP/PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Rutin	9	15 µL	Barley grass 1
2	4 µL	Hyperoside	**10**	**10 µL**	**Barley grass 2**
3	7 µL	Oat herb 1	11	7 µL	Wheat grass 1
4	10 µL	Oat herb 1	12	10 µL	Wheat grass 1
5	15 µL	Oat herb 1	13	15 µL	Wheat grass 1
6	10 µL	Oat herb 2	14	10 µL	Wheat grass 2
7	7 µL	Barley grass 1	15		Blank
8	**10 µL**	**Barley grass 1**			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.32
Hyperoside: orange fluorescent zone at Rf ~ 0.50

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a yellow zone at Rf ~ 0.18 (red arrow). Above it there are several faint orange zones. There may be an orange zone at the position of reference rutin. A prominent red zone is located close to the solvent front.

Test for adulteration

No blue or green zone is seen between the position of references rutin and hyperoside (Oat herb, yellow arrow). No green zone is seen at Rf ~ 0.44 (Wheat grass, blue arrow).

Isatis tinctoria
(Isatis root, ban lan gen)

1. Scope

This method identifies dried Isatis root (Isatis tinctoria L., syn. Isatis indigotica Fortune) by HPTLC fingerprint.

2. Source of method

Ph.Eur 7.3

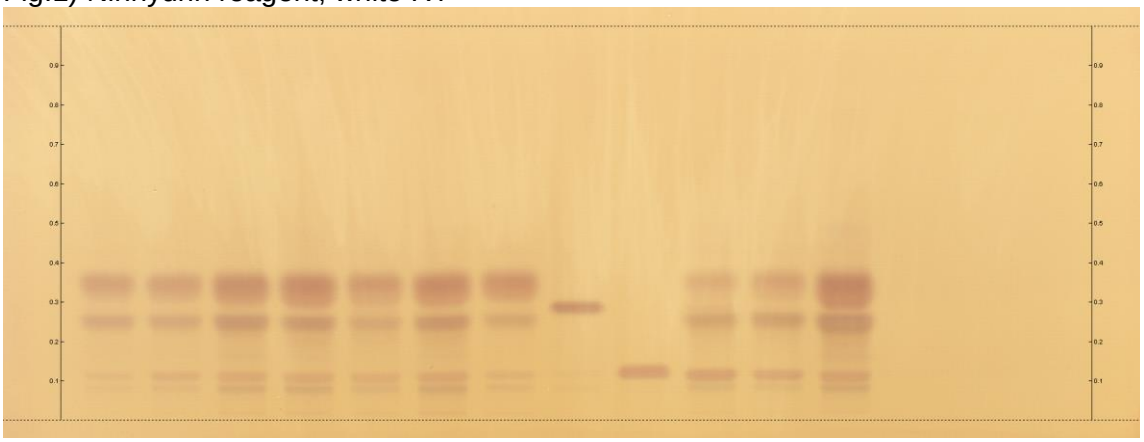
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of ethanol 70% and sonicate for 10 min, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 4 mg of L-arginine monohydrochloride in 1 mL of ethanol 70%. Dissolve 4 mg of L-cysteine hydrochloride monohydrate in 1 mL of ethanol 70%.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Acetonitrile, water, formic acid 30:8:2 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Before derivatization, treat plate with ammonia 25% vapor for 5 min. Ninyhdrin reagent Preparation: Dissolve 0.6 g of ninhydrin in 190 mL of isopropyl alcohol (2-propanol) and add 10 mL of glacial acetic acid. Use: Dip (time 0, speed 5), heat at 120°C for 3 min
Documentation:	1.) Clean plate, white RT 2.) Ninhydrin reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Ninhydrin reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Isatis root 1	9	2 µL	L-Arginine monohydrochloride
2	4 µL	Isatis root 2	10	4 µL	Isatis root 8
3	4 µL	**Isatis root 3**	11	4 µL	Isatis root 9
4	4 µL	Isatis root 4	12	4 µL	Isatis root 10
5	4 µL	Isatis root 5	13		Blank
6	4 µL	Isatis root 6	14		Blank
7	4 µL	Isatis root 7	15		Blank
8	2 µL	L-Cysteine HCl monohydrate			

Note: application volume of references is 2 µL instead of 4 µL

System suitability test

L-Cysteine hydrochloride monohydrate: a brown zone at Rf ~ 0.29.
L-Arginine monohydrochloride: a brown zone at Rf ~ 0.12.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a brown zone (Rf ~ 0.12) at the position of reference arginine. Right below it there is another faint brown zone. There is a brown zone at Rf ~ 0.25 right below the zone due to reference substance cysteine. Just above cysteine there is a prominent brown zone at Rf ~ 0.34.

Lavandula angustifolia (Lavender flower)

1. Scope

This method identifies dried Lavender flower (Lavandula angustifolia P. Mill.) by HPTLC fingerprint and discriminates Lavender oil (Lavandula angustifolia Mill.), Lavandin (Lavandula×intermedia Emeric ex Loisel, syn. Lavandula hybrida), and Spike lavender oil (Lavandula latifolia Medik).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references)

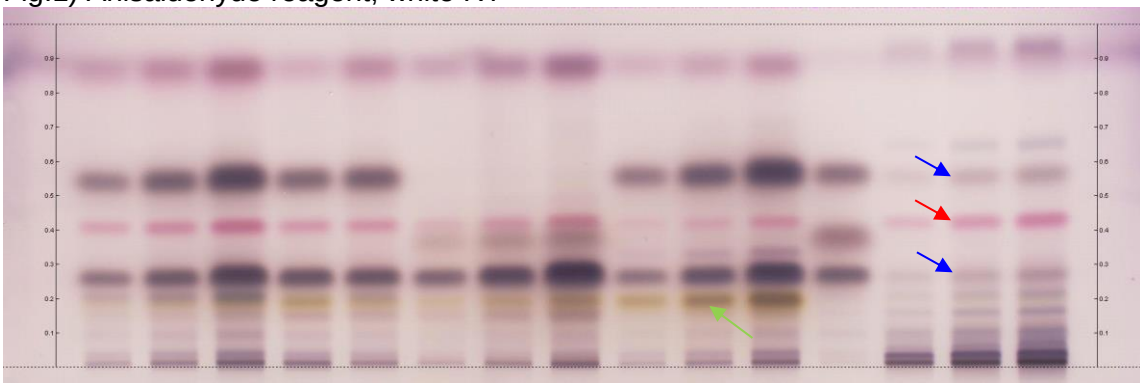
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of toluene and sonicate for 10 minutes, centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 µL of linalool in 1 mL of toluene. Dissolve 5 µL of linalyl acetate in 1mL of toluene. Optional: Dissolve 10 µL of cineole in 1 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 95:5 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Lavender oil 1	9	1 µL	Lavandin 1
2	2 µL	Lavender oil 1	10	2 µL	Lavandin 1
3	4 µL	Lavender oil 1	11	4 µL	Lavandin 1
4	2 µL	Lavender oil 2	12	2 µL	Linalool, Cineole, Linalyl acetate (with increasing Rf)
5	2 µL	Lavender oil 3	13	2 µL	Lavender flower 1
6	1 µL	Spike Lavender oil 1	**14**	**4 µL**	**Lavender flower 1**
7	2 µL	Spike Lavender oil 1	15	6 µL	Lavender flower 1
8	4 µL	Spike Lavender oil 1			

System suitability test
Linalyl acetate: violet zone at Rf ~ 0.57.
Linalool: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows a violet zone at Rf ~ 0.27 corresponding to reference linalool and a violet zone at Rf ~ 0.57 corresponding to linalyl acetate (blue arrows). Between these zones there is a reddish violet zone at Rf ~ 0.42 (red arrow). There is a grey zone at Rf ~ 0.66 above linalyl acetate.

Test for other species
No yellow zone is seen between the application position and reference linalool (green arrow) (Lavender oil, Lavandin, and Spike lavender oil).
Lavender oil, Lavandin, and Spike lavender oil all show a more intense violet zone at the position of linalool. Lavender oil and Lavandin also show a more intense violet zone at the position of linalyl acetate.

Lavandula angustifolia
(Lavender oil)

1. Scope

This method identifies Lavender essential oil (Lavandula angustifolia Mill.) by HPTLC fingerprint and discriminates Spike lavender oil (Lavandula latifolia Medik), Lavandin ((Lavandula ×intermedia Emeric ex Loisel, syn. Lavandula hybrida) and dried Lavender flower (Lavandula angustifolia P. Mill).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references)

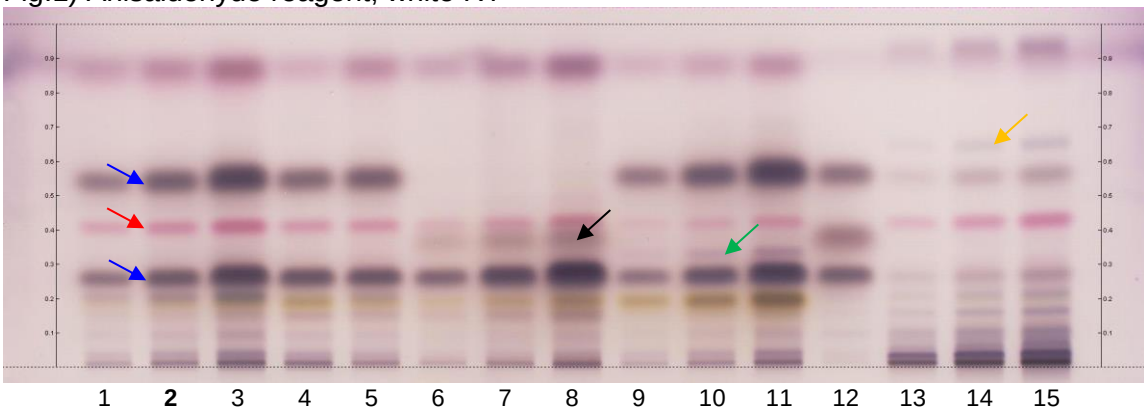
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of toluene and sonicate for 10 minutes, centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 µL of linalool in 1 mL of toluene. Dissolve 5 µL of linalyl acetate in 1mL of toluene. Optional: Dissolve 10 µL of cineole in 1 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 95:5 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Lavender oil 1	9	1 µL	Lavandin 1
2	**2 µL**	**Lavender oil 1**	10	2 µL	Lavandin 1
3	4 µL	Lavender oil 1	11	4 µL	Lavandin 1
4	2 µL	Lavender oil 2	12	2 µL	Linalool, Cineole, Linalyl acetate (with increasing Rf)
5	2 µL	Lavender oil 3	13	2 µL	Lavender flower 1
6	1 µL	Spike Lavender oil 1	14	4 µL	Lavender flower 1
7	2 µL	Spike Lavender oil 1	15	6 µL	Lavender flower 1
8	4 µL	Spike Lavender oil 1			

System suitability test
Linalyl acetate: violet zone at Rf ~ 0.57.
Linalool: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows an intense violet zone at Rf ~ 0.27 corresponding to reference linalool and an intense violet zone at Rf ~ 0.57 corresponding to linalyl acetate (blue arrows). Between these zones there is a violet-red zone at Rf ~ 0.42 (red arrow). Between the red zone and the zone due to linalool there may be a faint violet zone (cineole).

Test for other species
The chromatogram of Spike lavender oil does not show a zone at the position of linalyl acetate. There is a grey zone at the position of cineole (black arrow).
The chromatogram of Lavandin shows a narrow violet zone between linalool and cineole (green arrow).
Dried Lavender flower shows less intense violet zones due to linalool and linalyl acetate and an additional grey zone above linalyl acetate (orange arrow).

Lavandula latifolia
(Spike lavender oil)

1. Scope

This method identifies Spike lavender oil (Lavandula latifolia Medik) by HPTLC fingerprint and discriminates Lavender oil (Lavandula angustifolia Mill.), Lavandin (Lavandula×intermedia Emeric ex Loisel, syn. Lavandula hybrida) and dried Lavender flower (Lavandula angustifolia P. Mill).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references)

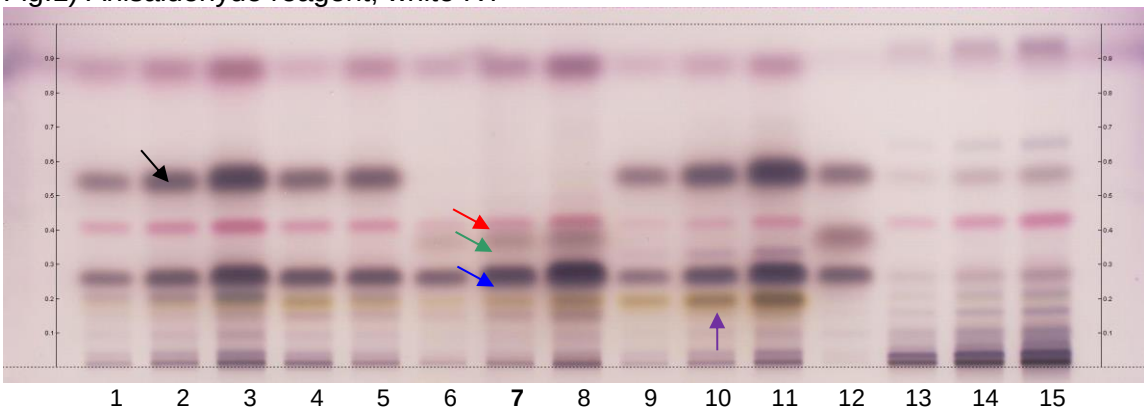
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of toluene and sonicate for 10 minutes, centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 µL of linalool in 1 mL of toluene. Dissolve 5 µL of linalyl acetate in 1mL of toluene. Optional: Dissolve 10 µL of cineole in 1 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 95:5 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Lavender oil 1	9	1 µL	Lavandin 1
2	2 µL	Lavender oil 1	10	2 µL	Lavandin 1
3	4 µL	Lavender oil 1	11	4 µL	Lavandin 1
4	2 µL	Lavender oil 2	12	2 µL	Linalool, Cineole, Linalyl acetate (with increasing Rf)
5	2 µL	Lavender oil 3	13	2 µL	Lavender flower 1
6	1 µL	Spike Lavender oil 1	14	4 µL	Lavender flower 1
7	**2 µL**	**Spike Lavender oil 1**	15	6 µL	Lavender flower 1
8	4 µL	Spike Lavender oil 1			

System suitability test
Linalyl acetate: violet zone at Rf ~ 0.57.
Linalool: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a violet zone at Rf ~ 0.27 corresponding to reference linalool (blue arrow) and a brownish zone due to cineole (green arrow). Above this zone there is a pink zone at Rf ~ 0.42 (red arrow). A faint violet zone at Rf ~ 0.57 corresponding to linalyl acetate may be present.

Test for other species
The chromatograms of Lavender oil and Lavanin show an intense violet zone at the position of linalyl acetate (black arrow). Lavender flower shows no yellow zone below the linalool reference (violet arrow).

Levisticum officinale
(Lovage root)

1. Scope

This method identifies dried Lovage root (Levisticum officinale W. Koch) by HPTLC fingerprint and detects the adulterants dried Chinese lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.), Angelica root (Angelica archangelica L.), Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.), and Chinese Angelica root (Angelica sinensis (Oliv.) Diels).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions **NOTE**: apply reference standards separately with method Reference standards ATS4/Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid with 180 mL methanol. Use: Dip for 1 s, heat at 100°C for 5 min
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) UV 366 nm 4.) Sulfuric acid, WRT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm

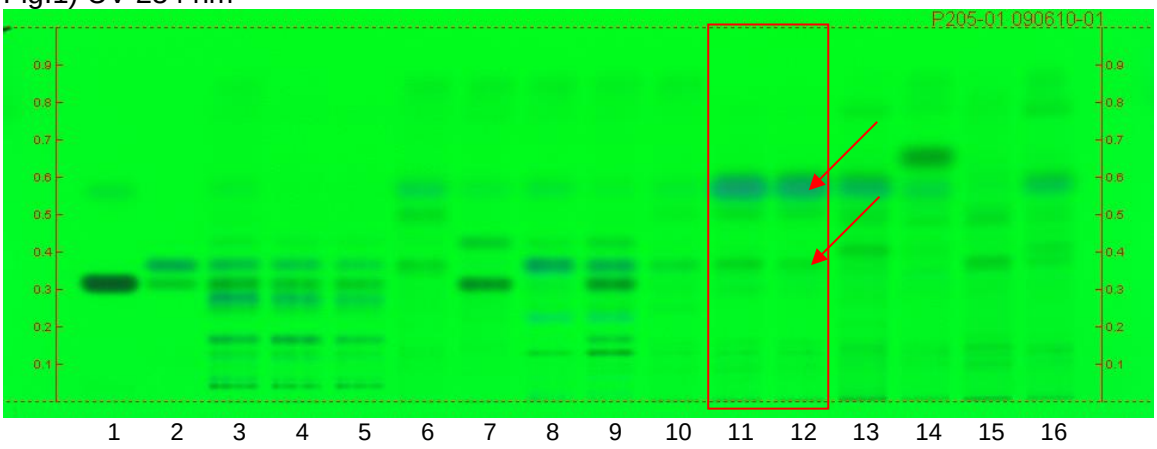


Fig.2) UV 366 nm

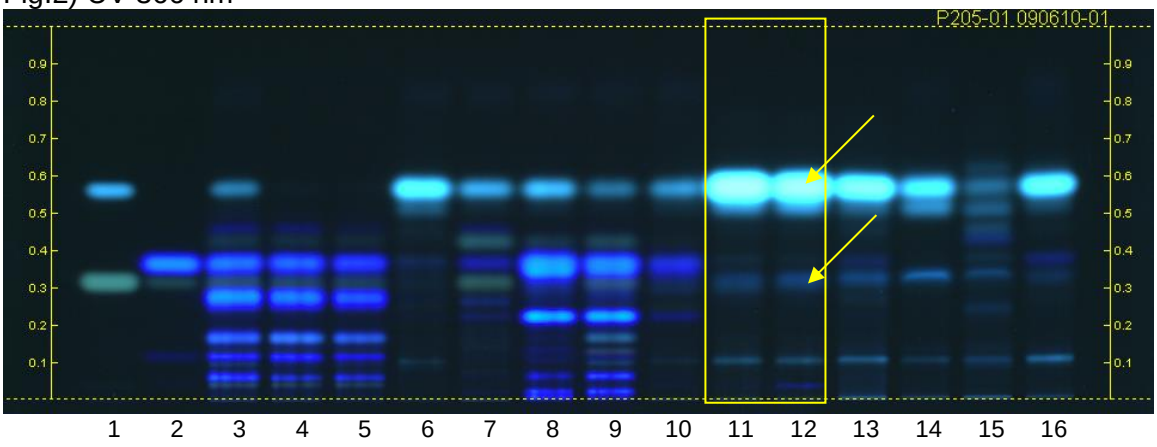
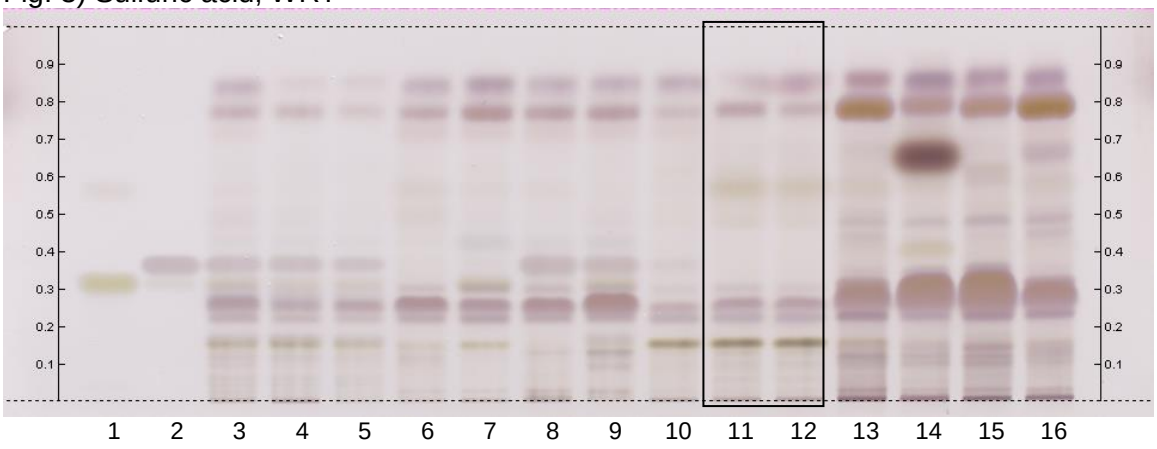


Fig. 3) Sulfuric acid, WRT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	9	4 µL	Doubleteeth pubescent Angelica root 2
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	**11**	**4 µL**	**Lovage root 2**
4	4 µL	Angelica root 2	**12**	**4 µL**	**Lovage root 3**
5	4 µL	Angelica root 3	13	4 µL	Chinese lovage root (*Ligusticum sinensis*)
6	4 µL	Chinese Angelica root	14	4 µL	Chinese lovage root 1 (*Ligusticum jeholense*)
7	4 µL	Dahurian Angelica root	15	4 µL	Chinese lovage root 2 (*Ligusticum jeholense*)
8	4 µL	Doubleteeth pubescent Angelica root 1	16	4 µL	Chinese lovage root (*Ligusticum chuanxiong*)

System suitability test
Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows an intense blue fluorescent zone at Rf ~ 0.57 and a weak quenching zone corresponding to reference osthole (Rf ~ 0.36) (red arrows).
Under UV 366 nm the chromatogram of the test solution shows an intense blue white fluorescent zone at Rf ~ 0.57 and a weak whitish fluorescent zone with similar Rf-value as reference imperatorin (yellow arrows).
After derivatization under white light there are two prominent purple zones below the solvent front. Below reference imperatorin there are two distinct purple zones and below them is an intense greenish zone.

Test for adulteration
Under UV 254 nm no zone is seen at or directly below the position of imperatorin. Under UV 366 nm there is no zone at the position of osthole. After derivatization under white light there are no zones at the position of imperatorin and osthole and just below the position of imperatorin there is no prominent diffuse purple zone (red arrow) (Angelica root, Chinese Angelica root, Doubleteeth pubescent root, Dahurian Angelica root, Chinese Lovage root).

Ligusticum sinense, Ligusticum jeholense (Chinese lovage root, gao ben)

1. Scope

This method identifies dried Chinese lovage root (Ligusticum sinense Oliv. or Ligusticum jeholense (Nakai & Kitag.) Nakai & Kitag.) by HPTLC fingerprint and detects the adulterants dried Lovage root (Levisticum officinale W. Koch), Angelica root (Angelica archangelica L.), Dahurian Angelica root (Angelica dahurica (Fisch. Ex Hoffm.) Benth. & Hook. F. ex Franch. & Sav.), Doubleteeth Pubescent Angelica root (Angelica pubescens Maxim.), and Chinese Angelica root (Angelica sinensis (Oliv.) Diels).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 4 mL of heptane and sonicate for 5 minutes, then centrifuge and filter the solutions and use the filtrates as test solutions.
Reference substances:	Dissolve 1 mg each of osthole and imperatorin in 10 mL of methanol. Optional: Dissolve 1 mg of Z-ligustilide in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references and of test solutions **NOTE**: apply reference standards separately with method Reference standards ATS4/Linomat5.cme
Mobile phase:	Toluene, ethyl acetate, glacial acetic acid 90:10:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid with 180 mL methanol. Use: Dip for 1 s, heat at 100°C for 5 min
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) UV 366 nm 4.) Sulfuric acid, WRT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm

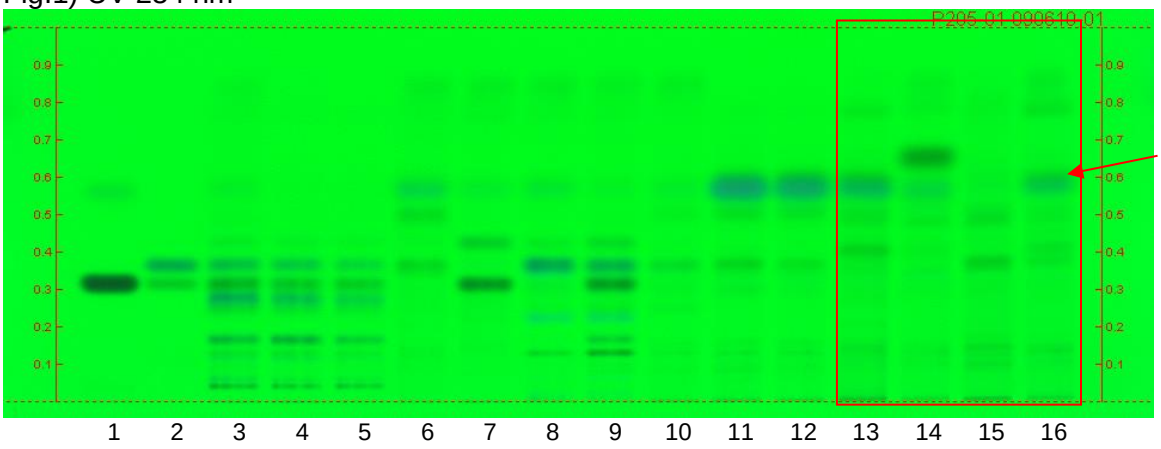


Fig.2) UV 366 nm

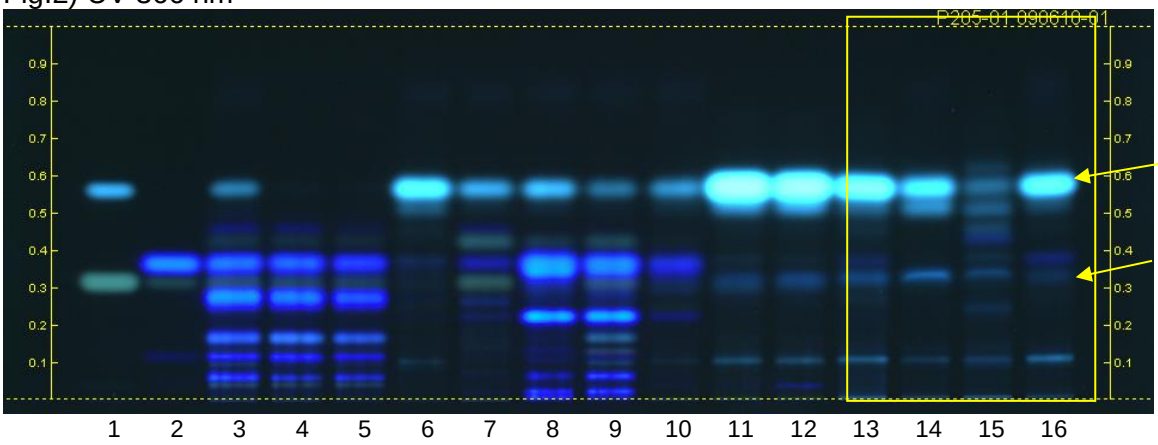
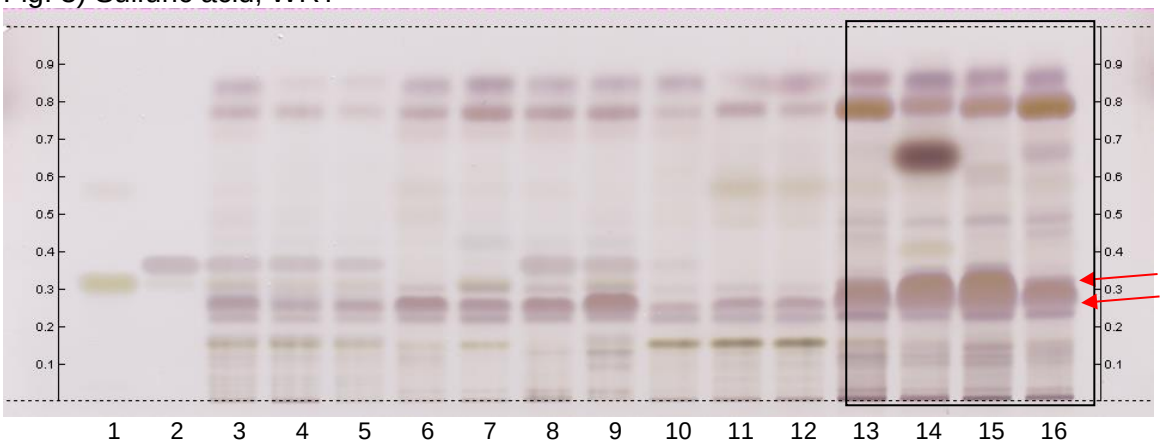


Fig. 3) Sulfuric acid, WRT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Imperatorin, z-Ligustilide (incr. Rf)	9	4 µL	Doubleteeth pubescent Angelica root 2
2	4 µL	Osthole	10	4 µL	Lovage root 1*
3	4 µL	Angelica root 1*	11	4 µL	Lovage root 2
4	4 µL	Angelica root 2	12	4 µL	Lovage root 3
5	4 µL	Angelica root 3	**13**	**4 µL**	**Chinese lovage root (*Ligusticum sinensis*)**
6	4 µL	Chinese Angelica root	**14**	**4 µL**	**Chinese lovage root 1 (*Ligusticum jeholense*)**
7	4 µL	Dahurian Angelica root	**15**	**4 µL**	**Chinese lovage root 2 (*Ligusticum jeholense*)**
8	4 µL	Doubleteeth pubescent Angelica root 1	**16**	**4 µL**	**Chinese lovage root (*Ligusticum chuanxiong*)**

System suitability test
Imperatorin: greenish fluorescent zone at Rf ~ 0.31 (UV 366 nm).
Osthole: blue fluorescent zone at Rf ~ 0.36 (UV 366 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. Under UV 254 nm the chromatogram of the test solution shows a weak blue fluorescent zone at Rf ~ 0.57 (red arrow). A weak quenching zone (Rf ~ 0.40) is seen above reference osthole. Under UV 366 nm the chromatogram of the test solution shows an intense light bluish fluorescent zone at Rf ~ 0.57 and a weak blue fluorescent zone with an Rf-value similar to reference imperatorin (yellow arrows).
After derivatization under white light there are two prominent purple zones below the solvent front. Below reference imperatorin there is a very prominent, diffuse purple zones and another distinct purple zone right below (red arrows).

Test for adulteration
Under UV 254 nm no zone is seen at or directly below the position of imperatorin. After derivatization under white light no zone is seen at the position of osthole and below the two prominent purple zones (red arrows) there is no intense greenish zone (Angelica root, Chinese Angelica root, Doubleteeth pubescent root, Dahurian Angelica root, Lovage root).

Malva sylvestris
(Mallow flower)

1. Scope

This method identifies the whole dried flower of mallow (Malva sylvestris L.) by HPTLC fingerprint and discriminates dried ripe Bilberry fruit (Vaccinium myrtillus L.) and dried Roselle flower (Hibiscus sabdariffa L.).

2. Source of method

CAMAG

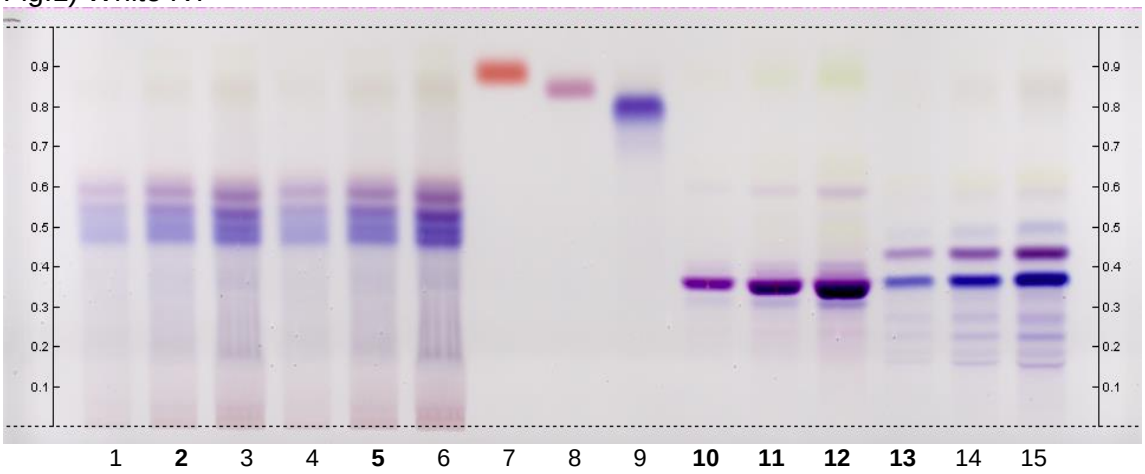
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2 mg of pelargonin in 5 mL of methanol. Dissolve 2 mg of delphinidin in 5 mL of methanol. Optional: dissolve 2 mg of malvidin in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	1-Butanol, formic acid, water 65:16:19 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	no derivatization
Documentation:	1.) Clean plate, white RT 2.) Developed plate, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) White RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Bilberry fruit (Ph.Eur. extraction)	9	2 µL	Delphinidin
2	3 µL	Bilberry fruit (Ph.Eur. extraction)	10	2 µL	Mallow flower
3	4 µL	Bilberry fruit (Ph.Eur. extraction)	**11**	**4 µL**	**Mallow flower**
4	2 µL	Bilberry fruit	12	6 µL	Mallow flower
5	3 µL	Bilberry fruit	13	2 µL	Roselle flower
6	4 µL	Bilberry fruit	14	4 µL	Roselle flower
7	2 µL	Pelargonin	15	6 µL	Roselle flower
8	2 µL	Malvidin			

System suitability test
Pelargonin: red to orange zone at Rf ~ 0.89.
Delphinidin: violet zone at Rf ~ 0.80.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows one intensive violet zone at Rf ~ 0.35.

Test for other species
No intense violet-blue zones are seen between Rf ~ 0.46 and 0.55 (Bilberry fruit) and no violet zone is present at Rf ~ 0.44 (Roselle flower).

Matricaria recutita (Chamomile flower)

1. Scope

This method identifies dried Chamomile flower (Matricaria recutita L.) by HPTLC fingerprint and discriminates the adulterants Chamomile flower oil (Matricaria recutita L.), dried Feverfew flower from Mexico (Tanacetum parthenium) dried Feverfew flower (Tanacetum parthenium (L.) Sch. Bip.) and dried Roman chamomile flower (Chamaemelum nobile (L.) All.).

2. Source of method

CAMAG Method of Analysis MOA009

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Dissolve 10 µL of the essential oil in 1 mL of toluene.
Reference substances:	Dissolve 1.5 mg of apigenin in 5 mL of methanol. Dissolve 1 mg of parthenolide in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, cyclohexane 1:1 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 4 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, UV 366 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, UV 366 nm

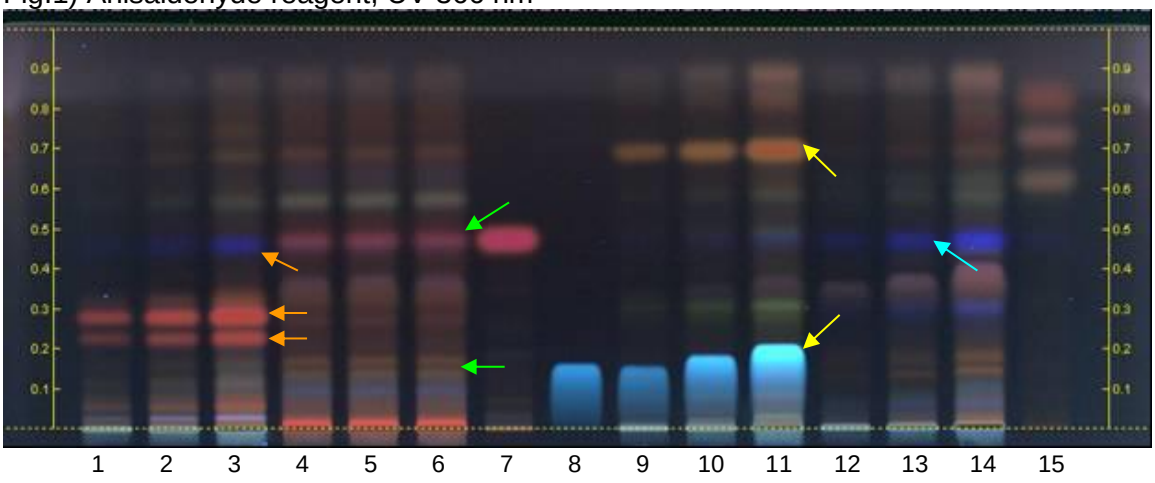
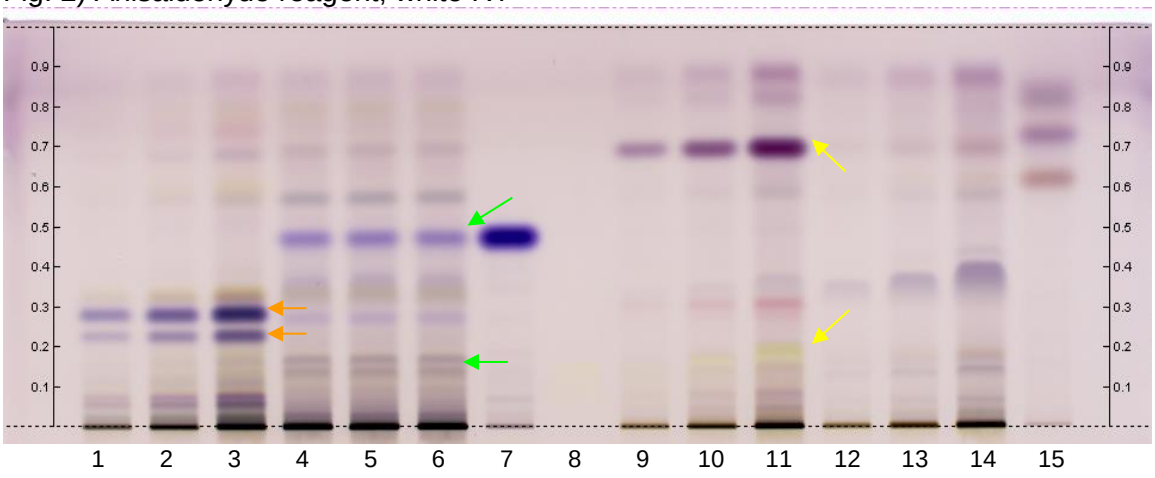


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Feverfew flower from Mexico	9	1 µL	Roman Chamomile flower
2	2 µL	Feverfew flower from Mexico	10	2 µL	Roman Chamomile flower
3	4 µL	Feverfew flower from Mexico	11	4 µL	Roman Chamomile flower
4	3 µL	Feverfew flower	12	1 µL	Chamomile flower
5	3.5 µL	Feverfew flower	**13**	**2 µL**	**Chamomile flower**
6	4 µL	Feverfew flower	14	4 µL	Chamomile flower
7	2 µL	Parthenolide	15	1 µL	Chamomile flower oil
8	2 µL	Apigenin			

System suitability test
Apigenin: blue zone at Rf ~ 0.20.
Parthenolide: pink zone at Rf ~ 0.48.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 366 nm the chromatogram of the test solution shows a dark blue zone at Rf ~ 0.46 (blue arrow; not visible under white light) a brown zone between Rf ~ 0.30 and 0.40 and another weak blue zone at Rf ~ 0.30. At the position of apigenin reference substance (Rf ~ 0.20) two brown zones are present (violet under white light).

Test for adulteration
Under UV 366 nm no pink zone (violet zone under white RT) at Rf ~ 0.48 corresponding to reference substance parthenolide is seen and there are no brown zones at the position of apigenin at Rf ~ 0.20 (green arrows, Feverfew flower).
Under UV 366 nm there are no intense red zones (violet zones under white RT) between Rf ~ 0.20 and 0.30. No blue zone is seen at the position of parthenolide (orange arrows; Feverfew flower from Mexico).
Under UV 366 nm no blue zone at the position of apigenin (Rf ~ 0.20) and no orange zone at Rf ~ 0.70 (purple under white light) is seen (yellow arrows; Roman Chamomile flower).
Chamomile flower oil does not show any zones below Rf ~ 0.60.

Melaleuca quinquenervia, Melaleuca viridiflora, Melaleuca leucadendra (Niaouli oil)

1. Scope

This method identifies Niaouli essential oil (Melaleuca quinquenervia (Cav.) S.T.Blake, Melaleuca viridiflora Sol. ex Gaertn. and Melaleuca leucadendra L.) by HPTLC fingerprint and discriminates tea tree oil (Melaleuca alternifolia), cajuput oil (Melaleuca leucadendron), kanuka oil (Kunzea ericoides), manuka oil (Leptospermum scoparium), eucalyptus oil (Eucalyptus globules), and neroli oil (Citrus aurantium var. amara).

2. Source of method

CAMAG

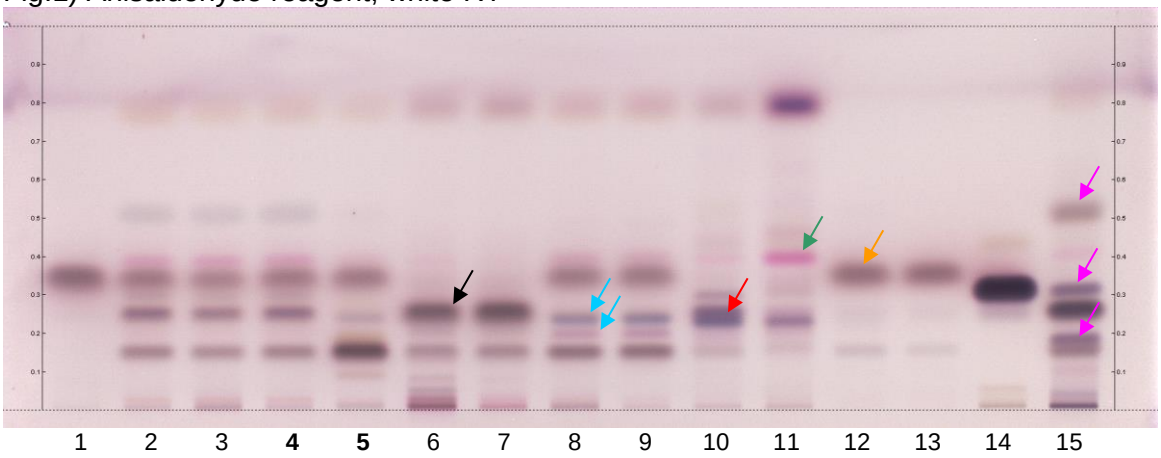
3. Procedure

Sample preparation:	Dissolve 100 mg of sample in toluene and dilute to 10 mL with toluene.
Reference substances:	Dissolve 50 µL of cineole and 25 µL of trans-nerolidol in toluene and dilute to 5.0 mL with toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, toluene 5:95 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2µL	Cineole	9	2µL	Cajuput oil 2
2	2µL	Niaouli oil CT Cineole 1	10	2µL	Kanuka oil
3	2µL	Niaouli oil CT Cineole 2	11	2µL	Manuka oil
4	**2µL**	**Niaouli oil CT Cineole 3**	12	2µL	Eucalyptus oil 1
5	**2µL**	**Niaouli oil**	13	2µL	Eucalyptus oil 2
6	2µL	Tea tree oil 1	14	2µL	Nerolidol
7	2µL	Tea tree oil 2	15	2µL	Neroli oil
8	2µL	Cajuput oil 1			

System suitability test
Cineole: brown zone at Rf ~ 0.35.
Nerolidol: violet zone at Rf ~ 0.32.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The test solution shows three brown zones between Rf ~ 0.16 and 0.35, the upper zone corresponding to reference substance cineole. Right above this zone there is a weak purple zone. A characteristic grey zone is seen at Rf ~ 0.51.

Test for other species
No broad brownish purple zone is seen at Rf ~ 0.26 (tea tree oil, black arrow).
No blue violet double zone is seen at Rf ~ 0.23 and 0.25 (cajuput oil, blue arrow).
No blue double zone is seen at Rf ~ 0.26 (kanuka oil, red arrow).
No intense purple zone is seen at Rf ~ 0.40 (manuka oil, green arrow).
Eucalyptus oil only shows one diffuse brown zone at the position of cineole (orange arrow).
No violet double zone at Rf ~ 0.19, no violet zone at the position of nerolidol and no brown zone at 0.50 is seen (Neroli oil, pink arrows).

Melissa officinalis
(Melissa leaf dry extract)

1. Scope

This method identifies the dry extract from Melissa leaf (Melissa officinalis L.) by HPTLC fingerprint and discriminates the dry extract from peppermint leaf (Mentha x piperita L.).

2. Source of method

Ph.Eur. 7.0

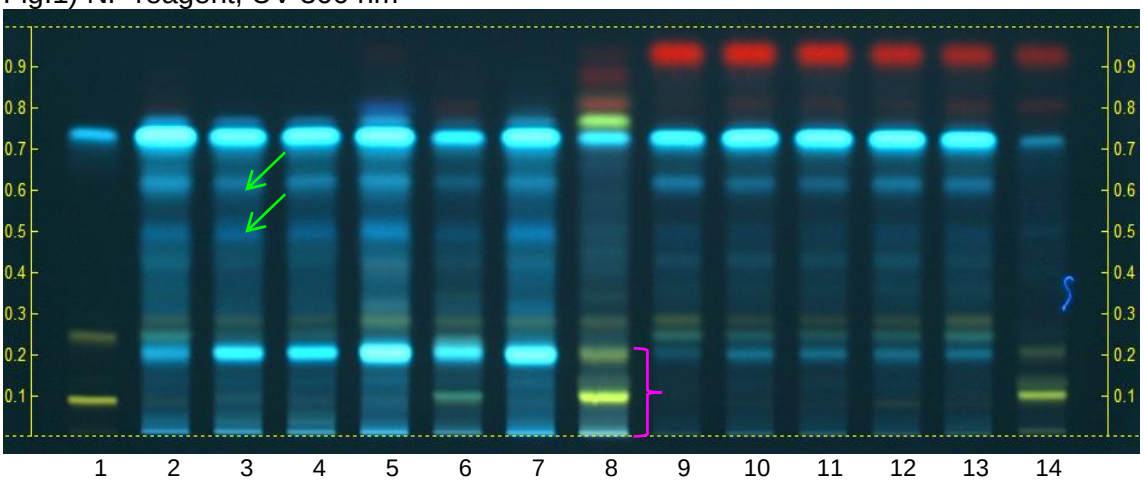
3. Procedure

Sample preparation:	Mix 0.2 g of extract with 5 mL of methanol and sonicate for 5 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Optional: Mix 0.5 g of dried Melissa leaf with 5 mL methanol and proceed as above
Reference substances:	Dissolve 1 mg of hyperoside, 1 mg of rutin and 5 mg of rosmarinic acid individually in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Formic acid, water, ethyl acetate 1:1:15 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent, dry in air
Documentation:	1.) NP reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Rutin, hyperoside, rosmarinic acid (with increasing Rf)	9	2 µL	Melissa leaf 1
2	2 µL	**Melissa leaf dry extract 1**	10	2 µL	Melissa leaf 2
3	2 µL	**Melissa leaf dry extract 2**	11	2 µL	Melissa leaf 3
4	2 µL	Melissa leaf dry extract 3	12	2 µL	Melissa leaf 4
5	2 µL	Melissa leaf dry extract 4	13	2 µL	Melissa leaf 5
6	2 µL	Melissa leaf dry extract 5 (adulterated with Peppermint leaf)	14	2 µL	Peppermint leaf
7	2 µL	Melissa leaf dry extract 6			
8	2 µL	Peppermint leaf dry extract			

System suitability test
Rutin: yellow fluorescent zone Rf ~ 0.09
Hyperoside: yellow fluorescent zone at Rf ~ 0.25
Rosmarinic acid: light blue fluorescent zone at Rf ~ 0.74

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a light blue fluorescent zone just below the position of reference hyperoside. At the position of rosmarinic acid there is an intense light blue fluorescent zone. Between this zone and reference hyperoside there are two bluoe fluorescent zones (green arrows).

Test for other species
No yellow zones are seen below the position of reference hyperoside (pink arrow, Peppermint dry extract).

Monarda didyma
(Oswego tea)

1. Scope

This method identifies whole or fragmented dried herb of Oswego tea (syn. Scarlet beebalm) (Monarda didyma L.) and discriminates mixtures with flowers of the same plant by HPTLC fingerprint.

2. Source of method

ZHAW, under evaluation by Ph.Eur

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Individually dissolve 2 mg of rutin and 2 mg of hyperoside each in 10 mL of methanol. Optional: Individually dissolve 4 mg of quercetin, 1.5 mg of chlorogenic acid, 10 mg of naringin, 10 mg of naringenin and 10 mg of narirutin each in 10 mL of methanol. Dissolve 2 mg of rosmarinic acid in 10 mL of ethanol 50% (v/v).
Application:	2 µL of references (except rosmarinic acid 5 µL), 4 µL of test solutions
Mobile phase:	Anhydrous formic acid, water, methyl ethyl ketone, ethyl acetate 10:10:30:50 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP reagent and PEG reagent, UV 366 nm 2.) NP reagent and PEG reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent and PEG reagent, UV 366 nm

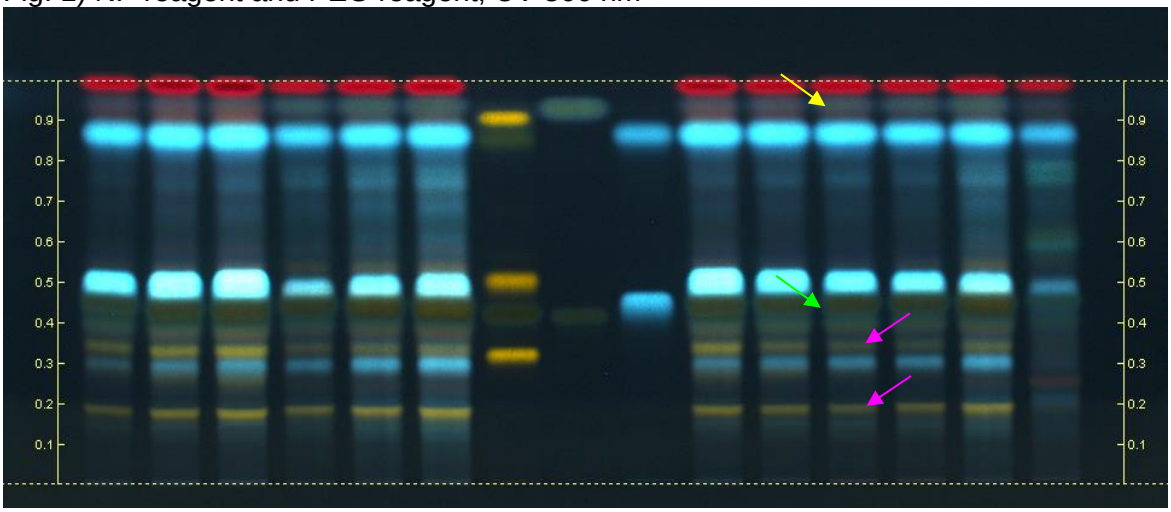
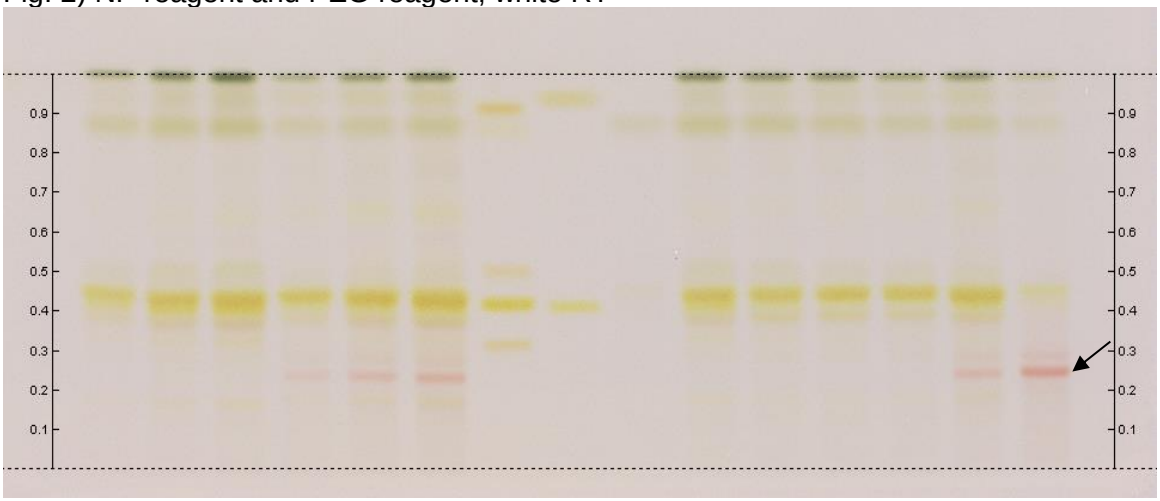


Fig. 2) NP reagent and PEG reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Oswego tea; herb 1	9	2 / 5 µL	Chlorogenic acid, rosmarinic acid (with increasing Rf)
2	4 µL	Oswego tea; herb 1	10	4 µL	Oswego tea; herb 1
3	6 µL	Oswego tea; herb 1	11	4 µL	Oswego tea; herb 2
4	2 µL	Oswego tea; herb with flower	**12**	**4 µL**	**Oswego tea; herb 3**
5	4 µL	Oswego tea; herb with flower	13	4 µL	Oswego tea; herb 4
6	6 µL	Oswego tea; herb with flower	14	4 µL	Oswego tea; herb with flower
7	2 µL	Rutin, narirutin, hyperoside, quercetin (with increasing Rf)	15	4 µL	Oswego tea; flower
8	2 µL	Naringin, naringenin (with increasing Rf)			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.32 (UV 366 nm).
Hyperoside: orange fluorescent zone at Rf ~ 0.50 (UV 366 nm).

Identification
Compare result under UV 366 nm with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an intense light blue zone at Rf ~ 0.50 corresponding in position to reference hyperosid and at Rf ~ 0.87 corresponding to reference rosmarinic acid. Faint brownish-yellow zones are seen slightly above the position of rutin and at Rf ~ 0.18 (pink arrows). A greenish grey zone is seen at Rf ~ 0.46 corresponding to the position of reference naringin. Close to the solvent front a red zone is seen. Just below it there is greenish grey zone corresponding to reference substance naringenin (yellow arrow).

Test for other species
Under white RT no red zone is seen at Rf~ 0.25 (black arrow, Oswego tea flower).

Paeonia lactiflora
(White paeony root, bai shao)

1. Scope

This method identifies dried peeled root of White paeony (Paeonia lactiflora Pall.) by HPTLC fingerprint and discriminates Red paeony root (Paeonia lactiflora Pall. or Peaonia veitchii Lynch) and Tree paeony root bark (Paeonia suffruticosa Andr., syn. Paeonia moutan Sims).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

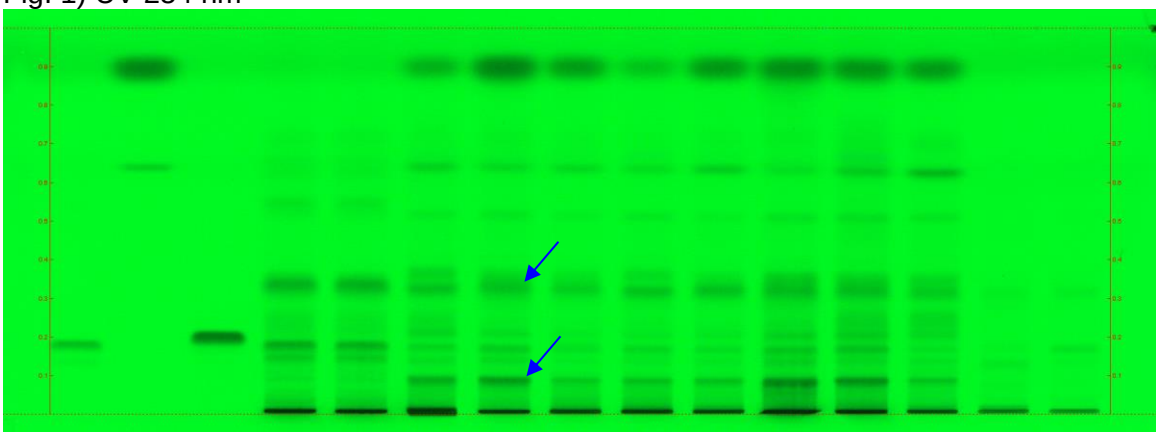
3. Procedure

Sample preparation:	Mix 0.5 g of powdered sample with 5 mL of methanol and heat on a water bath at 60°C for 1 minute, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of paeoniflorin and 1 mg of paeonol in 1 mL of methanol. Optional: Dissolve 1 mg of harpagoside and 1 mg of cholesterol in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	8 µL of references, 8 µL of test solutions
Mobile phase:	Methanol, ethyl acetate, formic acid, dichloromethane 5:5:3:35 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid in 180 mL methanol Use: The plate is immersed in sulfuric acid reagent for 1s, then heated for 5 min at 100°C
Documentation:	1.) Clean plate (white RT) 2.) UV 254 nm 3.) Sulfuric acid reagent, white RT

4. Results

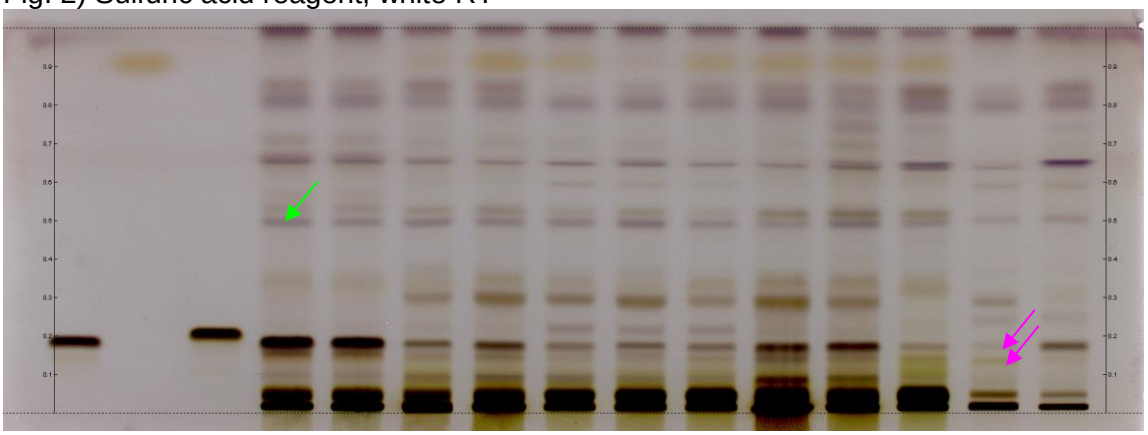
Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm



1 2 3 4 5 6 7 8 9 10 11 12 13 **14** 15

Fig. 2) Sulfuric acid reagent, white RT



1 2 3 4 5 6 7 8 9 10 11 12 13 **14** 15

Track	Volume	Sample	Track	Volume	Sample
1	8 µL	Paeoniflorin	9	8 µL	Tree paeony root bark 4
2	8 µL	Paeonol	10	8 µL	Tree paeony root bark 5
3	8 µL	Harpagoside	11	8 µL	Tree paeony root bark 6
4	8 µL	Red paeony root 1	12	8 µL	Tree paeony root bark 7
5	8 µL	Red paeony root 2	13	8 µL	Tree paeony root bark 8
6	8 µL	Tree paeony root bark 1	**14**	**10 µL**	**White paeony root 1**
7	8 µL	Tree paeony root bark 2	15	10 µL	White paeony root 2
8	8 µL	Tree paeony root bark 3			

System suitability test
Paeoniflorin: brown zone at Rf ~ 0.19 (white RT).
Paeonol: weak orange zone at Rf ~ 0.91 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows two weak quenching zones at Rf ~ 0.17 and 0.31.
Under white RT there is a weak brown zone at Rf ~ 0.17 and at Rf ~ 0.19 (pink arrows). Above it there are two brown zones, one at Rf ~ 0.24 and one at Rf ~ 0.29. There is a single brown zone at Rf ~ 0.50, a weak brown zone at Rf ~ 0.59 and a distinct violet zone at Rf ~0.65. Below the solvent front there are two diffuse brown zones and a third one right at the solvent front.

Test for adulteration and other species
Under UV 254 nm no (or only a very weak) zone is seen at Rf ~ 0.09 and at Rf ~ 0.30 (Tree peony root bark, blue arrows).
Under white RT no double but only a single zone is seen at Rf ~ 0.50 and no zone is seen at Rf ~ 0.53 (Red paeony root, green arrow).

Paeonia lactiflora or Paeonia veitchii (Red paeony root, chi shao)

1. Scope

This method identifies dried root of Red paeony (Paeonia lactiflora Pall. or Paeonia veitchii Lynch) by HPTLC fingerprint and discriminates White peony root (peeled root of Paeonia lactiflora Pall.) and Tree peony root bark (Paeonia suffruticosa Andr., syn. Paeonia moutan Sims).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

3. Procedure

Sample preparation:	Mix 0.5 g of powdered sample with 5 mL of methanol and heat on a water bath at 60°C for 1 minute, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of paeoniflorin and 1 mg of paeonol in 1 mL of methanol. Optional: Dissolve 1 mg of harpagoside and 1 mg of cholesterol in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	8 µL of references, 8 µL of test solutions
Mobile phase:	Methanol, ethyl acetate, formic acid, dichloromethane 5:5:3:35 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid in 180 mL methanol Use: The plate is immersed in sulfuric acid reagent for 1s, then heated for 5 min at 100°C
Documentation:	1.) Clean plate (white RT) 2.) UV 254 nm 3.) Sulfuric acid reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

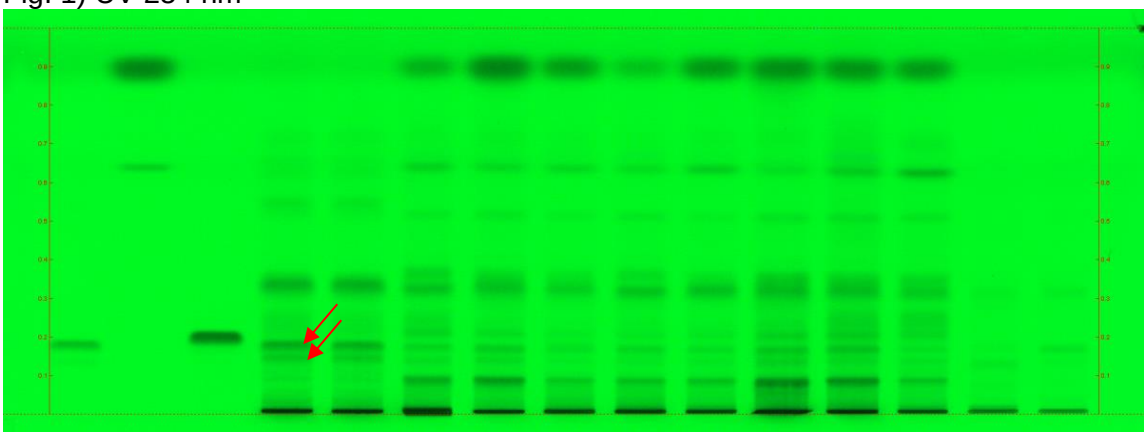
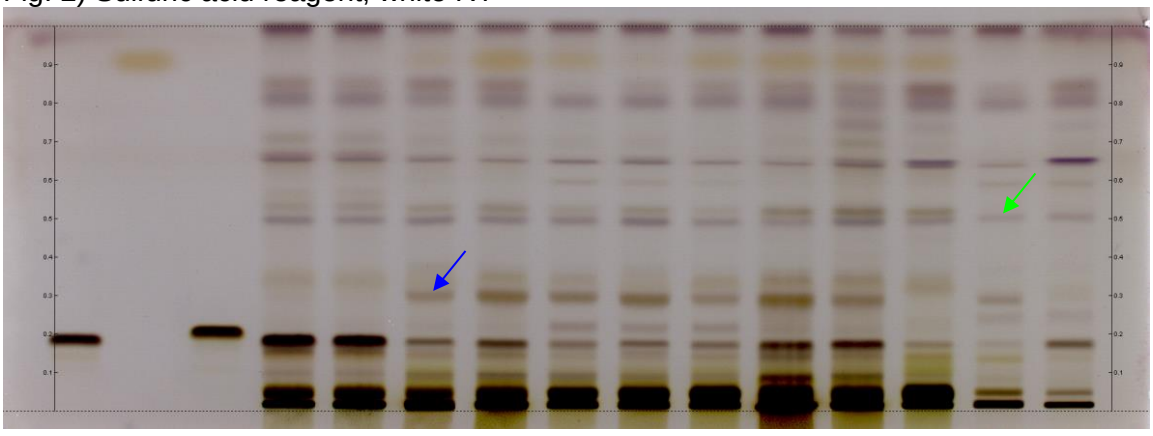


Fig. 2) Sulfuric acid reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	8 µL	Paeoniflorin	9	8 µL	Tree paeony root bark 4
2	8 µL	Paeonol	10	8 µL	Tree paeony root bark 5
3	8 µL	Harpagoside	11	8 µL	Tree paeony root bark 6
4	**8 µL**	**Red paeony root 1**	12	8 µL	Tree paeony root bark 7
5	8 µL	Red paeony root 2	13	8 µL	Tree paeony root bark 8
6	8 µL	Tree paeony root bark 1	14	10 µL	White paeony root 1
7	8 µL	Tree paeony root bark 2	15	10 µL	White paeony root 2
8	8 µL	Tree paeony root bark 3			

System suitability test
Paeoniflorin: brown zone at Rf ~ 0.19 (white RT).
Paeonol: weak orange zone at Rf ~ 0.91 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be

present.
Under UV 254 nm the test solution shows a quenching zone at Rf ~ 0.19 corresponding to reference substance paeoniflorin and right below it a weak quenching zone at Rf ~ 0.14 (red arrows). There is a quenching zone at Rf ~ 0.34.
Under white RT the test solution shows an intense brown zone at Rf ~ 0.19 corresponding to reference substance paeoniflorin. There is a violet zone at Rf ~ 0.50, a weak violet zone at Rf ~ 0.53 and an intense and distinct violet zone at Rf ~ 0.65, just above it there is a weak brown zone. Below the solvent front there are two diffuse brown zones and a third one right at the solvent front.

Test for adulteration and other species
Under white RT the test solution does neither show a brown zone at Rf ~ 0.30 (Tree peony root bark, blue arrow) nor only a single zone at Rf ~ 0.50 (White peony root, green arrow).

Paeonia suffruticosa
(Tree paeony root bark, mu dan pi)

1. Scope

This method identifies dried root bark of Tree paeony (Paeonia suffruticosa Andr., syn. Paeonia moutan Sims) by HPTLC fingerprint and discriminates White paeony root (peeled root of Paeonia lactiflora Pall.) and Red paeony root (Paeonia lactiflora Pall. or Paeonia veitchii Lynch).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

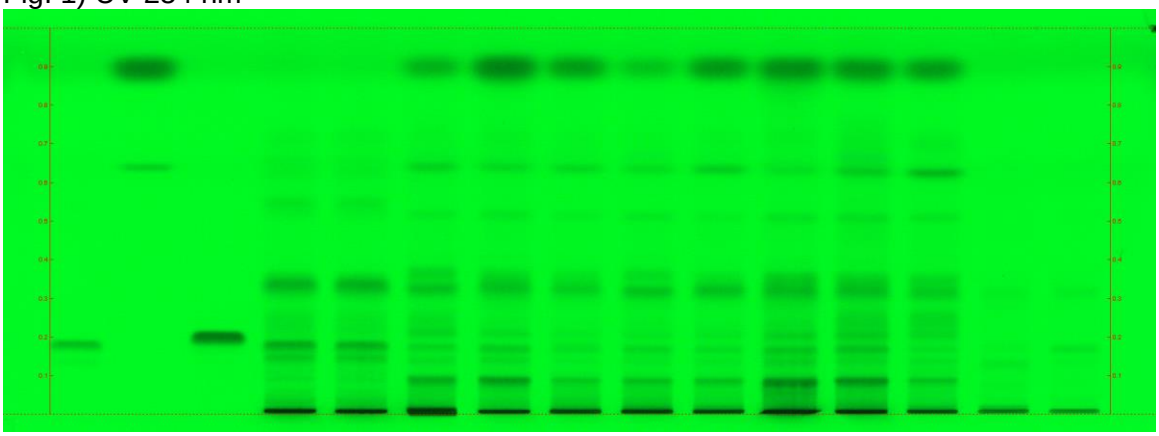
3. Procedure

Sample preparation:	Mix 0.5 g of powdered sample with 5 mL of methanol and heat on a water bath at 60°C for 1 minute, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of paeoniflorin and 1 mg of paeonol in 1 mL of methanol. Optional: Dissolve 1 mg of harpagoside and 1 mg of cholesterol in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	8 µL of references, 8 µL of test solutions
Mobile phase:	Methanol, ethyl acetate, formic acid, dichloromethane 5:5:3:35 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid in 180 mL methanol Use: : The plate is immersed in sulfuric acid reagent for 1s, then heated for 5 min at 100°C
Documentation:	1.) Clean plate (white RT) 2.) UV 254 nm 3.) Sulfuric acid reagent, white RT

4. Results

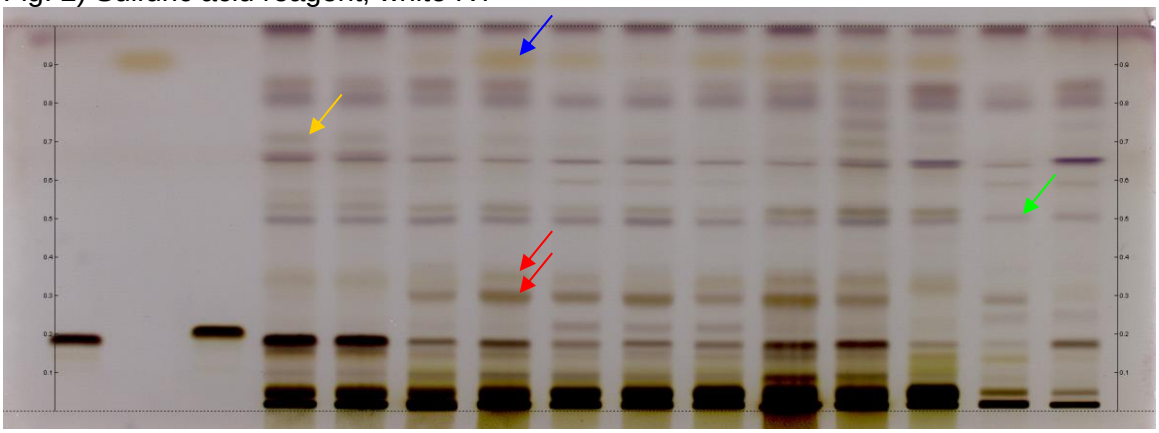
Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm



1 2 3 4 5 6 **7** 8 9 10 11 12 13 14 15

Fig. 2) Sulfuric acid reagent, white RT



1 2 3 4 5 6 **7** 8 9 10 11 12 13 14 15

Track	Volume	Sample	Track	Volume	Sample
1	8 µL	Paeoniflorin	9	8 µL	Tree paeony root bark 4
2	8 µL	Paeonol	10	8 µL	Tree paeony root bark 5
3	8 µL	Harpagoside	11	8 µL	Tree paeony root bark 6
4	8 µL	Red paeony root 1	12	8 µL	Tree paeony root bark 7
5	8 µL	Red paeony root 2	13	8 µL	Tree paeony root bark 8
6	8 µL	Tree paeony root bark 1	14	10 µL	White paeony root 1
7	**8 µL**	**Tree paeony root bark 2**	15	10 µL	White paeony root 2
8	8 µL	Tree paeony root bark 3			

System suitability test
Paeoniflorin: brown zone at Rf ~ 0.19 (white RT).
Paeonol: weak orange zone at Rf ~ 0.91 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows an intense quenching zone at Rf ~ 0.09 and a weak quenching zone at Rf ~ 0.19 corresponding to reference substance paeoniflorin. There is a diffuse double zone at Rf ~ 0.34 and several zones in the upper part of the chromatogram.
Under white RT the test solution shows a brown zone at Rf ~ 0.19 corresponding to reference substance paeoniflorin and a diffuse brown double zone at Rf ~ 0.32 (red arrows). There is a violet zone at Rf ~ 0.50, a weak violet zone at Rf ~0.53 and an intense and distinct violet zone at Rf ~ 0.65. Below the solvent front there are two diffuse brown zones and a third one right at the solvent front. At Rf ~ 0.91 there is a characteristic orange zone corresponding to reference substance paeonol (blue arrow).

Test for other species
Under white RT no zone is seen at Rf ~0.53 (Red paeony root, orange arrow). At Rf ~ 0.50 no single zone is seen (White peony root, green arrow).

Passiflora incarnate (Passion flower)

1. Scope

This method identifies dried Passion flower (Passiflora incarnata L.) by HPTLC fingerprint and discriminates the related species Passiflora biflora, Passiflora edulis, and Passiflora caerulea.

2. Source of method

Ph. Eur. 6.7 (change in preparation of samples and reference substances)

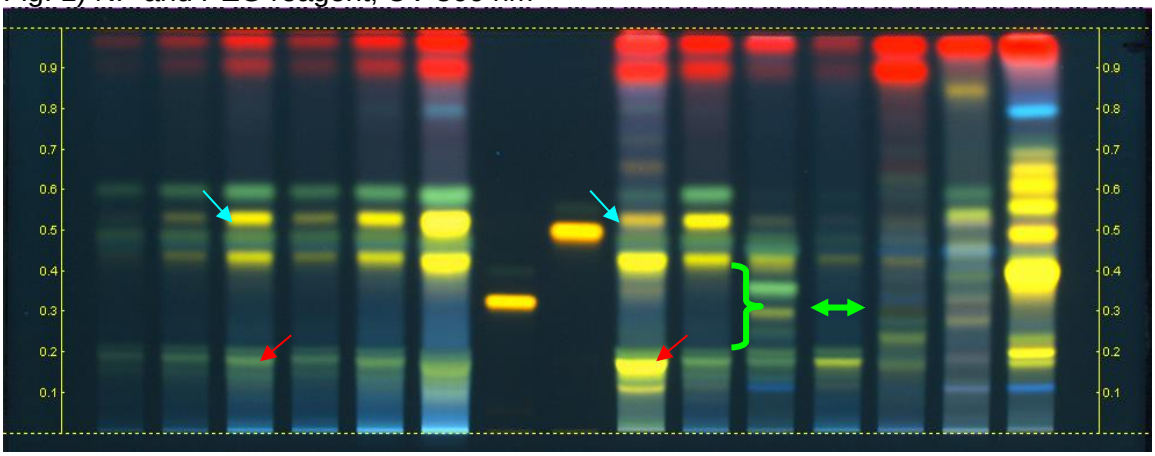
3. Procedure

Sample preparation:	Mix 2 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2.5 mg of rutin in 5 mL of methanol. Dissolve 4 mg of hyperoside in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water, ethyl methyl ketone 50:10:10:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: Dissolve 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: : Dissolve 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate for 3 min at 100 °C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Passion flower 1	**9**	**4 µL**	**Passion flower 2**
2	2 µL	Passion flower 1	10	4 µL	Passion flower 3
3	**4 µL**	**Passion flower 1**	11	4 µL	Passion flower 4 (adulterated?)
4	2 µL	Passion flower 1 (Ph. Eur extr.)	12	4 µL	*Passiflora caerulea* 1
5	4 µL	Passion flower 1 (Ph. Eur extr.)	13	4 µL	*Passiflora caerulea* 2
6	8 µL	Passion flower 1 (Ph. Eur extr.)	14	4 µL	*Passiflora edulis*
7	2 µL	Rutin	15	4 µL	*Passiflora biflora*
8	2 µL	Hyperoside			

System suitability test

Rutin: orange zone at Rf ~ 0.32.
Hyperoside: orange zone at Rf ~ 0.50.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a greenish to yellow zone at Rf ~ 0.16 (red arrows). In the middle of the chromatogram above the position of reference substance rutin there are four characteristic fluorescent zones: a yellow zone at Rf ~ 0.43, a green zone at Rf ~ 0.48, another yellow zone at Rf ~ 0.53 (blue arrow) right above the position of reference substance hyperoside and another green zone at Rf ~ 0.60. Right below the solvent front there are two red zones due to chlorophylls.

Test for adulteration

No zone is seen between Rf ~ 0.16 and Rf ~ 0.43 (green arrow) (Passiflora edulis, Passiflora caerulea). No intense yellow zones are detected between the upper green zone at Rf ~ 0.60 and the red zones below the solvent front (Passiflora biflora).

Peumus boldus
(Boldo leaf)

1. Scope

This method identifies dried Boldo leaf (Peumus boldus Molina) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation and derivatization reagent)

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2 mg of boldine in 5 mL of methanol. Dissolve 20 mg of hyoscine hydrobromide in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 6 µL of test solutions
Mobile phase:	Diethyl amine, methanol, toluene 10:10:80 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1% iodine reagent Preparation: 2 g iodine in 200 mL of ethanol Use: Dip (time 0, speed 5), heat at 100 °C for 3 minutes
Documentation:	1.) Clean plate, white RT 2.) Developed, UV 254 nm 3.) 1% iodine reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

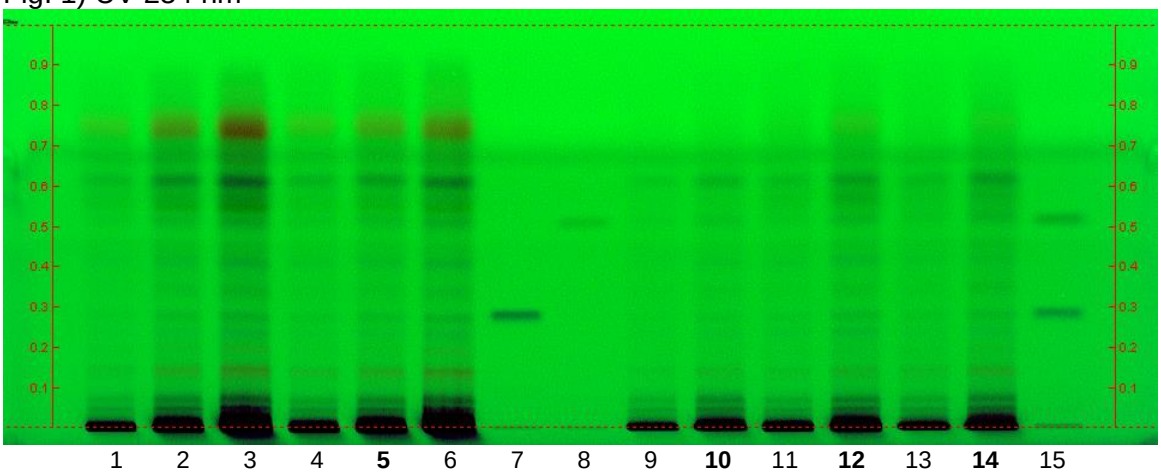
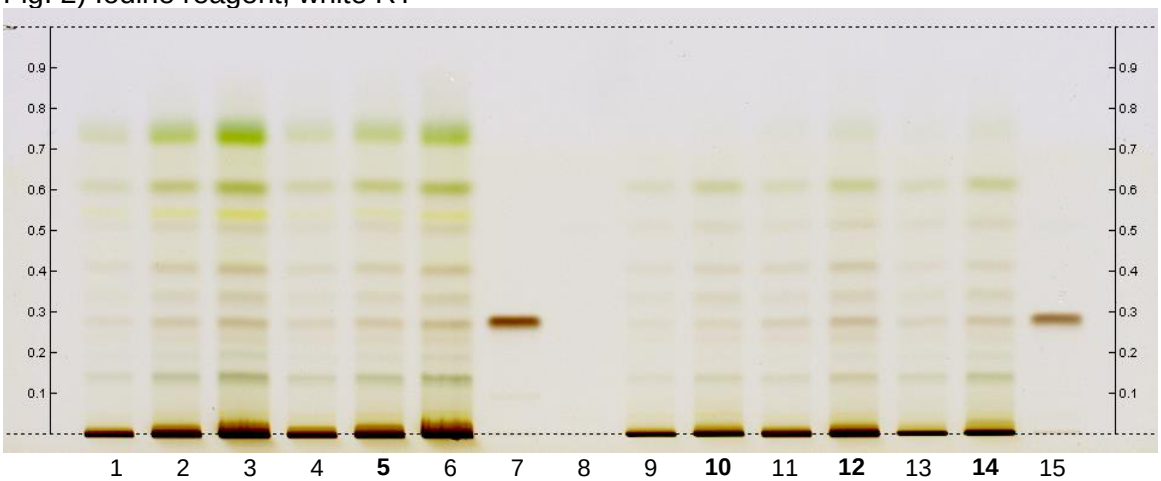


Fig. 2) Iodine reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Boldo leaf (Ph. Eur. extraction)	9	4 µL	Boldo leaf 1
2	4 µL	Boldo leaf (Ph. Eur. extraction)	**10**	**6 µL**	**Boldo leaf 1**
3	6 µL	Boldo leaf (Ph. Eur. extraction)	11	4 µL	Boldo leaf 2
4	4 µL	Boldo leaf	**12**	**6 µL**	**Boldo leaf 2**
5	**6 µL**	**Boldo leaf**	13	4 µL	Boldo leaf 3
6	8 µL	Boldo leaf	**14**	**6 µL**	**Boldo leaf 3**
7	2 µL	Boldine	15	2 µL	Boldine, hyoscine hydrobromide
8	2 µL	Hyoscine hydrobromide			

System suitability test
Boldine: a quenching zone at Rf ~ 0.28
Hyoscine hydrobromide: a weak quenching zone at Rf ~ 0.52

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.

Under white light the chromatogram of the test solution shows a brown zone corresponding to boldine at Rf ~ 0.28. Above this zone there are three brownish zones at Rf ~ 0.33, 0.40 and 0.50, and two green zones at Rf ~ 0.61 and 0.74.

Phyllanthus emblica
(Amla fruit, yu gan zi)

1. Scope

This method identifies dried Amla fruit (Phyllanthus emblica L.) and discriminates dried fruit of Belleric myrobalan (Terminalia bellerica (Gaertn.) Roxb) and Chebulic myrobalan (Terminalia chebula (Gaertn.) Retz) by HPTLC fingerprint.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chebulinic acid and 2 mg gallic acid in 10 mL of methanol. Optional: dissolve 1 mg of ellagic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 1 µL of test solutions
Mobile phase:	Ethyl formate, toluene, formic acid, water 30:1.5:4:3 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

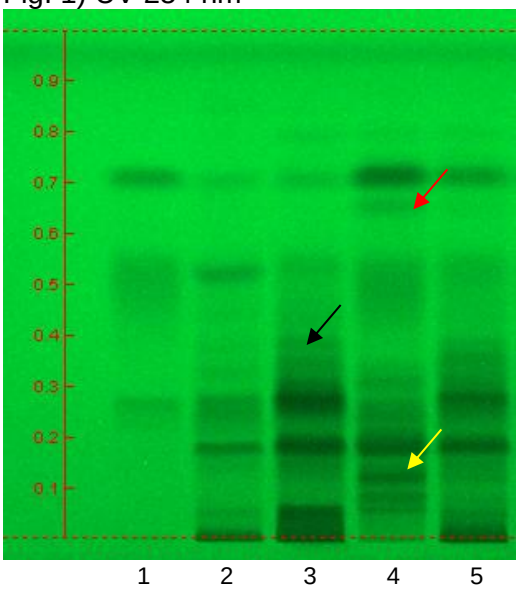
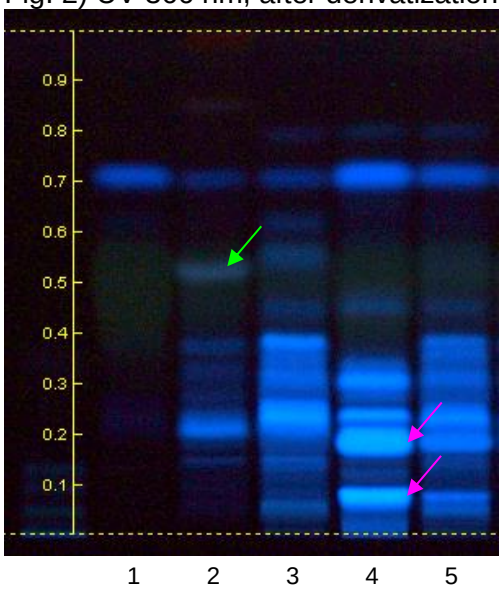


Fig. 2) UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Chebulinic acid, ellagic acid, and gallic acid (with increasing Rf)	9		Blank
2	1 µL	Belleric myrobalan fruit	10		Blank
3	1 µL	Chebulic myrobalan fruit	11		Blank
4	**1 µL**	**Amla fruit**	12		Blank
5	1 µL	Triphala powder	13		Blank
6		Blank	14		Blank
7		Blank	15		Blank
8		Blank			

System suitability test

Chebulinic acid: a quenching zone at Rf ~ 0.26 (UV 245 nm).
Gallic acid: a quenching zone at Rf ~ 0.71 (UV 245 nm).

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the test solution shows two characteristic quenching zones, one at Rf ~ 0.68 (red arrow) below the zone corresponding to gallic acid and the other at Rf ~ 0.14 (yellow arrow). After derivatization under UV 366 nm the test solution shows two characteristic and very intense fluorescent zones at Rf ~ 0.08 and 0.20 (pink arrows).

Test for other species

No quenching zone is seen under UV 254 nm at Rf ~ 0.39 (Chebulic myrobalan fruit; black arrow). No blue fluorescent zone is seen under UV 366 nm at Rf ~ 0.52 (Belleric myrobalan fruit, green arrow).

Fig. 3) Samples of Amla fruit (tracks 2-12), (track 1: reference substances)

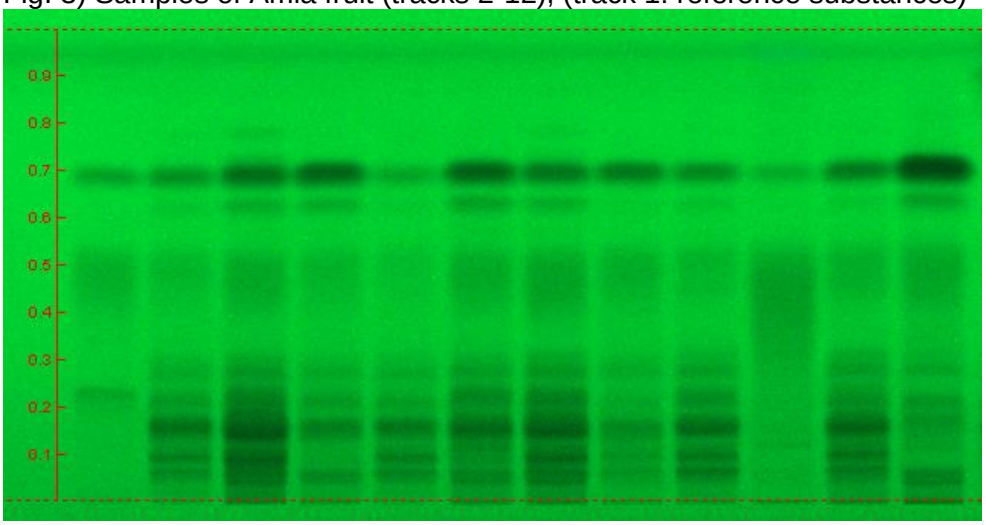
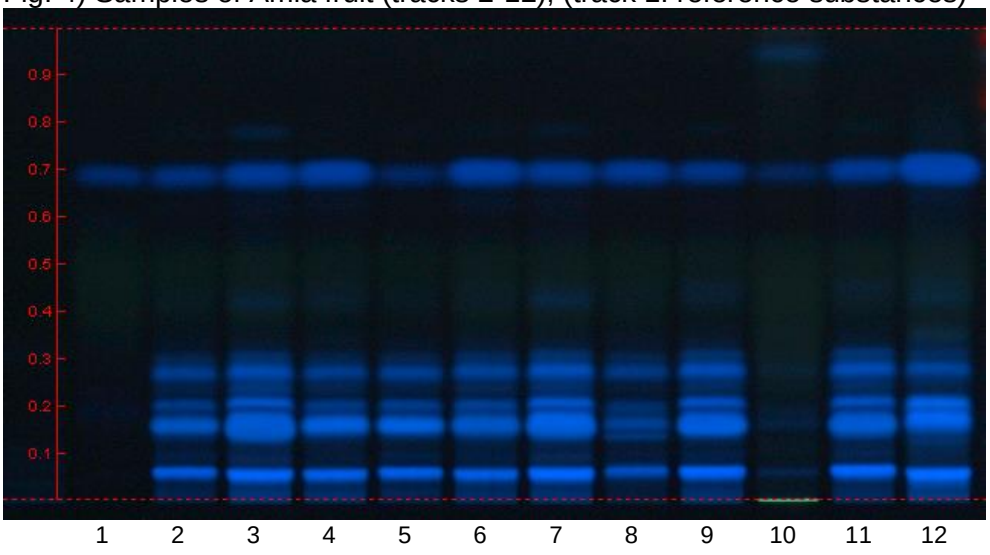


Fig. 4) Samples of Amla fruit (tracks 2-12), (track 1: reference substances)



Phyllanthus emblica, Terminalia bellerica, Terminalia chebula (Triphala powder)

1. Scope

This method identifies Triphala, a mixture of dried fruits from the three different plant species Phyllanthus emblica L., Terminalia bellerica (Gaertn.) Roxb., and Terminalia chebula (Gaertn.) Retz by HPTLC fingerprint.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chebulinic acid and 2 mg of gallic acid in 10 mL of methanol. Optional: dissolve 1 mg of ellagic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 1 µL of test solutions
Mobile phase:	Ethyl formate, toluene, formic acid, water 30:1.5:4:3 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of NP reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate 3min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

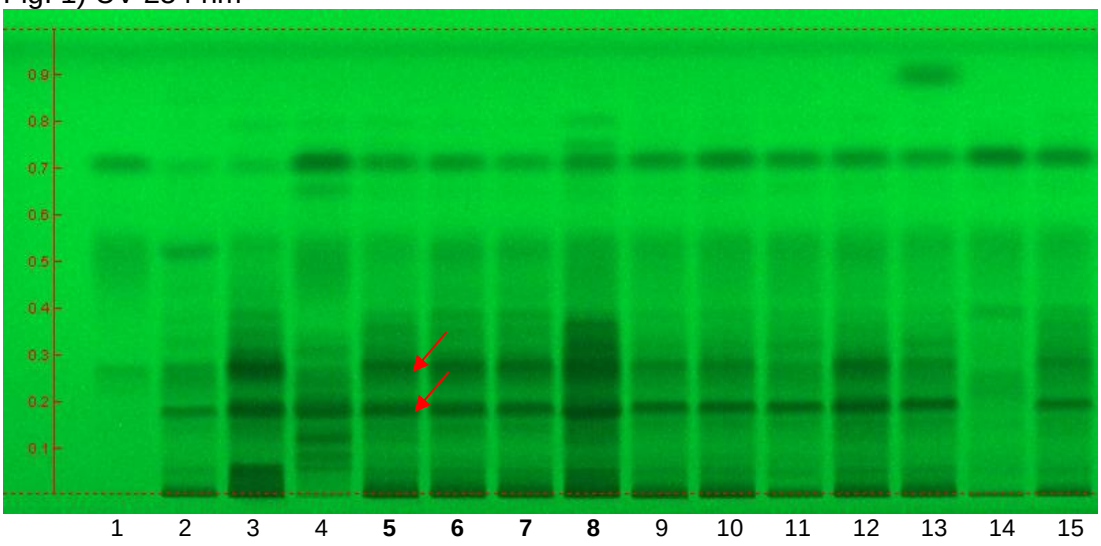
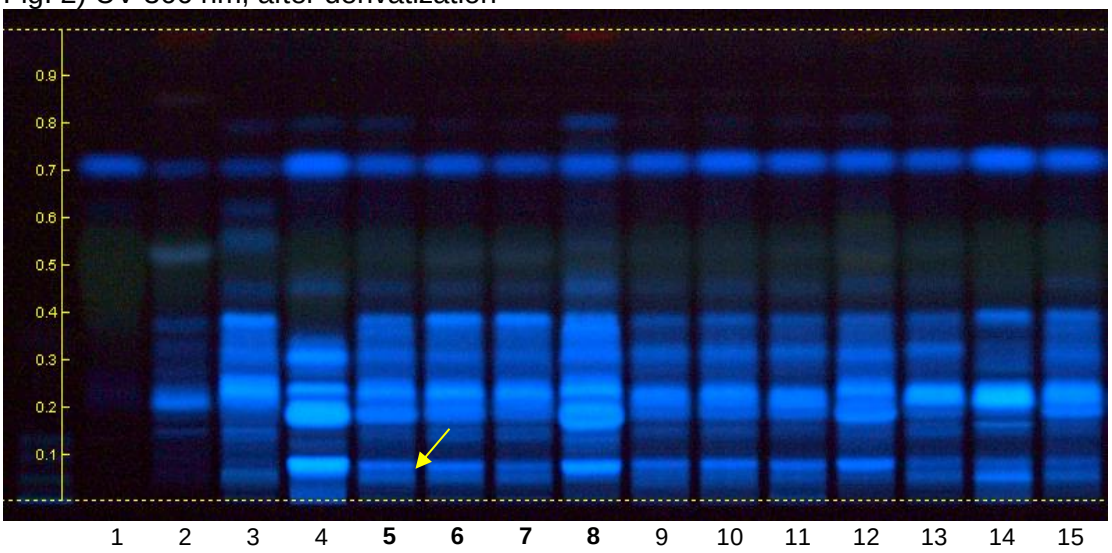


Fig. 2) UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Chebulinic acid, ellagic acid, and gallic acid (increasing Rf)	9	1 µL	Triphala tablets 1
2	1 µL	Belleric myrobalan fruit	10	1 µL	Triphala tablets 2
3	1 µL	Chebulic myrobalan fruit	11	1 µL	Triphala tablets 3
4	1 µL	Amla fruit	12	1 µL	Triphala tablets 4
5	**1 µL**	**Triphala powder 1**	13	1 µL	Triphala tablets 5
6	**1 µL**	**Triphala powder 2**	14	2 µL	Triphala powder extract
7	**1 µL**	**Triphala powder 3**	15	2 µL	Triphala tablets 6
8	**1 µL**	**Triphala powder 4**			

System suitability test
Chebulinic acid: a quenching zone at Rf ~ 0.26 (UV 245 nm).
Gallic acid: a quenching zone at Rf ~ 0.71 (UV 245 nm).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the test solution shows several diffuse quenching zones above the application position. The two main quenching zones are detected at Rf ~ 0.19 and Rf ~ 0.28 (red arrows). Above these zones there are additional diffuse quenching zones. There are quenching zones at the position of ellagic acid and of gallic acid.
Under UV 366 nm two blue fluorescent zones are detected right above the application position (yellow arrow). Several intense blue fluorescent zones are present between Rf ~ 0.16 and 0.40. At the position of gallic acid there is a fluorescent zone.

Pimpinella anisum
(Anise fruit (flavonoids))

1. Scope

This method identifies dried Anise fruit (Pimpinella anisum L.) by HPTLC fingerprint and discriminates dried Bitter Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. vulgare), Sweet Fennel fruit (Foeniculum vulgare Mill. ssp. vulgare var. dulce) and Caraway fruit (Carum carvi L.).

2. Source of method

CAMAG, proposed for Ph.Eur.

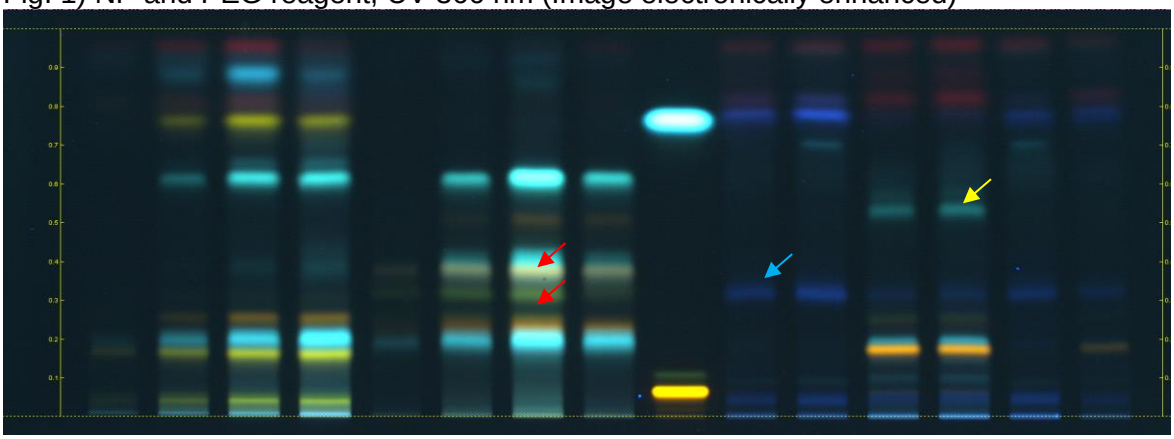
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3 mg of rutin in 1 mL of methanol. Dissolve 1 mg of caffeic acid in 1 mL of methanol
Stationary phase:	HPTLC Si 60 F_{254}
Application:	1 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	NP and PEG reagent Preparation NP reagent: 1 g of natural products reagent in 200 mL ethyl acetate Preparation PEG reagent: 10 g of polyethyleneglycole 400 in 200 mL dichloromethane Use: Heat for 3 min to 100°C, dip (time 0, speed 5) in NP reagent while still hot, dry, dip in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm (Image electronically enhanced)



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Anise fruit 1	9	3 / 1µL	Rutin, Caffeic acid (with incr. Rf)
2	**3 µL**	**Anise fruit 1**	10	3 µL	Bitter Fennel fruit 1
3	6 µL	Anise fruit 1	11	3 µL	Bitter Fennel fruit 2
4	3 µL	Anise fruit 2	12	3 µL	Sweet Fennel fruit 1
5	1 µL	Caraway fruit 1	13	3 µL	Sweet Fennel fruit 2
6	3 µL	Caraway fruit 1	14	3 µL	Wild Fennel fruit
7	6 µL	Caraway fruit 1	15	3 µL	Fennel tea
8	3 µL	Caraway fruit 2			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.07
Caffeic acid: light bluish fluorescent zone at Rf ~ 0.77

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. In the upper part of the chromatogram there are three prominent zones: a light blue zone at Rf ~ 0.88, a yellow zone at Rf ~ 0.76 and a light blue zone at Rf ~ 0.61. In the lower part of the chromatogram there is a sequence of three zones (yellow, light blue, yellowish) between Rf ~ 0.15 and 0.26. Right above the application position there is a yellow zone.

Test for adulteration
In the middle of the chromatogram there are neither yellow zones at Rf ~ 0.32 and Rf ~ 0.38 (red arrows, Caraway fruit) nor a faint dark blue zone at Rf ~ 0.32 (blue arrow, Bitter Fennel fruit) nor a light blue zone at Rf ~ 0.53 (yellow arrow, Sweet Fennel fruit).

Pimpinella anisum
(Anise oil)

1. Scope

This method identifies the essential oil from the dry ripe fruits of Pimpinella anisum L. by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation)

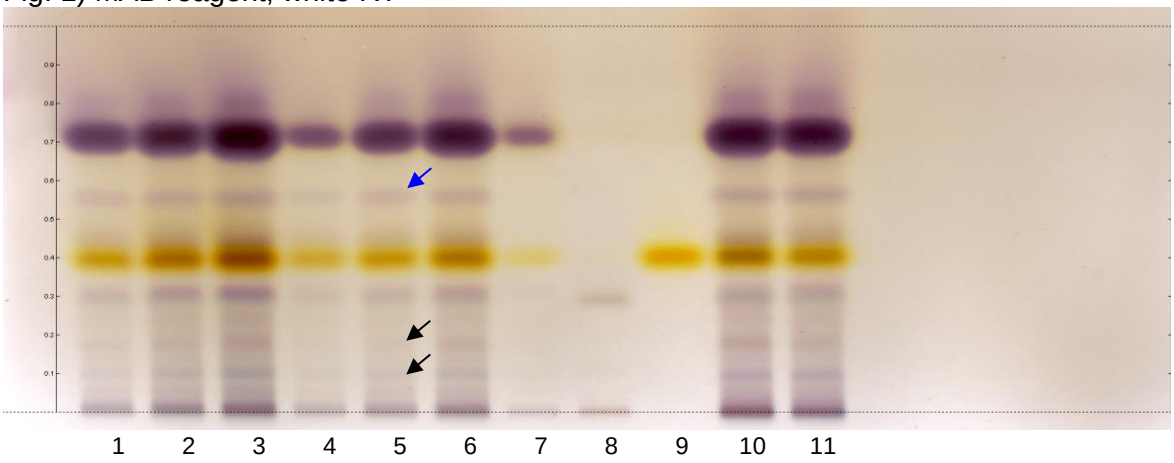
3. Procedure

Sample preparation:	Dissolve 50 µL of sample in 1mL of toluene.
Reference substances:	Dissolve 65 µL of anethole in 5 mL of toluene Dissolve 15 µL of linalool in 5 mL of toluene Optional: Dissolve 10 µL of anisaldehyde in 5 mL of toluene
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Toluene, ethyl acetate 93:7 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	MAB reagent Preparation: 0.25 g methyl 4-acetylbenzoate in a mix of 65 mL cold methanol and 5 mL sulfuric acid Use: Spray, heat at 100 °C for 8 min
Documentation:	1.) Clean plate, white RT 2.) MAB reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) MAB reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Anise oil 1 (Ph.Eur.)	9	2 µL	Anisaldehyde
2	2 µL	Anise oil 1 (Ph.Eur.)	10	2 µL	Anise oil 2 (Ph.Eur.)
3	3 µL	Anise oil 1 (Ph.Eur.)	11	2 µL	Anise oil 3 (Ph.Eur.)
4	1 µL	Anise oil 1	12		Blank
5	**2 µL**	**Anise oil 1**	13		Blank
6	3 µL	Anise oil 1	14		Blank
7	2 µL	Anethole	15		Blank
8	2 µL	Linalool			

System suitability test
Anethole: yellow zone at Rf ~ 0.71
Linalool: violet zone at Rf ~0.29

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. The chromatogram of the test solution shows an intense purple zone corresponding to the zone of reference substance anethole, a yellow zone corresponding to reference anisaldehyde and a grey zone corresponding to reference linalool. Between the zones of anethole and anisaldehyde a grey zone is seen at Rf ~ 0.56 (blue arrow). Two other grey zones are detected below the zone of linalool at Rf ~ 0.10 and 0.18 (black arrows).

Pimpinella anisum
(Aniseed)

1. Scope

This method identifies Aniseed (Pimpinella anisum L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 7.0 (change in sample preparation)

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 10 µL of olive oil in 1 mL of toluene. Dissolve 3 µL of anethole in 1 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of anethole, 0.5 µL of olive oil, 2 µL of test solutions
Mobile phase:	Toluene
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Phosphomolybdic acid reagent Preparation: 5 g phosphomolybdic acid in 200 mL ethanol Use: Dip (time 0, speed 5), heat at 120°C for 5 min
Documentation:	1.) Clean plate, white RT and UV 254nm 2.) UV 254 nm 3.) Phosphomolybdic acid reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254nm

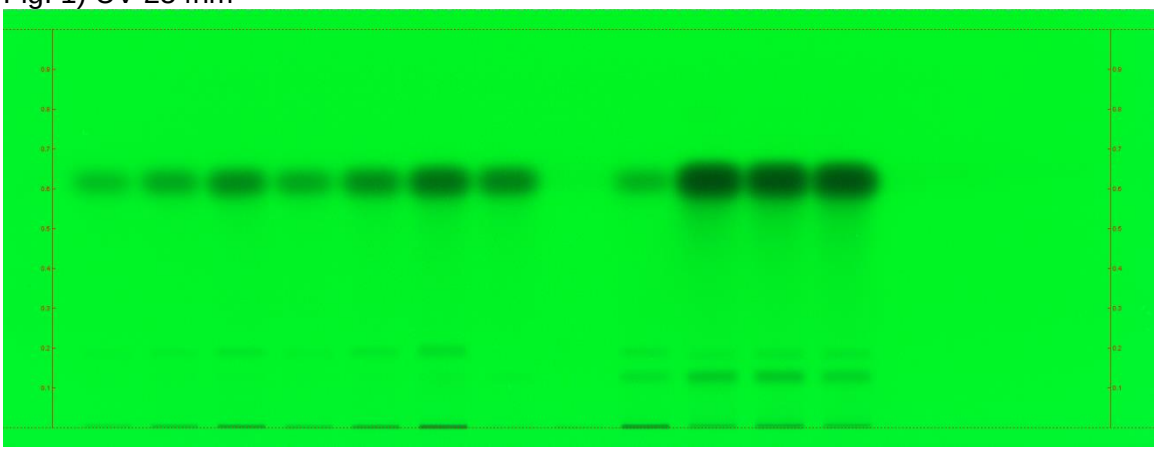
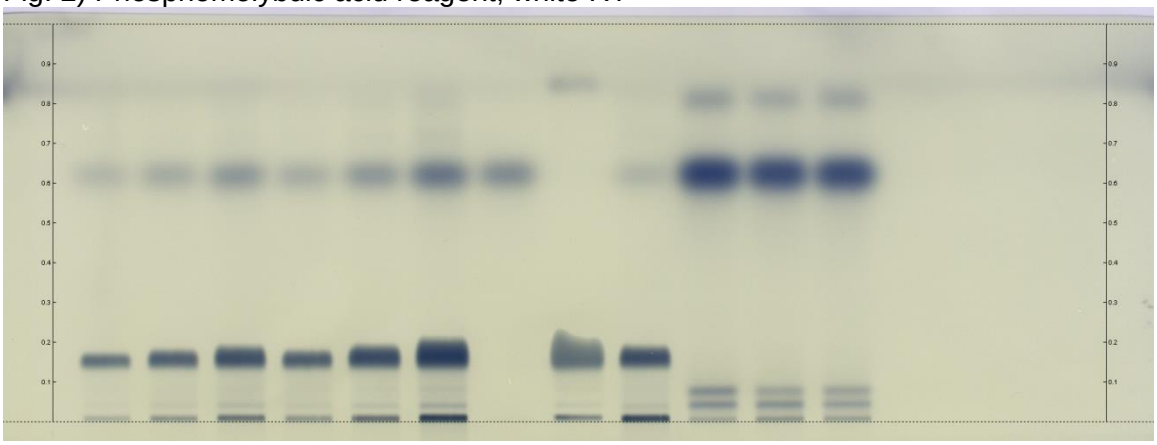


Fig. 2) Phosphomolybdic acid reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Aniseed 1 (Ph.Eur. method)	**9**	**2 µL**	**Aniseed 2**
2	2 µL	Aniseed 1 (Ph.Eur. method)	10	2 µL	Anise oil 1
3	3 µL	Aniseed 1 (Ph.Eur. method)	11	2 µL	Anise oil 2
4	1 µL	Aniseed 1	12	2 µL	Anise oil 3
5	**2 µL**	**Aniseed 1**	13		Blank
6	3 µL	Aniseed 1	14		Blank
7	2 µL	Anethole	15		Blank
8	2 µL	Olive oil			

System suitability test
Anethole: under UV 254 nm; a quenching zone at Rf ~ 0.61
Olive oil: after derivatization; a blue spot at Rf ~ 0.17

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a prominent zone corresponding to the zone anethole. After derivatization a bluish zone corresponding to anethole and a zone corresponding to the zone at Rf ~ 0.17 obtained with the reference solution (olive oil) are seen.

NOTE: After derivatization anise oil shows a blue zone at Rf ~ 0.81. No zone is seen at Rf ~ 0.17, but below this position two blue zones are detectable.

Piper methysticum (Kava rhizome)

1. Scope

This method identifies dried Kava rhizome (Piper methysticum G. Forst.) by HPTLC fingerprint.

2. Source of method

CAMAG Method of Analysis MOA007

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of kavain in 2 mL of toluene. Dissolve 1 mg of desmethoxyyangonin in 2 mL of toluene. Optional: dissolve 1 mg of dihydrokavain, 1 mg of methysticin, 1 mg of dihydromethysticin, and 1 mg of yangonin each in 2 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}, caffeine impregnated (see below)
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	tert-butyl methyl ether, n-hexane 7:3 (v/v)
Development:	**Unsaturated** chamber Developing distance 70 mm from lower edge Relative humidity 33%
Plate preparation	Dissolve 8 g of caffeine in 200 mL of dichloromethane. Use: Dip (time 0, speed 5) plate, dry at room temperature for 5 minutes, then heat at 80°C for 5 minutes.
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 4min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, UV 366 nm (while still hot!) 3.) Anisaldehyde reagent, white RT (while still hot!)

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent UV 366 nm

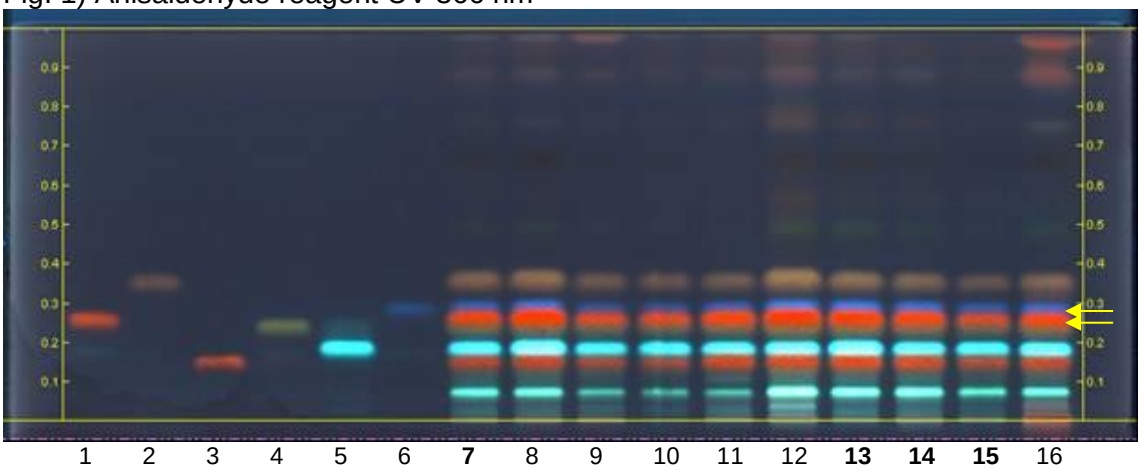
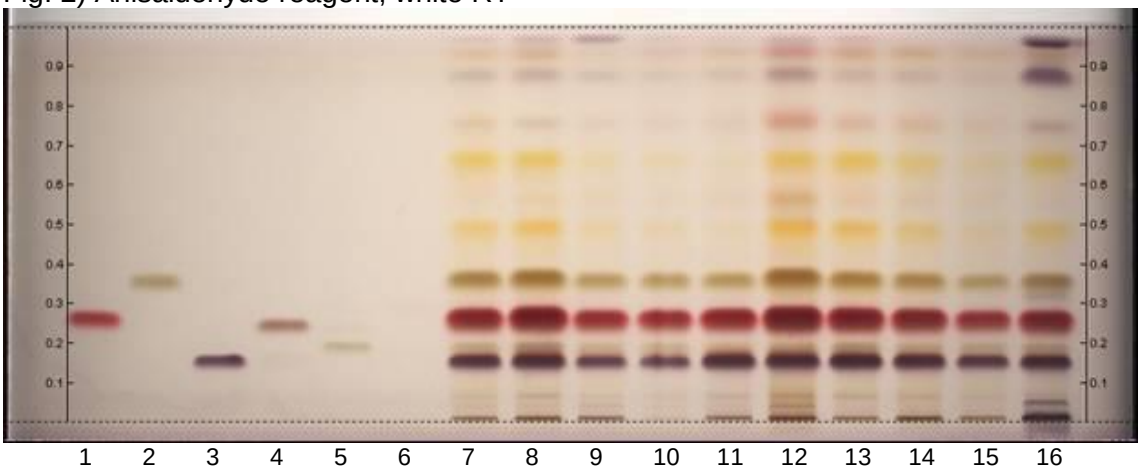


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Kavain	9	2 µL	Kava dry extract
2	2 µL	Dehydrokavain	10	2 µL	Kava root paste
3	2 µL	Methysticin	11	2 µL	Kava acetone extract
4	2 µL	Dehydromethysticin	12	2 µL	Kava liquid extract
5	2 µL	Yangonin	**13**	**2 µL**	**Kava rhizome 2**
6	2 µL	Desmethoxyyangonin	**14**	**2 µL**	**Kava rhizome 3**
7	**2 µL**	**Kava rhizome 1**	**15**	**2 µL**	**Kava rhizome 4**
8	2 µL	Kava capsules	16	2 µL	Kava liquid extract

System suitability test
Kavain: reddish zone at Rf ~ 0.27
Desmethoxyyangonin: blue zone at Rf ~ 0.30

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. Under UV 366 nm the chromatogram of the test solution shows a green zone (grey zone under white RT) at Rf ~ 0.08, a red zone (purple zone under white RT) at Rf ~ 0.16, another green zone (grayish and weak zone under white RT) at Rf ~ 0.19, another red zone (red and intense zone under white RT) at Rf ~ 0.27 corresponding to reference substance kavain, just above it a faint blue zone at Rf ~ 0.30 corresponding to reference substance desmethoxyyangonin (yellow arrows), and a brown zone (green zone under white RT) at Rf ~ 0.35. Under white light there are several yellow and reddish zones in the upper part of the chromatogram.

Plantago lanceolata
(Ribwort plantain leaf)

1. Scope

This method identifies dried Ribwort plantain (syn. Narrow-leaf plantain) leaf (Plantago lanceolata L.) by HPTLC fingerprint and detects the adulterants Grecian foxglove (Digitalis lanata Ehrh.) and Greater plantain leaf (Plantago major L).

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of aucubin in 10 mL of methanol. Dissolve 1 mg of acteoside (verbascoside) in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	6 µL of references, 8 µL of test solutions
Mobile phase:	Ethyl acetate, methanol, water 81:11:8 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Chloramine - trichloroacetic acid reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: Solution A: Dissolve 300 mg of sodium-N-chloro-(4-methylbenzene)sulfonamide in 10 mL of water. Solution B: Dissolve 10 g of trichloroacetic acid in ethanol and fill up to a volume of 40 mL. Mix solution A and B and shake. Use: Dip (time 0, speed 5), heat at 100°C for 8 min
Documentation:	1.) Clean plate, white RT 2.) Chloramine - trichloroacetic acid, UV 366 nm 3.) Chloramine - trichloroacetic acid, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Chloramine - trichloroacetic acid, UV 366 nm

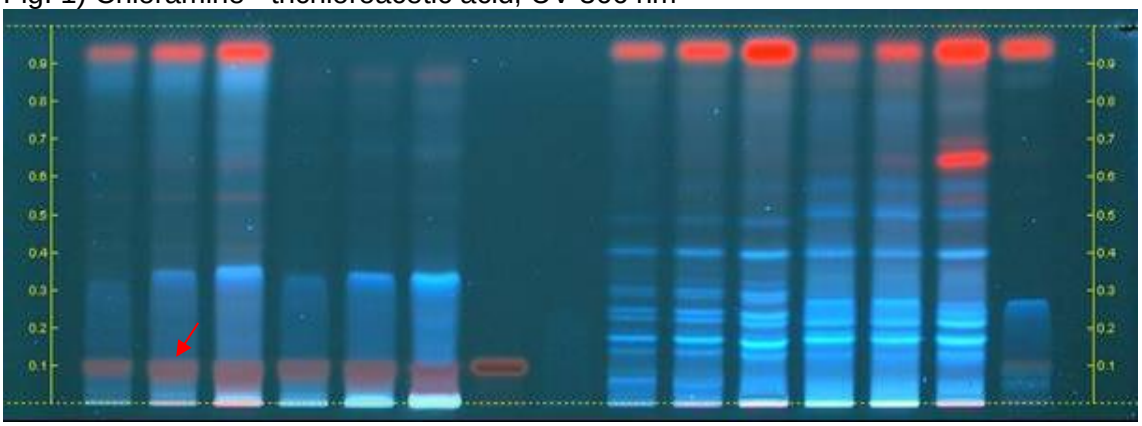


Fig. 2) Chloramine - trichloroacetic acid, white RT

Track	Volume	Sample	Track	Volume	Sample
1	4 µl	Ribwort plantain leaf 1	9	4 µl	Grecian foxglove leaf 1
2	**8 µl**	**Ribwort plantain leaf 1**	10	8 µl	Grecian foxglove leaf 1
3	16 µl	Ribwort plantain leaf 1	11	16 µl	Grecian foxglove leaf 1
4	4 µl	Ribwort plantain leaf 1 (extracted by shaking)	12	5 µl	Grecian foxglove leaf 2
5	8 µl	Ribwort plantain leaf 1 (extracted by shaking)	13	5 µl	Grecian foxglove leaf 3
6	16 µl	Ribwort plantain leaf 1 (extracted by shaking)	14	5 µl	Grecian foxglove leaf 4
7	5 µl	Aucubin	15	10 µl	Greater plantain leaf
8	10 µl	Acteoside			

System suitability test
Aucubin: black zone at Rf ~ 0.10 (white RT)

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under white RT the chromatogram of the test solution shows a black zone at Rf ~ 0.10 corresponding to aucubin; under UV 366 nm this zone appears brown (red arrows).
Under UV 366 nm there is a blue zone at Rf ~ 0.35 and a red zone due to chlorophylls right below the solvent front.

Test for adulteration
Under UV 366 nm no brown zone is seen at Rf ~ 0.10; under white RT no black zone is seen at Rf ~ 0.10 (Grecian foxglove leaf, Greater plantain leaf).

Polygonum multiflorum (Fleeceflower, he shou wu)

1. Scope

This method identifies dried Fleeceflower root (Polygonum mulitflorum Thunb.) by HPTLC fingerprint.

2. Source of method

Pharmeuropa 20.1

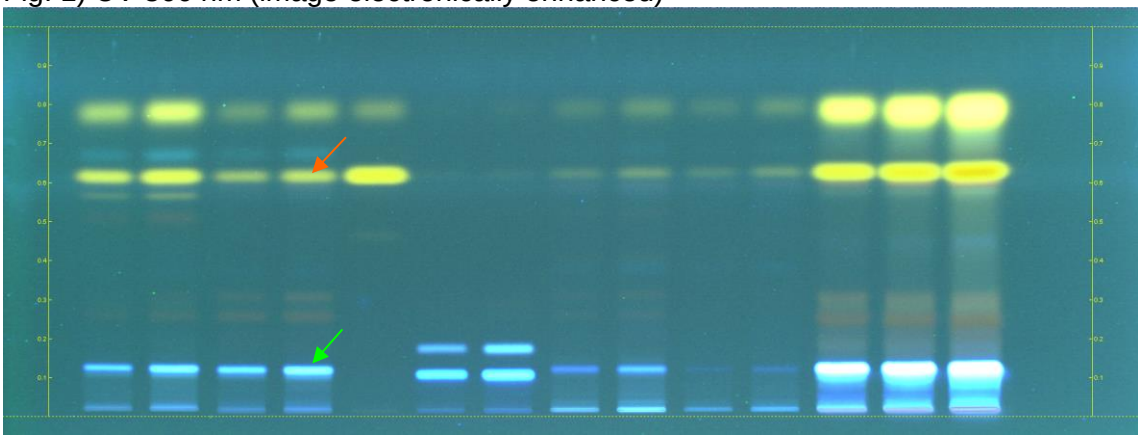
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 0.3 mg of emodin in 1 mL of methanol. Dissolve 1 mg of rhaponticin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	7 µL of references, 7 µL of test solutions
Mobile phase:	Toluene, ethanol, glacial acetic acid 8:2:0.5 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 366 nm (image electronically enhanced)



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Fleeceflower root #1	9	7 µL	Fleeceflower root #3
2	7 µL	Fleeceflower root #1	10	5 µL	Fleeceflower root #4
3	5 µL	Fleeceflower root #2	11	7 µL	Fleeceflower root #4
4	**7 µL**	**Fleeceflower root #2**	12	5 µL	Fleeceflower root #5
5	2 µL	Emodin	13	7 µL	Fleeceflower root #5
6	5 µL	Rhaponticin	14	10 µL	Fleeceflower root #5
7	7 µL	Rhaponticin	15		Blank
8	5 µL	Fleeceflower root #3			

System suitability test

Emodin: yellow fluorescent zone at Rf ~ 0.60 (additional zone at Rf ~ 0.80 due to impurity).
Rhaponticin: blue fluorescent zone at Rf ~ 0.11 (additional zone at Rf ~ 0.20 due to impurity).

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a yellow zone at Rf ~ 0.60 corresponding to reference emodin (red arrow) and a blue zone at Rf ~ 0.11 corresponding to reference rhaponticin (green arrow). Another yellow zone is seen at Rf ~ 0.80. Between emodin and rhaponticin there are two faint brown zones.

Primula veris
(Cowslip Primrose Flower)

1. Scope

This method identifies dried Cowslip primrose flower (Primula veris L.) by HPTLC fingerprint.

2. Source of method

CAMAG, under evaluation by Ph.Eur

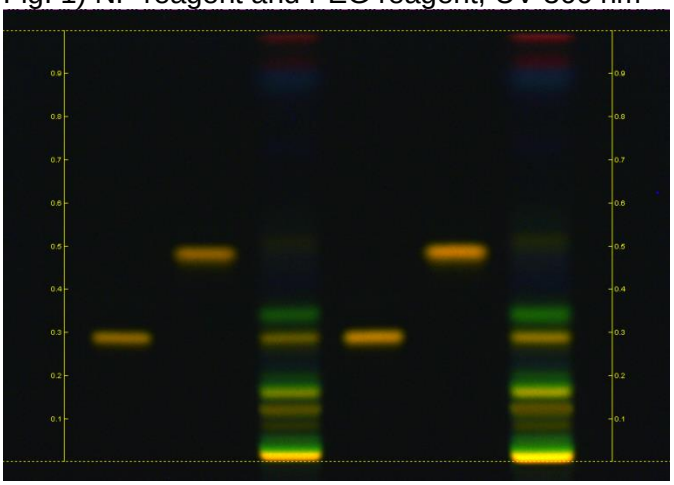
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol, sonicate for 10 minutes, then centrifuge or filter the solution and use the supernatant / filtrate as the test solution.
Reference substances:	Dissolve 2 mg of rutin and 2 mg of hyperoside individually in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, water, anhydrous formic acid, acetic acid (100:27:11:11 v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of diphenylboric acid 2-aminoethyl ester is dissolved in 200 mL of ethyl acetate. 2.) PEG reagent Preparation: 10 g of polyethylene glycol (Macrogol) 400 is dissolved in 200 mL of dichloromethane. Use: Heat plate at 100°C for 5 min, then dip (time 0, speed 5) in NP reagent, dry in a stream of cold air, dip (time 0, speed 5) in PEG reagent and dry in a stream of cold air.
Documentation:	NP reagent and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent and PEG reagent, UV 366 nm



1 2 **3** 4 5 6

Track	Volume	Sample
1	2 µL	Rutin
2	2 µL	Hyperoside
3	**2 µL**	**Primula veris flower**
4	3 µL	Rutin
5	3 µL	Hyperoside
6	3 µL	Primula veris flower

System suitability test
Rutin: orange zone at Rf ~ 0.29
Hyperoside: orange zone at Rf ~ 0.49

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a green fluorescent zone just above reference substance rutin. At the position of rutin an orange to yellow fluorescent zone is detected. Below the position of rutin there are two orange to yellow fluorescent zones. Below a weak greenish-blue zone is detected and further below a yellow to orange zone is present.

Pueraria montana var. chinensis (Thomson's kudzu root, gen ge)

1. Scope

This method identifies Thomson's Kudzu root (Pueraria montana (Lour.) Merr var. chinense Maesen & S.M. Almeida, syn. Pueraria thomsonii Benth.) by HPTLC fingerprint and detects the adulterant Kudzu root (Pueraria montana (Lour.) Merr. var lobata (Willd.) Maesen & S.M. Almeida, syn: Pueraria lobata (Willd.) Ohwi).

2. Source of method

AHP

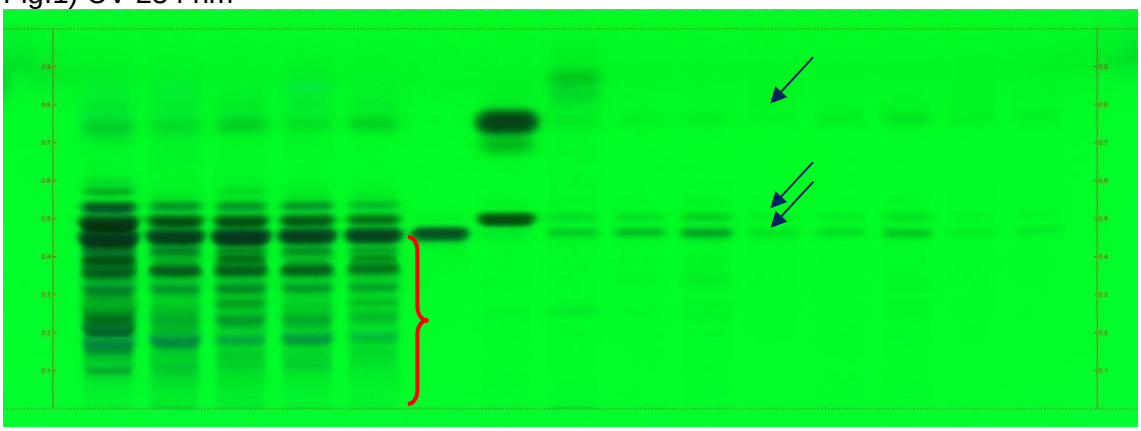
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of daidzin and 1 mg of puerarin individually in 1 mL of methanol. Optional: Dissolve 1 mg of daidzein in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	7 µL of references, 7 µL of test solutions
Mobile phase:	Dichloromethane, ethyl acetate, methanol, water 20:40:22:10 (v/v/v/v). **NOTE**: to avoid the formation of two phases respect this order when mixing the mobile phase.
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate UV 254nm 2.) UV 254 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm



Track	Volume	Sample	Track	Volume	Sample
1	7 µL	Kudzu root 1	9	7 µL	Thomson's kudzu root 1
2	7 µL	Kudzu root 2	**10**	**7 µL**	**Thomson's kudzu root 2**
3	7 µL	Kudzu root 3	11	7 µL	Kudzu root 7 (mislabeled)
4	7 µL	Kudzu root 4	12	7 µL	Thomson's kudzu root 3
5	7 µL	Kudzu root 5	13	7 µL	Thomson's kudzu root 4
6	7 µL	Puerarin	14	7 µL	Thomson's kudzu root 5
7	7 µL	Daidzin, Daidzein (with increasing Rf)	15	7 µL	Thomson's kudzu root 6
8	7 µL	Kudzu root 6			

System suitability test

Puerarin: dark quenching zone at Rf ~ 0.48
Daidzin: dark quenching zone at Rf ~ 0.51

Identification

Compare result with reference image. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows weak quenching zones corresponding to reference standards puerarin and daidzin (black arrows). Below the zone corresponding to puerarin there are no or only very weak quenching zones. At Rf 0.75 there may be a weak zone corresponding to reference standard daidzein.

Test for other species

No intense zone is seen between the zone corresponding to puerarin and the start position (red arrow, Kudzu root).

Pueraria montana var. lobata (Kudzu root, ge gen)

1. Scope

This method identifies dried Kudzu root (Pueraria montana (Lour.) Merr. var lobata (Willd.) Maesen & S.M. Almeida, syn: Pueraria lobata (Willd.) Ohwi) by HPTLC fingerprint and discriminates Thomson's Kudzu root (Pueraria montana (Lour.) Merr var. chinense Maesen & S.M. Almeida, syn. Pueraria thomsonii Benth.)

2. Source of method

AHP

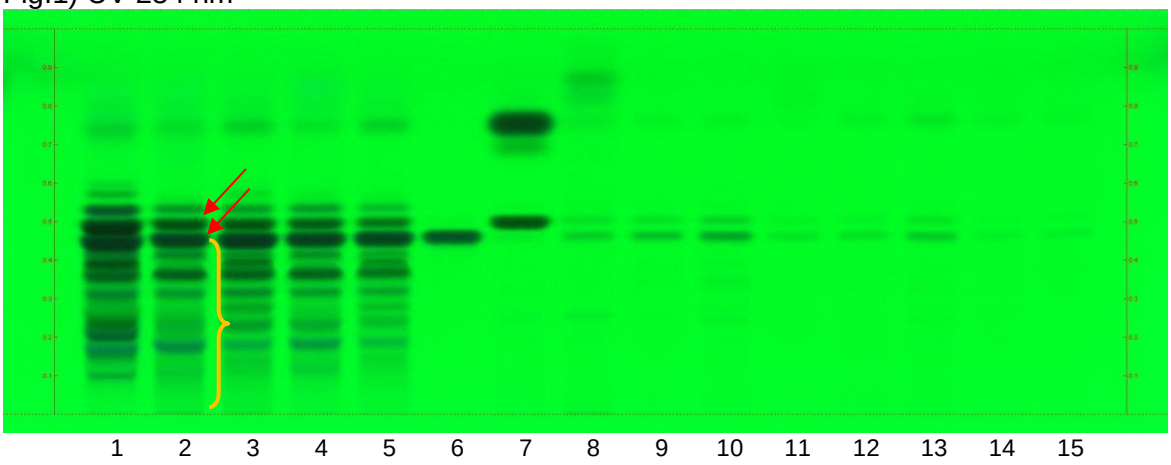
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of daidzin and 1 mg of puerarin individually in 1 mL of methanol. Optional: Dissolve 1 mg of daidzein in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	7 µL of references, 7 µL of test solutions
Mobile phase:	Dichloromethane, ethyl acetate, methanol, water 20:40:22:10 (v/v/v/v). **NOTE**: to avoid the formation of two phases respect this order when mixing the mobile phase.
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Documentation:	1.) Clean plate UV 254nm 2.) UV 254 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm



Track	Volume	Sample	Track	Volume	Sample
1	7 µL	Kudzu root #1	9	7 µL	Thomson's kudzu root #1
2	**7 µL**	**Kudzu root #2**	10	7 µL	Thomson's kudzu root #2
3	7 µL	Kudzu root #3	11	7 µL	Kudzu root #7 (mislabeled
4	7 µL	Kudzu root #4	12	7 µL	Thomson's kudzu root #3
5	7 µL	Kudzu root #5	13	7 µL	Thomson's kudzu root #4
6	7 µL	Puerarin	14	7 µL	Thomson's kudzu root #5
7	7 µL	Daidzin, Daidzein (with increasing Rf)	15	7 µL	Thomson's kudzu root #6
8	7 µL	Kudzu root #6			

System suitability test

Puerarin: dark quenching zone at Rf ~ 0.48
Daidzin: dark quenching zone at Rf ~ 0.51

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows intense quenching zones corresponding to reference substances puerarin and daidzin (red arrows). Below the zone corresponding to puerarin there are at least 5 quenching zones of medium intensity (orange arrow). Above the zone corresponding to daidzin there are 1 to 2 distinct quenching zones. At Rf ~ 0.75 there is a weak zone corresponding to reference standard daidzein.

Test for other species

The chromatogram of Thomson's kudzu root shows only two weak quenching zones corresponding to reference substances puerarin and daidzin.

Rosmarinus officinalis (Rosemary leaf)

1. Scope

This method identifies dried Rosemary leaf (Rosmarinus officinalis L.) by HPTLC fingerprint and discriminates dried Oregano leaf (Origanum vulgare L. ssp. hirtum), Thyme leaf (Thymus vulgaris L.), Holy basil (Ocimum sanctum L. / O. tenuiflorum L.) and Sweet basil (Ocimum basilicum).

2. Source of method

CAMAG

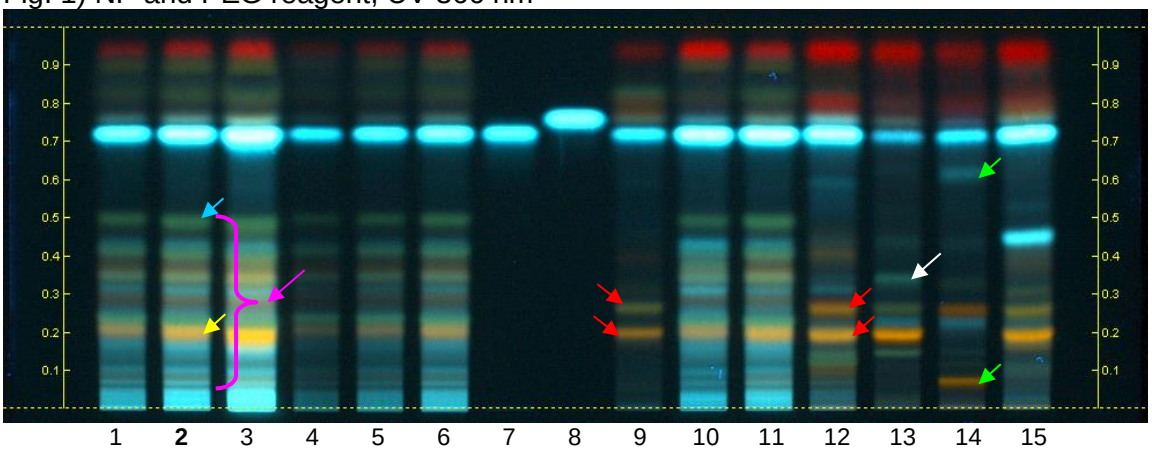
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 mg of rosmarinic acid in 10 mL of methanol. Dissolve 6 mg of caffeic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 8 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: : 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Rosemary leaf	9	8 µL	Oregano leaf (old sample)
2	**8 µL**	**Rosemary leaf**	10	8 µL	Rosemary leaf 2
3	16 µL	Rosemary leaf	11	8 µL	Rosemary leaf 3
4	1 µL	Rosemary leaf 1 (Ph. Eur extract.)	12	8 µL	Thyme leaf
5	2 µL	Rosemary leaf 1 (Ph. Eur extract.)	13	8 µL	Holy basil leaf
6	4 µL	Rosemary leaf 1 (Ph. Eur extract.)	14	8 µL	Sweet basil leaf
7	2 µL	Rosmarinic acid	15	8 µL	Marjoram leaf
8	2 µL	Caffeic acid			

System suitability test

Rosmarinic acid: light blue fluorescent zone at Rf ~ 0.73.
Caffeic acid: light blue fluorescent zone at Rf ~ 0.76.

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a red zone right below the solvent front. At the position of reference substance rosmarinic acid an intense light blue zone is seen. A green zone is detected at Rf ~ 0.50 (blue arrow). Below this zone there is a pattern of characteristically colored zones of low intensity (pink arrow). An intense orange zone is detected at Rf ~ 0.21 (yellow arrow).

Test for other species

There are no two orange zones at Rf ~ 0.27 and 0.20 (red arrows, Oregano leaf or Thyme leaf). No light blue zone is seen at Rf ~ 0.32 (white arrow, Holy basil leaf). No orange zone is seen at Rf ~ 0.27 and no zone is seen at Rf ~ 0.63 (green arrows, Sweet basil leaf).

Rosmarinus officinalis (Rosemary oil)

1. Scope

This method identifies the essential oil from the flowering aerial parts of Rosemary (Rosmarinus officinalis L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 7.0 (change in preparation of samples/references and derivatization reagent).

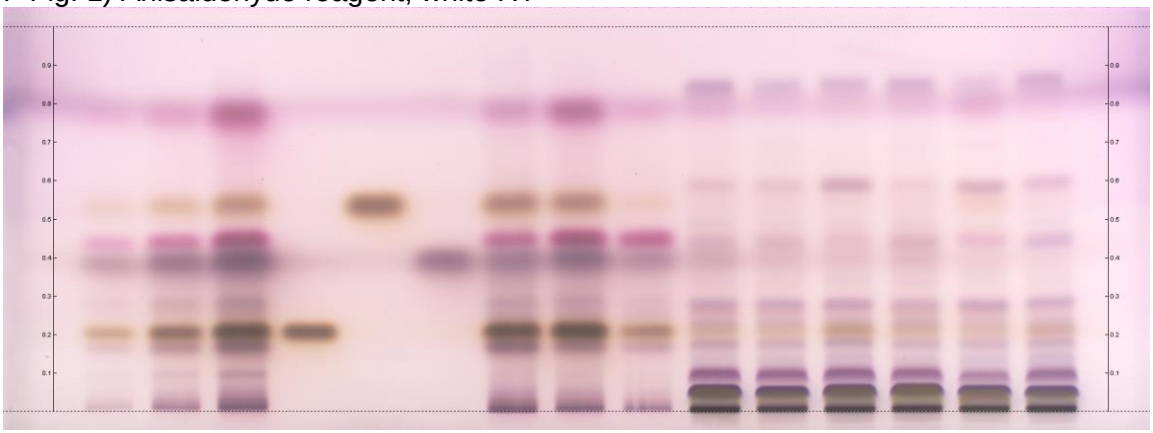
3. Procedure

Sample preparation:	Dissolve 20 µL of essential oil in 1 mL of toluene. Optional: mix 0.5 g of powdered dry Rosemary leaf with 5 mL of toluene and sonicate for 10 min, then centrifuge or filter the solutions and use the supernatants/filtrates as test solution.
Reference substances:	Dissolve 1 mg of hyperoside, 1 mg of rutin and 5 mg of rosmarinic acid individually in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	3 µL of references, 3 µL of test solutions
Mobile phase:	Ethyl acetate, toluene 5:95 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

F Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Rosemary oil	9	1 µL	Rosemary oil Ph.Eur 4.0
2	**3 µL**	**Rosemary oil**	10	5 µL	Rosemary leaf #1
3	5 µL	Rosemary oil	11	5 µL	Rosemary leaf #2
4	2 µL	Borneol	12	5 µL	Rosemary leaf #3
5	2 µL	Bornyl acetate	13	5 µL	Rosemary leaf #4
6	5 µL	Cineole	14	5 µL	Rosemary leaf from Peru
7	3 µL	Rosemary oil CT Campher Ph.Eur. 5.0	15	5 µL	Rosemary leaf from Croatia
8	3 µL	Rosemary oil CT Cineole			

System suitability test
Borneol: brownish zone at ~ 0.21.
Bornyl acetate: brown zone at ~ 0.54.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a purple zone in the upper third. At the position of reference bornyl acetate there is a yellowish brown zone. Below this zone a purple zone is detected. At the position of reference cineole there is a greyish violet zone. At the position of reference borneol a yellowish brown zone is seen, just above and below this zone there is each a greyish violet zone.

Salvia fruticosa, syn. S. triloba (Three-lobed sage leaf)

1. Scope

This method identifies dried Three-lobed sage leaf (Salvia fruticosa Mill., syn. Salvia triloba L. fil) by HPTLC fingerprint and discriminates dried Sage leaf (Salvia officinalis L.).

2. Source of method

CAMAG

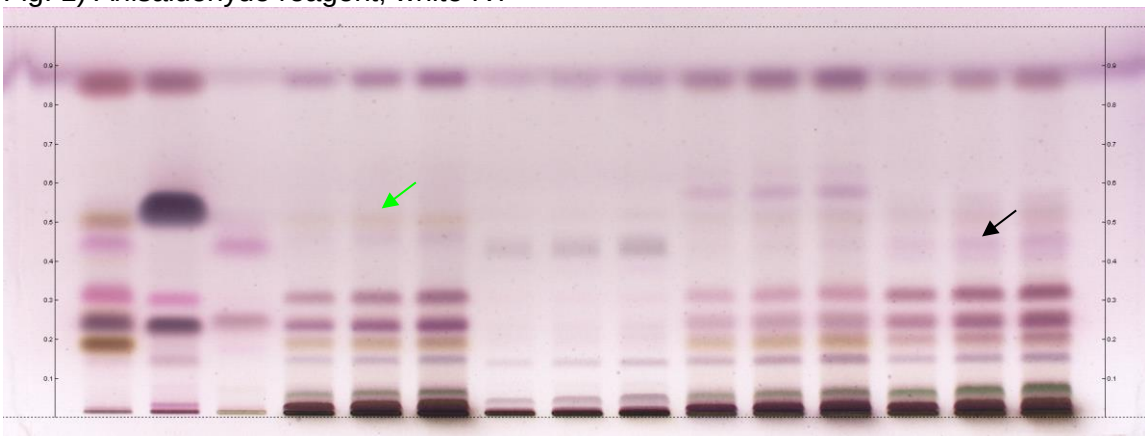
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 mg of thujon in 1 mL of methanol. Dissolve 25 µL of cineole in 10 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 7 µL of test solutions
Mobile phase:	Dichloromethane
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol add 20 mL of glacial acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: spray, heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldeyhde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	3µL	Sage oil	9	10µL	Clary sage leaf
2	3µL	Clary sage oil	10	5µL	Spanish sage leaf
3	10µL	Cineole, thujon (with increasing Rf)	11	7µL	Spanish sage leaf
4	5µL	Sage leaf	12	10µL	Spanish sage leaf
5	7µL	Sage leaf	13	5µL	Three-lobed sage leaf
6	10µL	Sage leaf	**14**	**7µL**	**Three-lobed sage leaf**
7	5µL	Clary sage leaf	15	10µL	Three-lobed sage leaf
8	7µL	Clary sage leaf			

System suitability test
Thujon: violet zone at Rf ~ 0.47.
Cineole: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a violet zone just below the solvent front. A diffuse violet zone is seen at the position of thujon (black arrow). There is a violet zone at Rf ~ 0.30 and slightly below reference cineole there is another violet zone. A brown zone is seen at Rf ~ 0.20 and there is a blue violet zone just below it.

Test for other species
No yellow zone is seen at Rf ~ 0.50 (Sage leaf, green arrow).

Salvia lavandulifolia (Spanish sage oil)

1. Scope

This method identifies the essential oil from Spanish sage (Salvia lavandulifolia Vahl) by HPTLC fingerprint and discriminates the essential oil from the other species Clary sage (Salvia sclarea L.), and Sage (Dalmatian Sage, Salvia officinalis L.).

2. Source of method

CAMAG

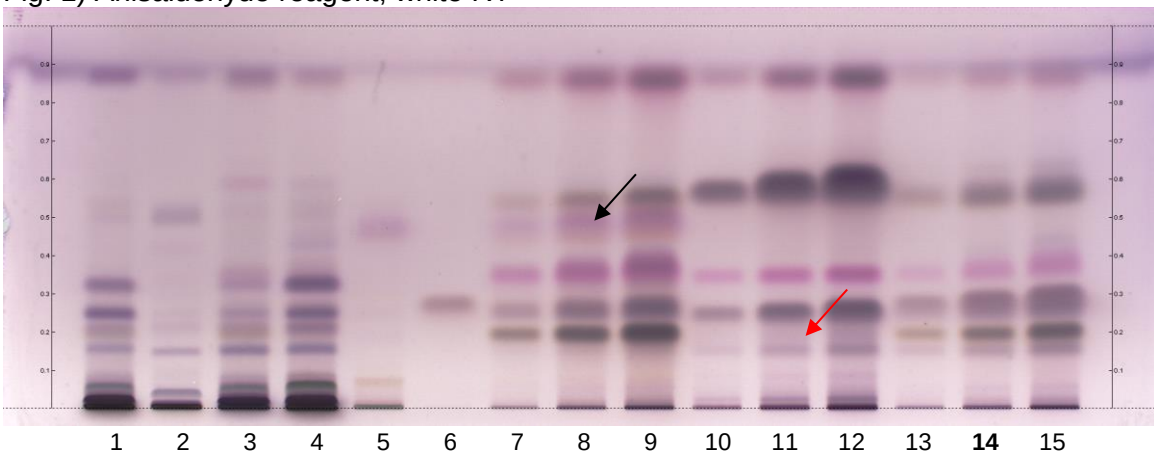
3. Procedure

Sample preparation:	Mix 100 µL of sample with 10 mL of toluene.
Reference substances:	Dissolve 1 mg of thujon in 1 mL of toluene. Dissolve 30 µL of cineole in 10 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 3 µL of test solutions
Mobile phase:	Dichloromethane
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: To 170 mL of ice-cooled methanol add 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: spray, heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	7µL	Sage leaf	9	5µL	Sage oil
2	7µL	Clary sage leaf	10	1µL	Clary sage oil
3	7µL	Spanish sage leaf	11	3µL	Clary sage oil
4	7µL	Three-lobed sage leaf	12	5µL	Clary sage oil
5	2µL	Thujon	13	1µL	Spanish sage oil
6	2µL	Cineole	**14**	**3µL**	**Spanish sage oil**
7	1µL	Sage oil	15	5µL	Spanish sage oil
8	3µL	Sage oil			

System suitability test
Thujon: weak pink zone at Rf ~ 0.47.
Cineole: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a dark grey zone at Rf ~ 0.54. At the position of reference cineole there is a diffuse violet zone (Rf ~ 0.27). Above it there is a pink zone (Rf ~ 0.34) and below the position of cineole there is an intense grey zone at Rf ~ 0.20.

Test for other species
No zone is seen at the position of reference thujon at Rf ~ 0.47 (black arrow, Sage oil).
The chromatogram of Clary sage oil lacks the grey zone at Rf ~ 0.20 (red arrow).

Salvia miltiorrhiza
(Chinese Sage, dan shen)

1. Scope

This method investigates the content of individual tanshinones in Chinese Sage root (Salvia miltiorrhiza Bunge) by HPTLC.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of ethyl acetate and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 0.6 mg each of tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone in 1.5 mL of ethyl acetate.
Stationary phase:	HPTLC Si 60 F254 caffeine impregnated (preparation: immerse plate into a solution of 80 g/L caffeine in dichloromethane for 1 s, dry at room temperature for 5 min, then at 80°C for 5 min).
Application:	2 µL of references, 10 µL of test solutions
Mobile phase:	Toluene, ethyl acetate, acetic acid 95:5:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid in 180 mL methanol 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: The plate is immersed in sulfuric acid reagent for 1 s, then heated for 5 min at 100°C
Documentation:	1.) Clean plate (white RT) 2.) White RT 3.) Sulfuric acid reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) White RT

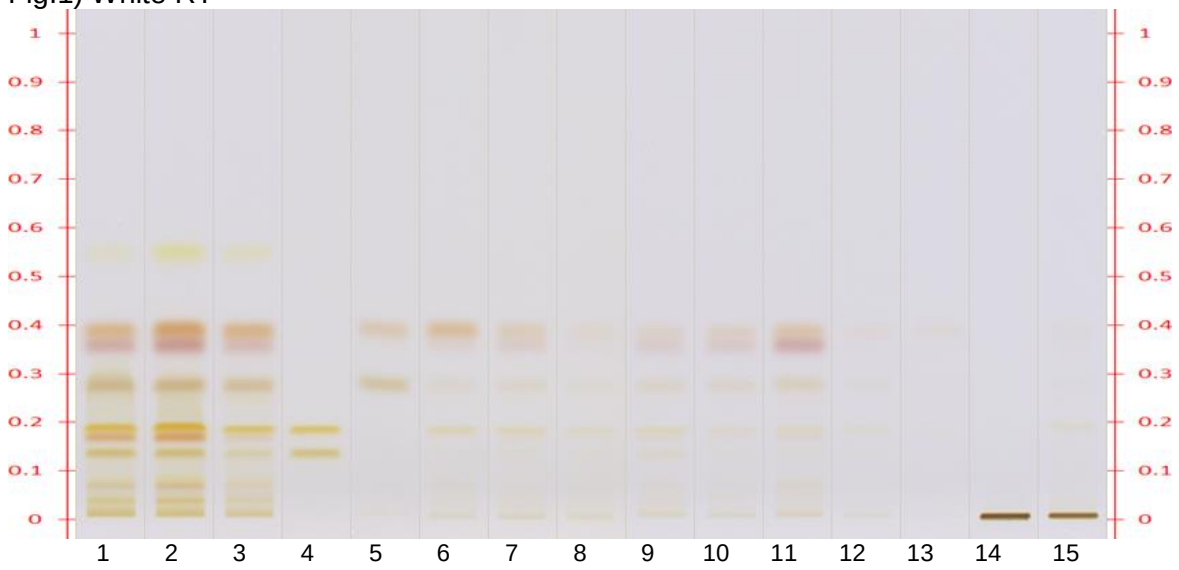
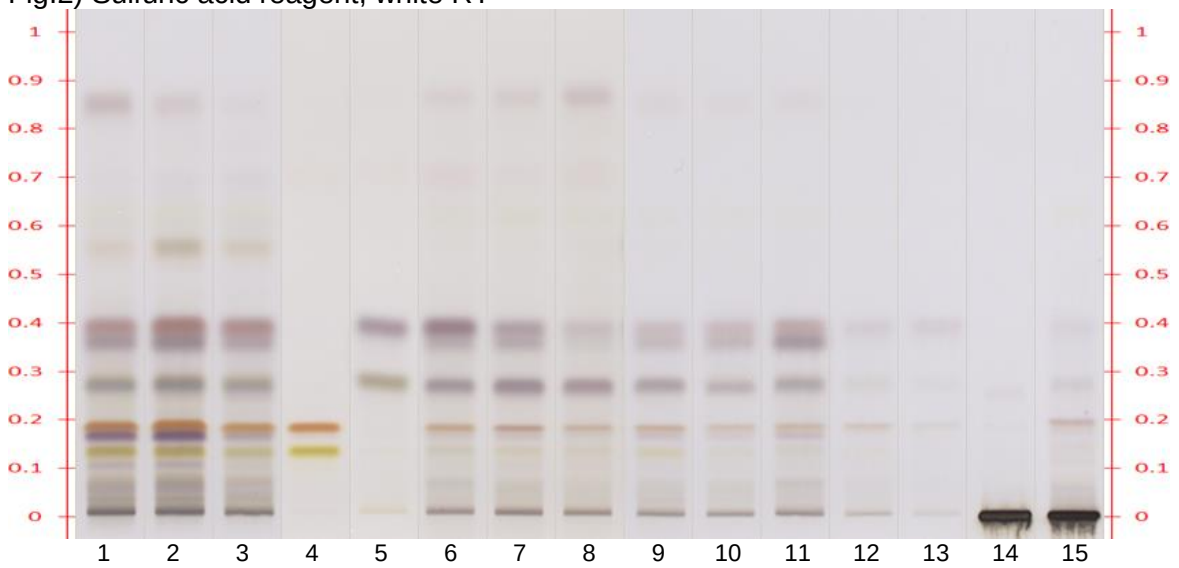


Fig.2) Sulfuric acid reagent, white RT



Track	Sample	Track	Sample
1	**Chinese Sage root 1**	9	Chinese Sage root 7
2	**Chinese Sage root 2**	10	Chinese Sage root 8
3	**Chinese Sage root 3**	11	Chinese Sage root 9
4	Dihydrotanshinone, cryptotanshinone (with increasing Rf)	12	Chinese Sage root granules 1
5	Tanshinone I, tanshinone IIA (with increasing Rf)	13	Chinese Sage root granules 2
6	Chinese Sage root 4	14	Chinese Sage root extract 1
7	Chinese Sage root 5	15	Chinese Sage root extract 2
8	Chinese Sage root 6		

System suitability test
Tanshinone I: brownish zone at Rf ~ 0.27 (derivatized, white RT).
Tanshinone IIA: violet zone at Rf ~ 0.39 (derivatized, white RT).
Dihydrotanshinone: yellow zone at Rf ~ 0.13 (derivatized, white RT).
Cryptotanshinone: reddish zone at Rf ~ 0.18 (derivatized, white RT).

Identification
Compare results with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under white light the chromatogram of the test solution shows distinct yellow to orange zones corresponding to reference substances dihydrotanshinone, cryptotanshinone, tanshinone I, and tanshinone IIA. Just below the position of cryptotanshinone there is another distinct yellow zone. Just below the position of tanshinone IIA a purple zone is seen. Additional yellow zones are detected between reference dihydrotanshinone and the start position.
After derivatization under white light the chromatogram of the test solution shows a yellow zone corresponding to reference dihydrotanshinone, an orange zone corresponding to reference cryptotanshinone, a greenish zone corresponding to reference tanshinone I and a purple zone corresponding to reference tanshinone IIA. Just below the position of cryptotanshinone there is a distinct violet zone. Just below the position of tanshinone IIA a bluish violet zone is seen. Weak grey zones are detected between reference dihydrotanshinone and the start position.

Salvia miltiorrhiza (Chinese Sage root, dan shen) Identification

1. Scope

This method identifies dried Chinese Sage root (Salvia miltiorrhiza Bunge) by HPTLC fingerprint (polar substances).

2. Source of method

Modified from PhEur 7 (change in preparation of sample/references and detection).

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 min, then centrifuge or filter the solutions and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of salvianolic acid B and 1 mg of tanshinone IIA in 1 mL of methanol. Optional: Dissolve 1 mg each of rutin and hyperoside in 1.5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references and test solutions
Mobile phase:	Toluene, dichloromethane, ethyl acetate, methanol, formic acid 4:6:8:1:4 (v/v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33% for optimum separation (the Rf-value of salvianolic acid B is influenced by the relative humidity)
Derivatization reagent:	Sulfuric acid reagent Preparation: 20 mL sulfuric acid in 180 mL methanol Use: The plate is immersed in sulfuric acid reagent for 1 s, then heated for 5 min at 100°C
Documentation:	1.) Clean plate (white RT and UV 254 nm) 2.) UV 254 nm 3.) Sulfuric acid reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 254 nm

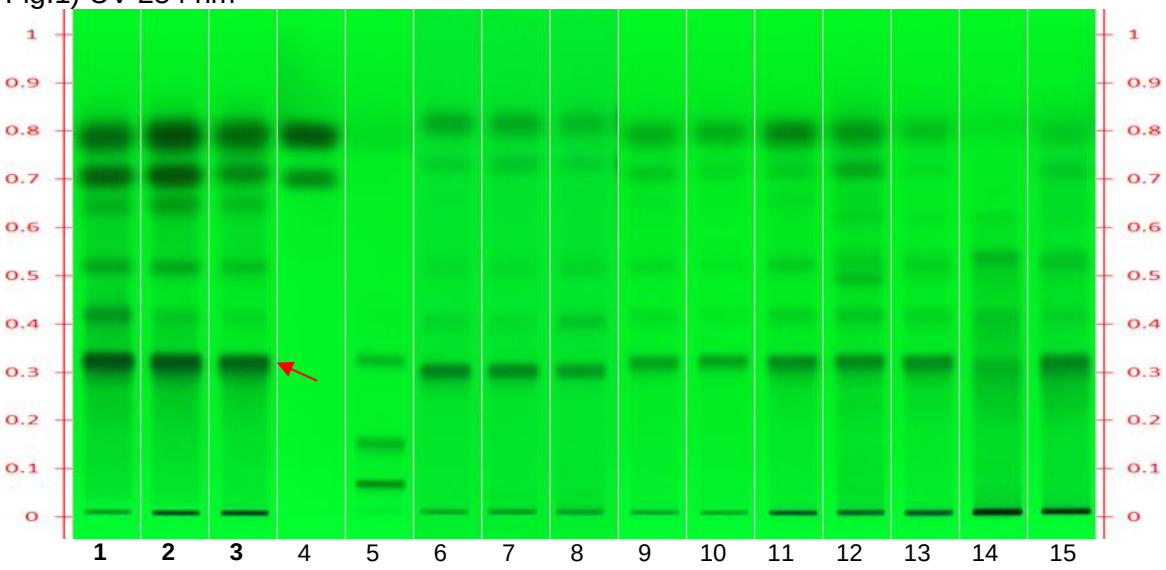
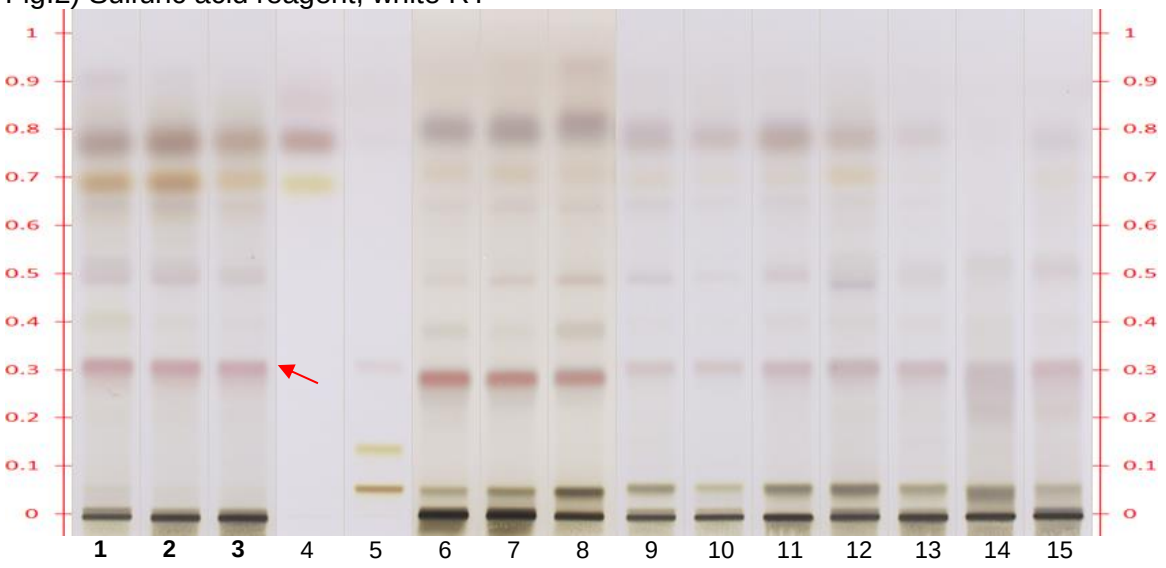


Fig.2) Sulfuric acid reagent, white RT



Track	Sample	Track	Sample
1	**Chinese Sage root 1**	9	Chinese Sage root 7
2	**Chinese Sage root 2**	10	Chinese Sage root 8
3	**Chinese Sage root 3**	11	Chinese Sage root 9
4	Dihydrotanshinone, tanshinone IIA (with increasing Rf)	12	Chinese Sage root granules 1
5	Rutin, hyperoside, salvianolic acid B (with increasing Rf)	13	Chinese Sage root granules 2
6	Chinese Sage root 4	14	Chinese Sage root extract 1
7	Chinese Sage root 5	15	Chinese Sage root extract 2
8	Chinese Sage root 6		

System suitability test
Salvianolic acid: a quenching zone at Rf ~ 0.30.
Tanshinone IIA: a quenching zone at Rf ~ 0.78.

Identification
Compare with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present. Under UV 254 nm a prominent quenching zone corresponding to salvianolic acid B is detected at Rf ~ 0.30 in all samples. After derivatization this zone appears reddish under white light (red arrows). Above the zone due to salvianolic acid there is a quenching zone at Rf ~0.51 (grey under white light) and possibly a weak quenching zone at Rf ~ 0.40. At the position of reference tanshinone IIA there is a prominent quenching zone which appears reddish under white light. Just below it there is another quenching zone which appears yellow under white light.
For detailed analysis of tanshinones and other apolar compounds method **Chinese Sage root (tanshinones)** can be used.

Salvia officinalis
(Sage leaf)

1. Scope

This method identifies dried Sage leaf (Salvia officinalis L.) by HPTLC fingerprint and discriminates dried Three-lobed sage leaf (Salvia fruticosa Mill. / Salvia triloba L. fil).

2. Source of method

CAMAG

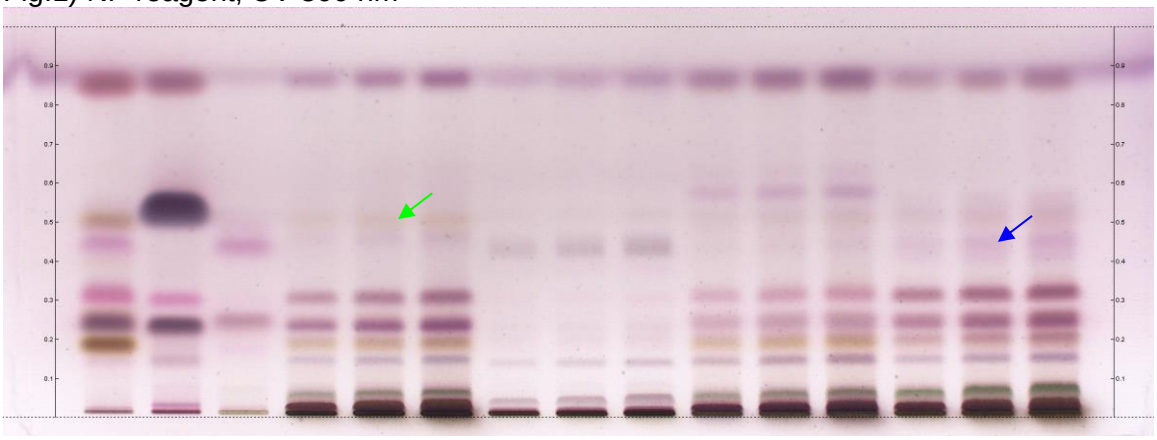
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 5 mg of thujon in 1 mL of methanol. Dissolve 25 µL of cineole in 10 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 7 µL of test solutions
Mobile phase:	Dichloromethane
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol add 20 mL of glacial acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: spray, heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldeyhde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	3µL	Sage oil	9	10µL	Clary sage leaf
2	3µL	Clary sage oil	10	5µL	Spanish sage leaf
3	10µL	Cineole, thujon (with increasing Rf)	11	7µL	Spanish sage leaf
4	5µL	Sage leaf	12	10µL	Spanish sage leaf
5	**7µL**	**Sage leaf**	13	5µL	Three-lobed sage leaf
6	10µL	Sage leaf	14	7µL	Three-lobed sage leaf
7	5µL	Clary sage leaf	15	10µL	Three-lobed sage leaf
8	7µL	Clary sage leaf			

System suitability test
Thujon: violet zone at Rf ~ 0.47.
Cineole: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a violet zone just below the solvent front. A weak yellow zone is seen at Rf ~ 0.50 (green arrow). There is a violet zone at Rf ~ 0.30 and slightly below reference cineole there is another violet zone. A yellow zone is seen at Rf ~ 0.20 and there is a blue violet zone just below it.

Test for other species
No violet zone is seen at the position of thujon (Three-lobed sage leaf, blue arrow).

Salvia officinalis
(Sage oil)

1. Scope

This method identifies the essential oil from Sage (Dalmatian Sage, Salvia officinalis L.) by HPTLC fingerprint and discriminates the essential oil from the other species Clary sage (Salvia sclarea L.), and Spanish sage (Salvia lavandulifolia Vahl).

2. Source of method

CAMAG

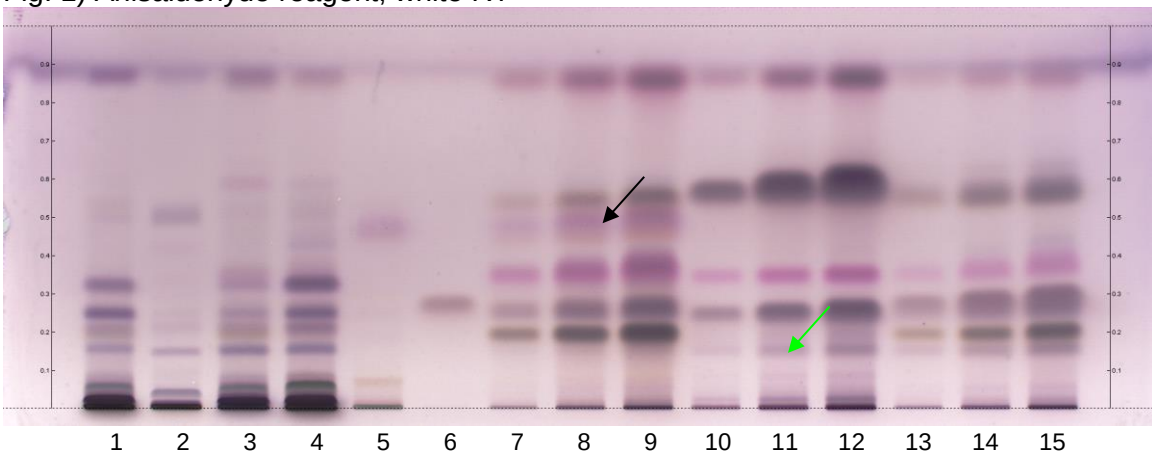
3. Procedure

Sample preparation:	Mix 100 µL of sample with 10 mL of toluene.
Reference substances:	Dissolve 1 mg of thujon in 1 mL of toluene. Dissolve 30 µL of cineole in 10 mL of toluene.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 3 µL of test solutions
Mobile phase:	Dichloromethane
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: To 170 mL of ice-cooled methanol add 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: spray, heat at 100°C for 5 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldeyhde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	7µL	Sage leaf	9	5µL	Sage oil
2	7µL	Clary sage leaf	10	1µL	Clary sage oil
3	7µL	Spanish sage leaf	11	3µL	Clary sage oil
4	7µL	Three-lobe sage leaf	12	5µL	Clary sage oil
5	2µL	Thujon	13	1µL	Spanish sage oil
6	2µL	Cineol	14	3µL	Spanish sage oil
7	1µL	Sage oil	15	5µL	Spanish sage oil
8	**3µL**	**Sage oil**			

System suitability test
Thujon: weak pink zone at Rf ~ 0.47.
Cineole: violet zone at Rf ~ 0.27.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a grey zone at Rf ~ 0.54. At the position of reference thujon there is a diffuse pink zone (black arrow). Below it there is another pink zone. Just below the position of reference cineole a violet zone is seen. Below this zone there is an intense grey zone at Rf ~ 0.20.

Test for other species
No zone is seen below the grey zone at Rf ~ 0.20 (green arrow, Clary sage oil).
The chromatograms of Spanish sage oil and Clary sage oil lack the zone at the position of thujon.

Saposhnikovia divaricate (Saposhnikovia root, Fang feng)

1. Scope

This method identifies dried Saposhnikovia root (Saposhnikovia divaricata (Turcz.) Schischk.) by HPTLC fingerprint and detects discriminates dried Caraway root (Carum carvi L.), dried Wild Carrot root (Daucus carota L.) and dried Seseli mairei H. Wolff root.

2. Source of method

University of Regensburg

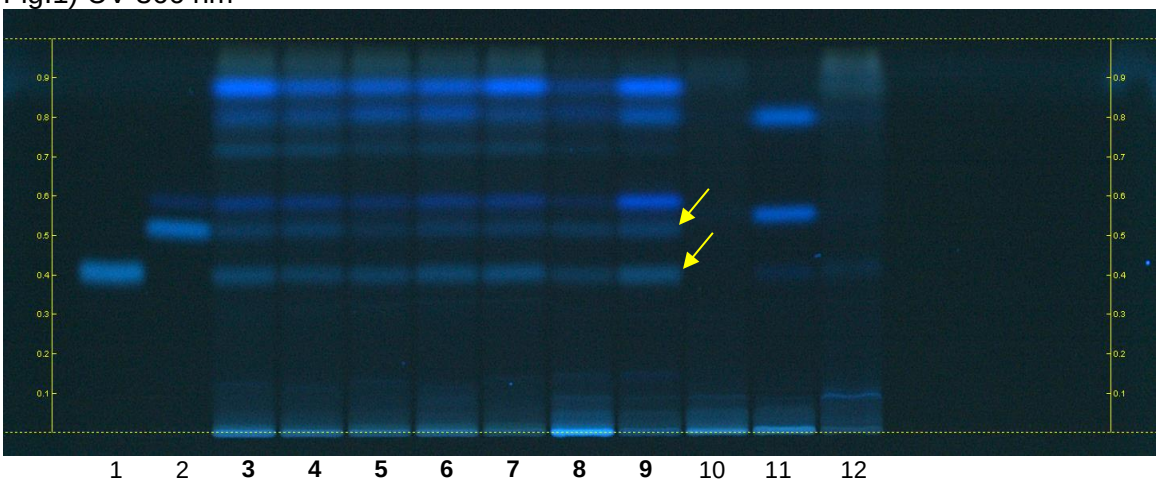
3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 10 mL of methanol and sonicate for 30 minutes, then centrifuge or filter the solutions and use the supernatant / filtrate as test solution.
Reference substances:	Dissolve 1.0 mg of Prim-O-glucosylcimifugin in 1 mL of methanol. Dissolve 1.0mg of 4'-O-β-D-Glucosyl-5-O-methylvisamminol acid in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	7.5 µL of references, 7.5 µL of test solutions
Mobile phase:	Dichloromethane, methanol, acetonitrile 4:2:1 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent, dry in air
Documentation:	1.) UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	7.5 µL	Prim-O-glucosylcimifugin	**7**	**7.5 µL**	**Saposhnikovia root, Chinese 5**
2	7.5 µL	4'-O-β-D-Glucosyl-5-O-methylvisamminol	**8**	**7.5 µL**	**Saposhnikovia root, Chinese 6**
3	**7.5 µL**	**Saposhnikovia root, Chinese 1, BRM**	**9**	**7.5 µL**	**Saposhnikovia root, German**
4	**7.5 µL**	**Saposhnikovia root, Chinese 2**	10	7.5 µL	Caraway root
5	**7.5 µL**	**Saposhnikovia root, Chinese 3**	11	7.5 µL	Wild Carrot root
6	**7.5 µL**	**Saposhnikovia root, Chinese 4**	12	7.5 µL	*Seseli mairei* root

System suitability test

Prim-O-glucosylcimifugin: fluorescent zone at Rf ~ 0.41
4'-O-β-D-Glucosyl-5-O-methylvisamminol: fluorescent zone at Rf ~ 0.51

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows blue fluorescent zones at Rf ~ 0.41 and Rf ~ 0.51 corresponding to reference substances prim-O-glucosylcimifugin and 4'-O-β-D-Glucosyl-5-O-methylvisamminol (yellow arrows). Several blue fluorescent zones are detected above the position of reference substance 4'-O-β-D-Glucosyl-5-O-methylvisamminol.

Test for adulteration

Caraway root, Wild Carrot root and Seseli mairei root show no blue zone at the position of reference substances prim-O-glucosylcimifugin and 4'-O-β-D-Glucosyl-5-O-methylvisamminol.

Silybum marianum
(Milk thistle fruit)

1. Scope

This method identifies Milk thistle fruit (Silybum marianum (L.) Gaertn.) by HPTLC fingerprint.

2. Source of method

CAMAG Method of Analysis MOA008

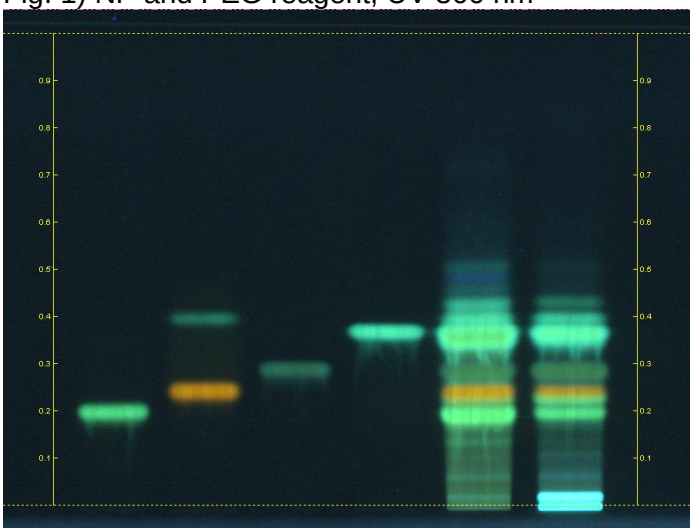
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and heat at 70°C for 5 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of silybin in 10 mL of methanol. Dissolve 1 mg of silychristin in 10 mL of methanol. Optional: individually dissolve 1 mg of silydianin and taxifolin each in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	10 µL of references, 10 µL of test solutions
Mobile phase:	Chloroform, acetone, formic acid 75:16.5:8.5 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of methylene chloride Use: Heat plate 3 min at 100 °C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	10 µL	Silychristin	9		Blank
2	10 µL	Taxifolin	10		Blank
3	10 µL	Silydianin	11		Blank
4	10 µL	Silybin	12		Blank
5	10 µL	Milk thistle extract	13		Blank
6	**10 µL**	**Milk thistle fruit**	14		Blank
7		Blank	15		Blank
8		Blank			

System suitability test

Silychristin: green fluorescent zone at Rf ~ 0.20
Silybin: green fluorescent zone at Rf ~ 0.38

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a green zone at Rf ~ 0.20 corresponding to reference substance silychristin. There is a green zone at Rf ~ 0.29 (silydianin) and another intense green zone at Rf ~ 0.38 corresponding to reference substance silybin. Reference substance taxifolin shows two zones, an orange zone at Rf ~ 0.26 and a green zone at Rf ~ 0.40 which are both visible in the chromatogram of the test solution.

Solidago gigantean
(Giant goldenrod herb)

1. Scope

This method identifies dried Giant goldenrod herb (Solidago gigantea Ait) by HPTLC fingerprint and distinguishes European goldenrod herb (Solidago virgaurea L.).

2. Source of method

Modified from Ph. Eur 6.7 (change in sample preparation and reference substances)

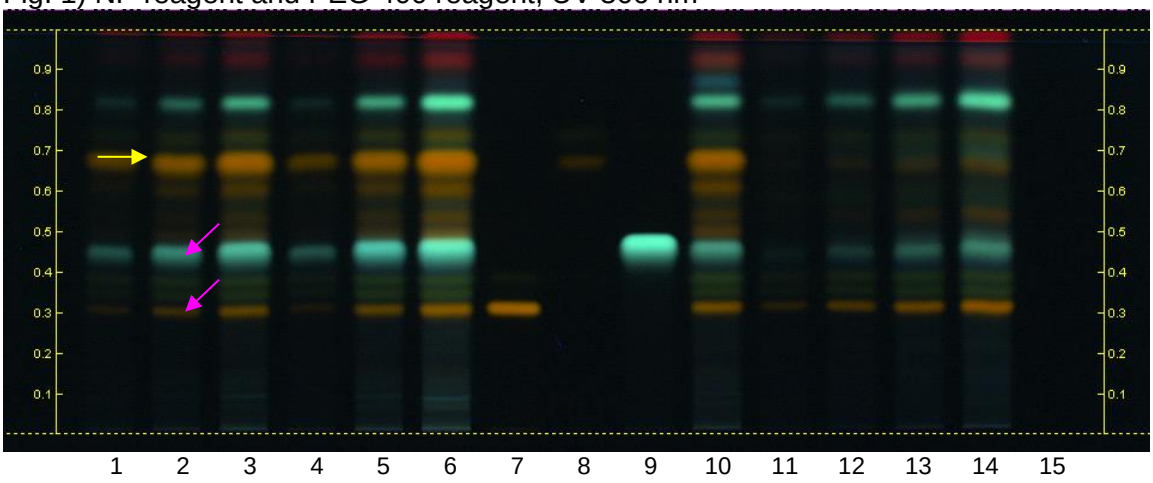
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	chlorogenic acid each in 1 mL of methanol. Optional: dissolve 1 mg of quercitrin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Formic acid, water, methyl ethyl ketone, ethyl acetate 6:6:18:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate. 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane. Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP reagent and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent and PEG 400 reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Giant goldenrod herb 1	9	5 µL	Chlorogenic acid
2	**5 µL**	**Giant goldenrod herb 1**	10	5 µL	Giant goldenrod herb 2
3	7 µL	Giant goldenrod herb 1	11	5 µL	Goldenrod herb (old sample)
4	1 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	12	7 µL	Goldenrod herb (old sample)
5	3 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	13	5 µL	European goldenrod herb 1
6	5 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	14	7 µL	European goldenrod herb 1
7	5 µL	Rutin	15		Blank
8	5 µL	Quercitrin			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.30.
Chlorogenic acid: green fluorescent zone at Rf ~ 0.48.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an orange zone at Rf ~ 0.30 corresponding to reference substance rutin and a green zone at Rf ~ 0.48 corresponding to chlorogenic acid (pink arrows). Above it there are several faint brown zones and an intense brown zone (Rf ~ 0.67) at the position of quercitrin (yellow arrow). A green zone is seen at Rf ~ 0.82.

Test for other species
European goldenrod herb does not show an intense brown zone at Rf ~ 0.67.

Solidago virgaurea
(European goldenrod herb)

1. Scope

This method identifies dried European goldenrod herb (Solidago virgaurea L.) by HPTLC fingerprint and detects the adulterants dried Giant goldenrod herb (Solidago gigantea Ait) and Canadian goldenrod herb (Solidago canadensis L. var. canadensis).

2. Source of method

Modified from Ph. Eur 6.7 (change in sample preparation and reference substances)

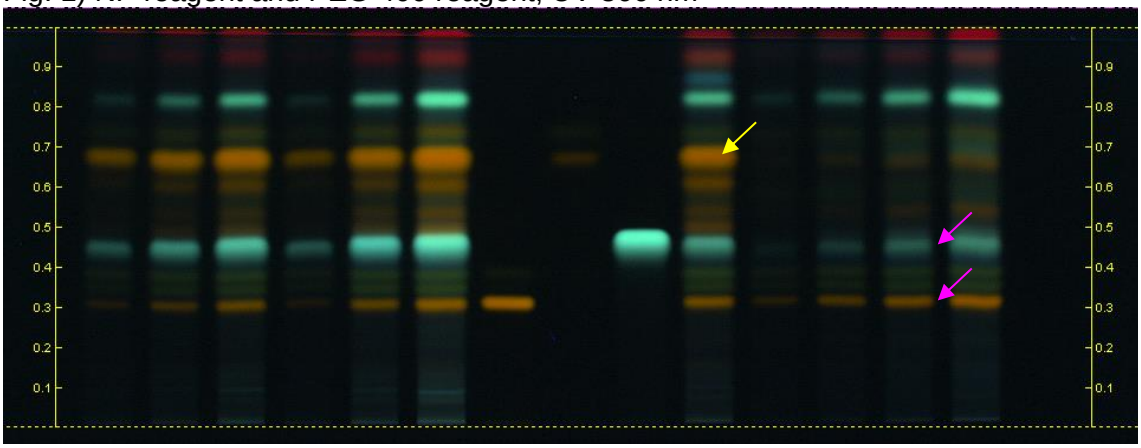
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Individually dissolve 1 mg of rutin and 1 mg of chlorogenic acid each in 1 mL of methanol. Optional: dissolve 1 mg of quercitrin in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Formic acid, water, methyl ethyl ketone, ethyl acetate 6:6:18:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: : 1 g of natural products reagent in 200 mL of ethyl acetate. 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane. Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP reagent and PEG reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP reagent and PEG 400 reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	Giant goldenrod herb 1	9	5 µL	Chlorogenic acid
2	5 µL	Giant goldenrod herb 1	10	5 µL	Giant goldenrod herb 2
3	7 µL	Giant goldenrod herb 1	11	5 µL	Goldenrod herb (old sample)
4	1 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	12	7 µL	Goldenrod herb (old sample)
5	3 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	**13**	**5 µL**	**European goldenrod herb 1**
6	5 µL	Giant goldenrod herb 1 (Ph.Eur. extraction)	14	7 µL	European goldenrod herb 1
7	5 µL	Rutin	15		Blank
8	5 µL	Quercitrin			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.30.
Chlorogenic acid: green fluorescent zone at Rf ~ 0.48.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an orange zone at Rf ~ 0.30 corresponding to reference substance rutin and a green zone at Rf ~ 0.48 corresponding to chlorogenic acid (pink arrows). Above it there are two faint brown zones, the upper one at Rf ~ 0.67 at the position of reference quercitrin (faint brown zone). Another green zone is seen at Rf ~ 0.82.

Test for other species
No intense orange zone is seen at Rf ~ 0.67 (Giant goldenrod herb).

Tanacetum parthenium (Feverfew flower)

1. Scope

This method identifies dried Feverfew flower (Tanacetum parthenium (L.) Sch. Bip.) by HPTLC fingerprint and discriminates the adulterants dried Feverfew flower from Mexico (Tanacetum parthenium), Roman Chamomile flower (Chamaemelum nobile (L.) All.), Chamomile flower (Matricaria recutita L.) and Chamomile flower oil (Matricaria recutita L.).

2. Source of method

CAMAG Method of Analysis MOA009

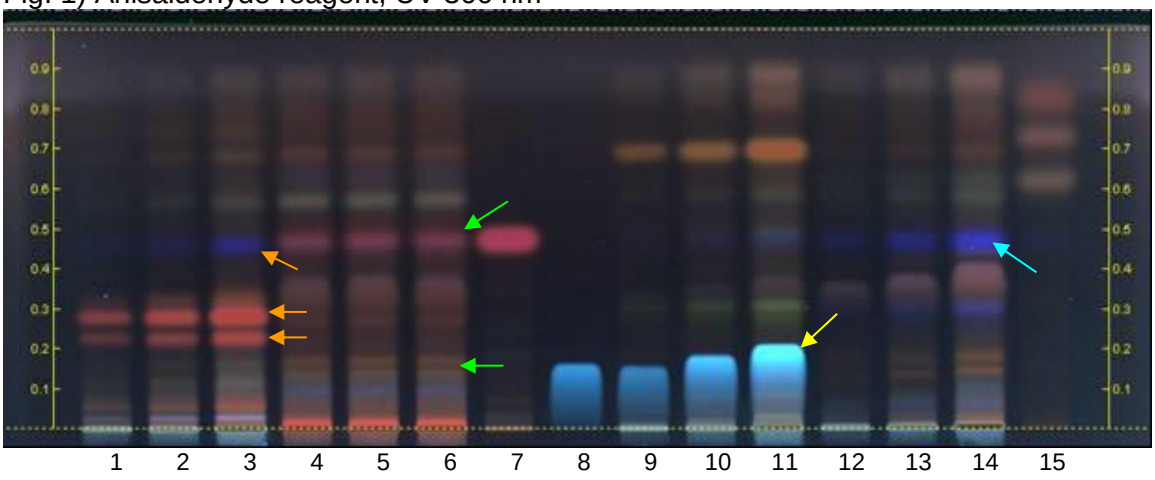
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions. Dissolve 10 µL of the essential oil in 1 mL of toluene.
Reference substances:	Dissolve 1.5 mg of apigenin in 5 mL of methanol. Dissolve 1 mg of parthenolide in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 2 µL of test solutions
Mobile phase:	Ethyl acetate, cyclohexane 1:1 (v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 4 min
Documentation:	1.) Clean plate, white RT 2.) Anisaldehyde reagent, UV 366 nm 3.) Anisaldehyde reagent, white RT

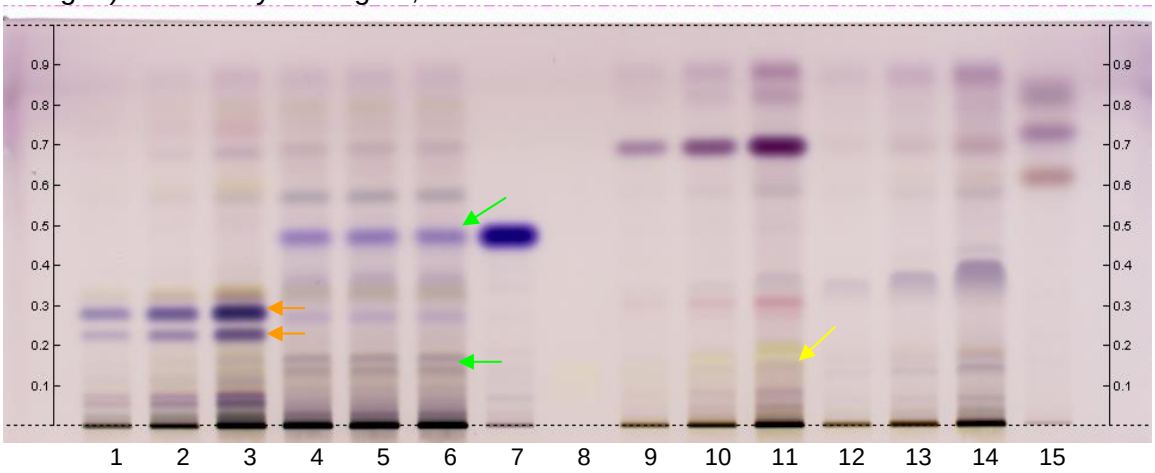
4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, UV 366 nm



Fi Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	1 µL	Feverfew flower from Mexico	9	1 µL	Roman Chamomile flower
2	2 µL	Feverfew flower from Mexico	10	2 µL	Roman Chamomile flower
3	4 µL	Feverfew flower from Mexico	11	4 µL	Roman Chamomile flower
4	**3 µL**	**Feverfew flower**	12	1 µL	Chamomile flower
5	3.5 µL	Feverfew flower	13	2 µL	Chamomile flower
6	4 µL	Feverfew flower	14	4 µL	Chamomile flower
7	2 µL	Parthenolide	15	1 µL	Chamomile flower oil
8	2 µL	Apigenin			

System suitability test
Apigenin: blue zone at Rf ~ 0.20
Parthenolide: pink zone at Rf ~ 0.48

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 366 nm the chromatogram of the test solution shows a prominent pink zone (violet zone under white light) at Rf ~ 0.48 corresponding to reference substance parthenolide. Above it there is a greenish zone (grey zone under white light) and a weak brownish zone (violet zone under white light). At the position of reference substance apigenin (Rf ~ 0.20) there are two brown zones (grey zone under white light) (green arrows).

Test for adulteration
Under UV 366 nm there are no intense red zones (violet zone under white light) between Rf ~ 0.20 and 0.30. No blue zone is seen at the position of parthenolide (orange arrows; Feverfew flower from Mexico).
Under UV 366 nm no blue zone at the position of apigenin (Rf ~ 0.20) is seen (yellow arrow; Roman Chamomile flower).
Under UV 366 nm no blue zone is seen at the position of parthenolide (blue arrow; Chamomile flower).
Chamomile flower oil does not show any zones below Rf ~ 0.60.

Terminalia belleric
(Belleric myrobalan fruit, mao he zi)

1. Scope

This method identifies dried fruit of Belleric myrobalan (Terminalia bellerica (Gaertn.) Roxb.) and discriminates dried fruit of Chebulic myrobalan (Terminalia chebula (Gaertn.) Retz) and Amla (Phyllanthus emblica L.) by HPTLC fingerprint.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chebulinic acid and 2 mg gallic acid in 10 mL of methanol. Optional: dissolve 1 mg of ellagic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 1 µL of test solutions
Mobile phase:	Ethyl formate, toluene, formic acid, water 30:1.5:4:3 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry, dip (time 0, speed 5) in PEG reagent
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

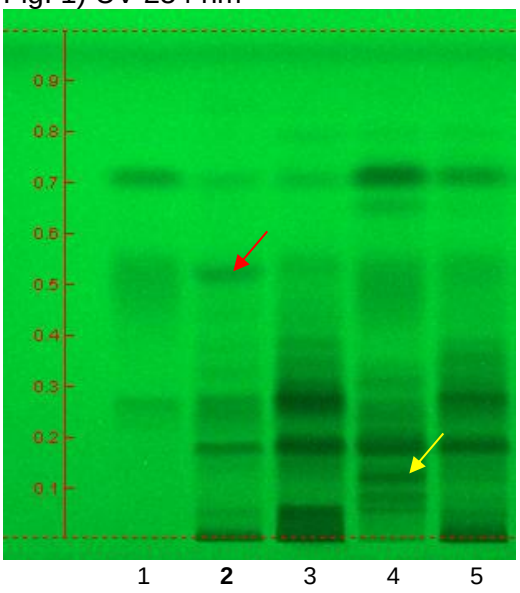
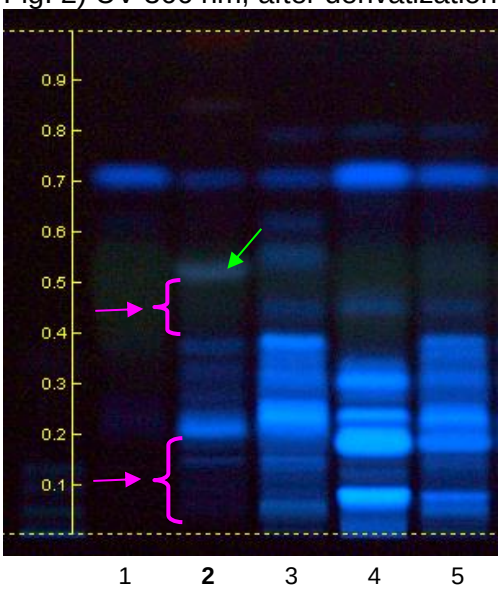


Fig. 2) UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Chebulinic acid, ellagic acid, and gallic acid (increasing Rf)	9		Blank
2	**1 µL**	**Belleric myrobalan fruit**	10		Blank
3	1 µL	Chebulic myrobalan fruit	11		Blank
4	1 µL	Amla fruit	12		Blank
5	1 µL	Triphala powder	13		Blank
6		Blank	14		Blank
7		Blank	15		Blank
8		Blank			

System suitability test

Chebulinic acid: a quenching zone at Rf ~ 0.26 (UV 245 nm).
Gallic acid: a quenching zone at Rf ~ 0.71 (UV 245 nm).

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the test solution shows several quenching zones between Rf ~ 0.18 and 0.28. A characteristic well defined quenching zone at Rf ~ 0.53 is present (red arrow). At the position of gallic acid a faint dark zone is visible. Under UV 366 nm there is a blue fluorescent zone at Rf ~ 0.21 and a characteristic fluorescent zone at Rf ~ 0.53 (green arrow).

Test for other species

Under UV 366 nm there are no fluorescent zones between Rf ~ 0.40 and 0.52 and below Rf ~ 0.14 (Chebulic myrobalan fruit, Amla fruit, pink arrows).

Terminalia chebula
(Chebulic myrobalan fruit, he zi)

1. Scope

This method identifies dried fruit of Chebulic myrobalan (Terminalia chebula (Gaertn) Retz) and discriminates dried fruit of Belleric myrobalan (Terminalia bellerica (Gaertn) Roxb) and Amla (Phyllanthus emblica L.) by HPTLC fingerprint.

2. Source of method

CAMAG

3. Procedure

Sample preparation:	Mix 1.0 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter and use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 1 mg of chebulinic acid and 2 mg gallic acid in 10 mL of methanol. Optional: dissolve 1 mg of ellagic acid in 10 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 1 µL of test solutions
Mobile phase:	Ethyl formate, toluene, formic acid, water 30:1.5:4:3 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry, dip (time 0, speed 5) in PEG reagent
Documentation:	1.) Clean plate, UV 254 nm 2.) UV 254 nm 3.) NP reagent, UV 366 nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

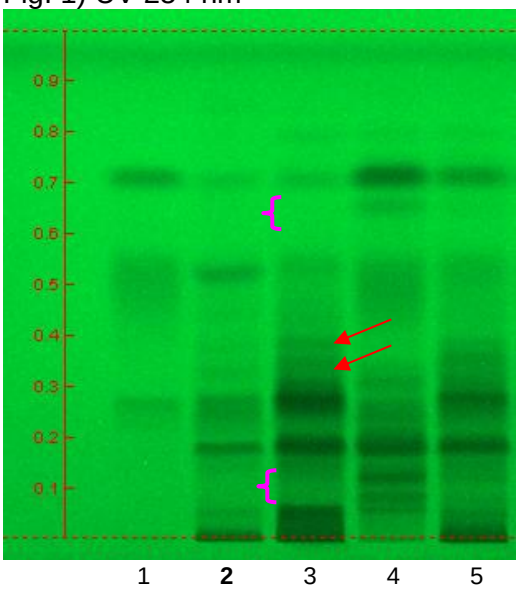
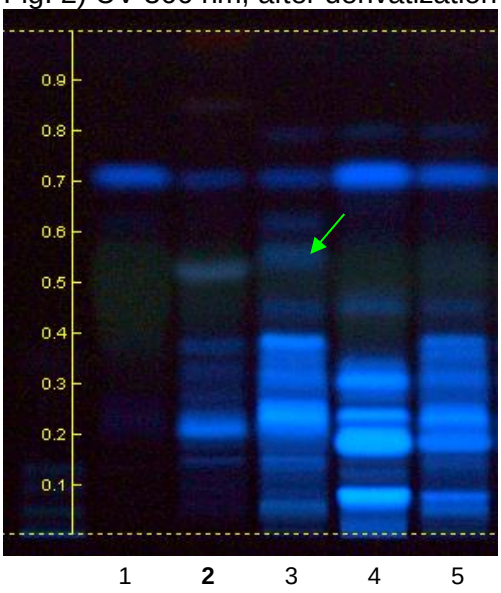


Fig. 2) UV 366 nm, after derivatization



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Chebulinic acid, ellagic acid, and gallic acid (increasing Rf)	9		Blank
2	1 µL	Belleric myrobalan fruit	10		Blank
3	**1 µL**	**Chebulic myrobalan fruit**	11		Blank
4	1 µL	Amla fruit	12		Blank
5	1 µL	Triphala powder	13		Blank
6		Blank	14		Blank
7		Blank	15		Blank
8		Blank			

System suitability test

Chebulinic acid: a quenching zone at Rf ~ 0.26 (UV 245 nm).
Gallic acid: a quenching zone at Rf ~ 0.71(UV 245 nm).

Identification

Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the test solution shows three intense quenching zones at Rf ~ 0.05, 0.19 and 0.28. A characteristic quenching double zone is present at Rf ~ 0.40/0.38 (red arrows). At the position of gallic acid a quenching zone is visible. Under UV 366 nm there are blue fluorescent zones at Rf ~ 0.23 and Rf ~ 0.38 and a characteristic fluorescent zone at Rf ~ 0.53 (green arrow).

Test for other species

Under UV 254 nm there is no zone between Rf ~ 0.08 and 0.12 and no zone at Rf ~ 0.66 (Amla fruit, pink arrows). Under UV 366 nm no fluorescent zone is detectable at Rf ~ 0.52 (Belleric myrobalan fruit).

Tilia cordata, Tilia platyphyllos, Tilia × vulgaris, or mixtures (Lime flower)

1. Scope

This method identifies dried Lime flower (Tilia cordata Miller, Tilia platyphyllos Scop., Tilia x vulgaris, or mixtures) by HPTLC fingerprint.

2. Source of method

Modified from Ph.Eur. 6.7 (change in sample preparation).

3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter, use the supernatants/filtrates as test solutions.
Reference substances:	Dissolve 2 mg of caffeic acid in 5 mL of methanol. Dissolve 2.5 mg of rutin in 5 mL of methanol. Optional: 4 mg of hyperoside in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 8 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water, ethyl methyl ketone 50:10:10:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of NP reagent in 200 ml ethyl acetate 2.) PEG reagent Preparation10 g of polyethylene glycol 400 in 200 mL methylene chloride Use: Heat plate 3 min at 100 °C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) Clean plate, white RT 2.) NP and PEG reagent, UV 366 nm 3.) NP and PEG reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm

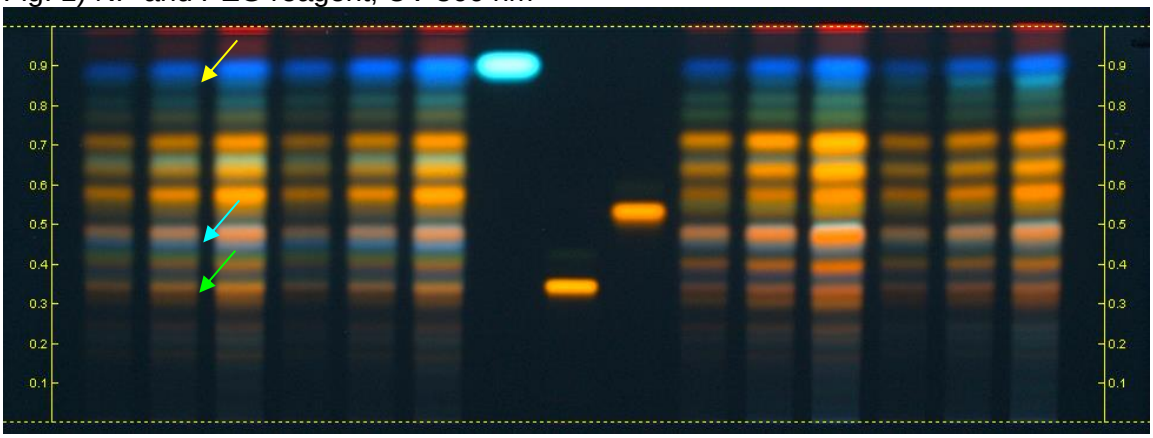
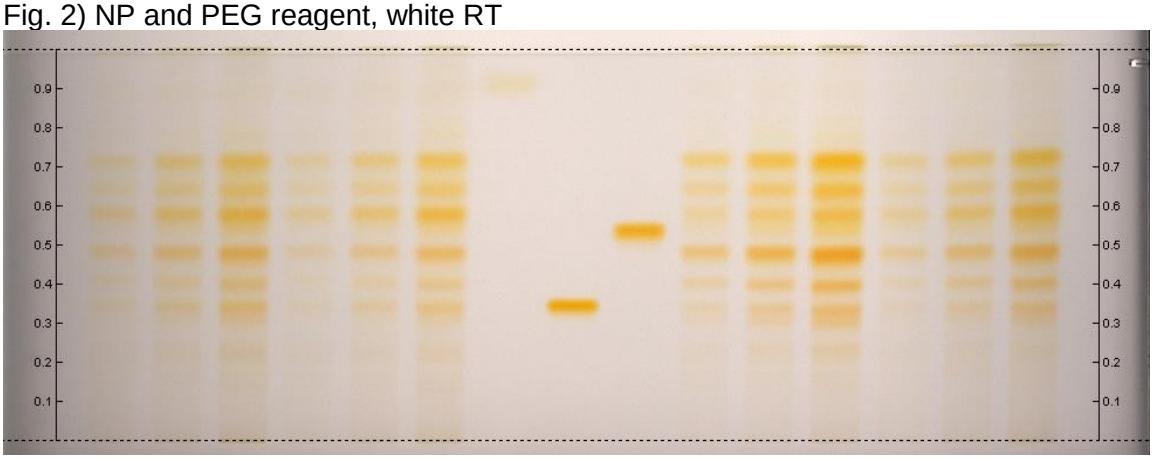


Fig. 2) NP and PEG reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Lime flower 1	9	2 µL	Hyperoside
2	**8 µL**	**Lime flower 1**	10	4 µL	Lime flower 2
3	16 µL	Lime flower 1	**11**	**8 µL**	**Lime flower 2**
4	1 µL	Lime flower 1 (Ph. Eur extraction)	12	16 µL	Lime flower 2
5	2 µL	Lime flower 1 (Ph. Eur extraction)	13	4 µL	Lime flower 3
6	4 µL	Lime flower 1 (Ph. Eur extraction)	**14**	**8 µL**	**Lime flower 3**
7	2 µL	Caffeic acid	15	16 µL	Lime flower 3
8	2 µL	Rutin			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.35
Caffeic acid: bluish fluorescent zone at Rf ~ 0.90

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 366 nm the chromatogram of the test solution shows an orange zone at Rf ~ 0.35 corresponding to reference substance rutin (green arrow). Above it there are two orange zones at Rf ~ 0.40 and Rf ~ 0.49 (blue arrow) and a blue zone in between at Rf ~ 0.46. At the position of reference substance hyperoside there may be a faint orange zone. Above the position of hyperoside there are three orange zones. Right below the solvent front and just below the position of reference substance caffeic acid there is a blue zone at Rf ~ 0.89 (yellow arrow). Under white light the orange fluorescent zones are detected as yellow zones.

Triticum aestivum (Wheat grass)

1. Scope

This method identifies dried Wheat grass (Triticum aestivum L.) by HPTLC fingerprint and detects dried Oat herb (Avena sativa L.) and dried Barley grass (Hordeum vulgare L.).

2. Source of method

CAMAG, under evaluation by Ph. Eur

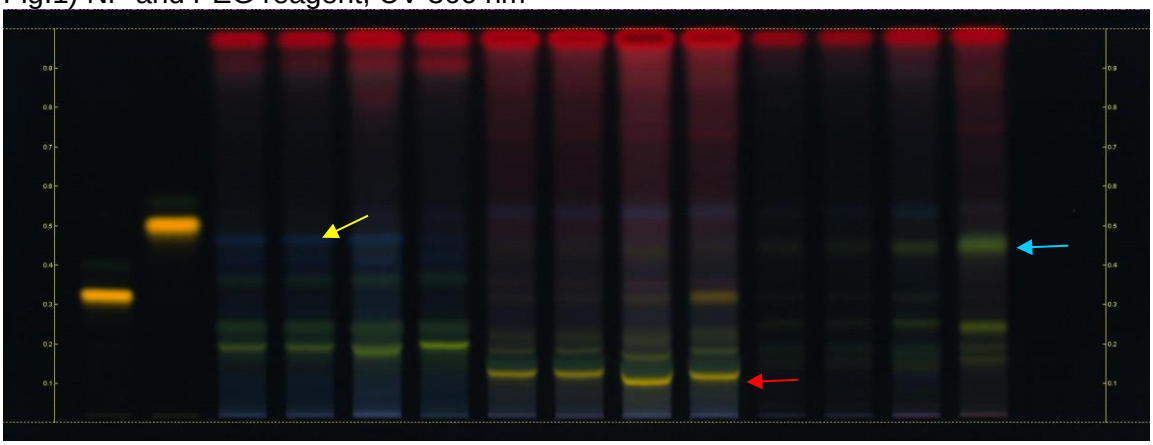
3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of rutin in 1mL of methanol. Dissolve 1 mg of hyperoside in 1mLof methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	4 µL of references, 10 µL of test solutions
Mobile phase:	Formic acid, water, methyl ethyl ketone, ethyl acetate 10 :10 :30 :50 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL ethyl acetate 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL dichloromethane Use: Heat plate 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent
Documentation:	1.) NP/PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	4 µL	Rutin	9	15 µL	Barley grass #1
2	4 µL	Hyperoside	10	10 µL	Barley grass #2
3	7 µL	Oat herb #1	11	7 µL	Wheat grass #1
4	10 µL	Oat herb #1	12	10 µL	Wheat grass #1
5	15 µL	Oat herb #1	13	15 µL	Wheat grass #1
6	10 µL	Oat herb #2	**14**	**10 µL**	**Wheat grass #2**
7	7 µL	Barley grass #1	15		Blank
8	10 µL	Barley grass #1			

System suitability test
Rutin: orange fluorescent zone at Rf ~ 0.32.
Hyperoside: orange fluorescent zone at Rf ~ 0.50.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a green zone at Rf ~ 0.46 (blue arrow). There are three green zones between Rf ~ 0.25 and Rf ~ 0.15.

Test for other species
No blue zone is seen between the position of references rutin and hyperoside (Oat herb, yellow arrow). No zone is seen between the application position and the green zone at Rf ~ 0.15 (Barley grass, red arrow).

Malva sylvestris
(Mallow flower)

1. Scope

This method identifies the whole dried flower of mallow (Malva sylvestris L.) by HPTLC fingerprint and discriminates dried ripe Bilberry fruit (Vaccinium myrtillus L.) and dried Roselle flower (Hibiscus sabdariffa L.).

2. Source of method

CAMAG

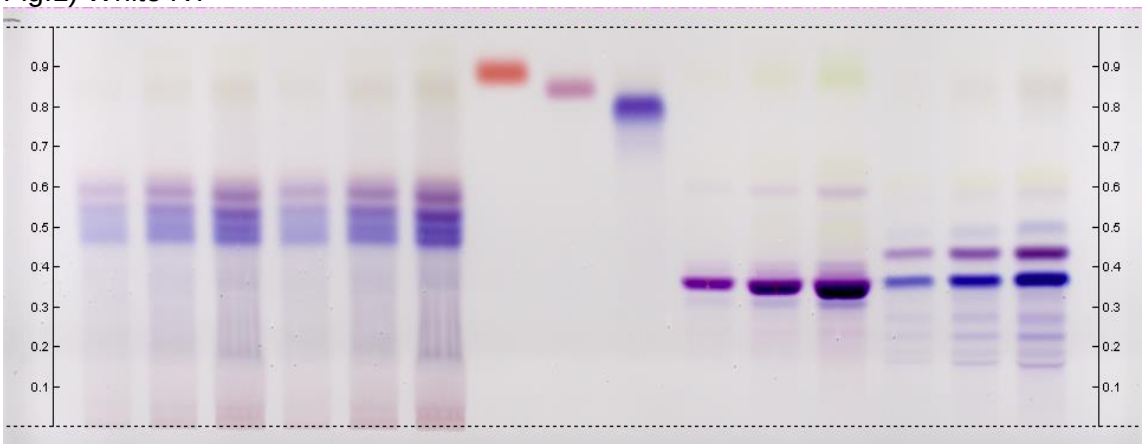
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 2 mg of pelargonin in 5 mL of methanol. Dissolve 2 mg of delphinidin in 5 mL of methanol. Optional: dissolve 2 mg of malvidin in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	1-Butanol, formic acid, water 65:16:19 (v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	no derivatization
Documentation:	1.) Clean plate, white RT 2.) Developed plate, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig.1) White RT



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Bilberry fruit (Ph.Eur. extraction)	9	2 µL	Delphinidin
2	3 µL	Bilberry fruit (Ph.Eur. extraction)	10	2 µL	Mallow flower
3	4 µL	Bilberry fruit (Ph.Eur. extraction)	11	4 µL	Mallow flower
4	2 µL	Bilberry fruit	12	6 µL	Mallow flower
5	**3 µL**	**Bilberry fruit**	13	2 µL	Roselle flower
6	4 µL	Bilberry fruit	14	4 µL	Roselle flower
7	2 µL	Pelargonin	15	6 µL	Roselle flower
8	2 µL	Malvidin			

System suitability test
Pelargonin: red to orange zone at Rf ~ 0.89
Delphinidin: violet zone at Rf ~ 0.80.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows four violet to violet-blue zones between Rf ~ 0.46 and 0.58.

Test for adulteration
No intense violet zone is seen at Rf ~ 0.35 (Mallow flower). There is neither a blue zone at Rf ~ 0.37 nor a violet zone at Rf ~ 0.44 (Roselle flower).

Verbascum thapsus L., V. densiflorum Bertol. syn. V. thapsiforme Schrad, V. phlomoides L. (Mullein flower)

1. Scope

This method identifies dried Mullein flower (Verbascum thapsus L., V. densiflorum Bertol. syn. V. thapsiforme Schrad, and V. phlomoides L.) by HPTLC fingerprint.

2. Source of method

Ph.Eur 6.7 (change in preparation of samples and reference substances)

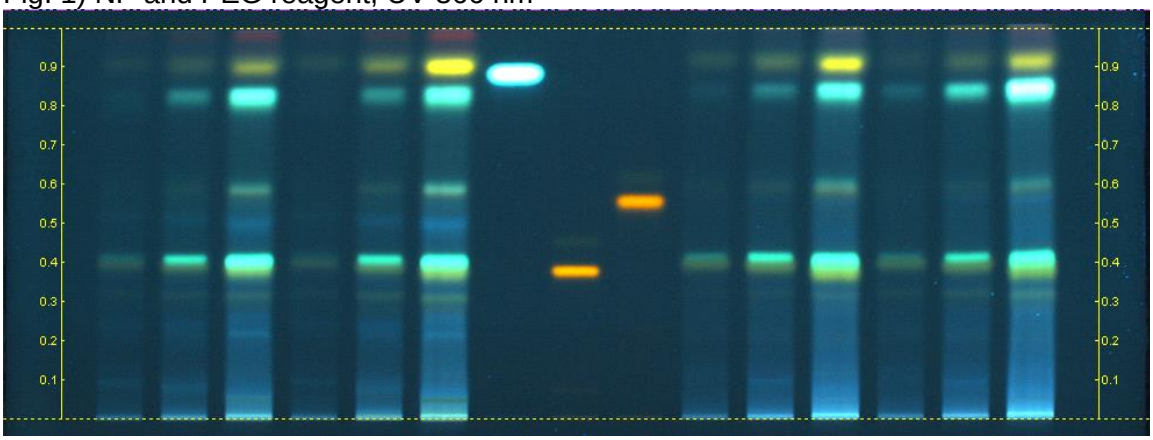
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 10 mL of methanol and sonicate at 60°C for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 3.5 mg of rutin in 5 mL of methanol. Dissolve 2 mg of caffeic acid in 5 mL of methanol. Optional: 4 mg of hyperoside in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 4 µL of test solutions
Mobile phase:	Ethyl acetate, formic acid, water, ethyl methyl ketone 50:10:10:30 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of NP reagent in 200 mL ethyl acetate. 2.) PEG reagent Preparation: 10g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, then dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent.
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Mullein flower 1	9	2 µL	Hyperoside
2	**4 µL**	**Mullein flower 1**	10	2 µL	Mullein flower 2
3	8 µL	Mullein flower 1	11	4 µL	Mullein flower 2
4	1 µL	Mullein flower 1 (Ph. Eur extr.)	12	8 µL	Mullein flower 2
5	3 µL	Mullein flower 1 (Ph. Eur extr.)	13	2 µL	Mullein flower 3
6	6 µL	Mullein flower 1 (Ph. Eur extr.)	14	4 µL	Mullein flower 3
7	2 µL	Caffeic acid	15	8 µL	Mullein flower 3
8	2 µL	Rutin			

System suitability test

Rutin: orange fluorescent zone at Rf ~ 0.38.
Caffeic acid: blue fluorescent zone at Rf ~ 0.89.

Identification

Compare result with reference images in Image Comparison Viewer. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows an intense bluish zone at Rf ~ 0.40 just above the position of reference substance rutin. There is another bluish zone at Rf ~ 0.60 slightly higher than the position of reference substance hyperoside. Just below the position of reference substance caffeic acid there is an intense bluish zone at Rf ~ 0.82 and just above caffeic acid there is a yellow zone at Rf ~ 0.90.

Verbena officinalis
(European vervain herb)

1. Scope

This method identifies dried European vervain herb (Verbena officinalis L.) by HPTLC fingerprint and detects the adulterant Lemon verbena leaf (Aloysia citriodora Palau (syn. Aloysia triphylla (L'Hér.) Kuntze; Verbena triphylla L'Hér.; Lippia citriodora Kunth.).

2. Source of method

Modified from Ph. Eur 6.7 (change in preparation of samples and references).

3. Procedure

Sample preparation:	Mix 500 mg of powdered sample with 5 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Dissolve 1 mg of rutin in 5 mL of methanol. Dissolve 1 mg of arbutin in 5 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	5 µL of references, 5 µL of test solutions
Mobile phase:	Formic acid, acetic acid, water, ethyl acetate 11:11:27:100 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of anisaldehyde. Use: Dip (time 0, speed 5), heat at 100°C for 5 min.
Documentation:	1.) Clean plate, white RT and UV 254 nm 2.) UV 254 nm 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) UV 254 nm

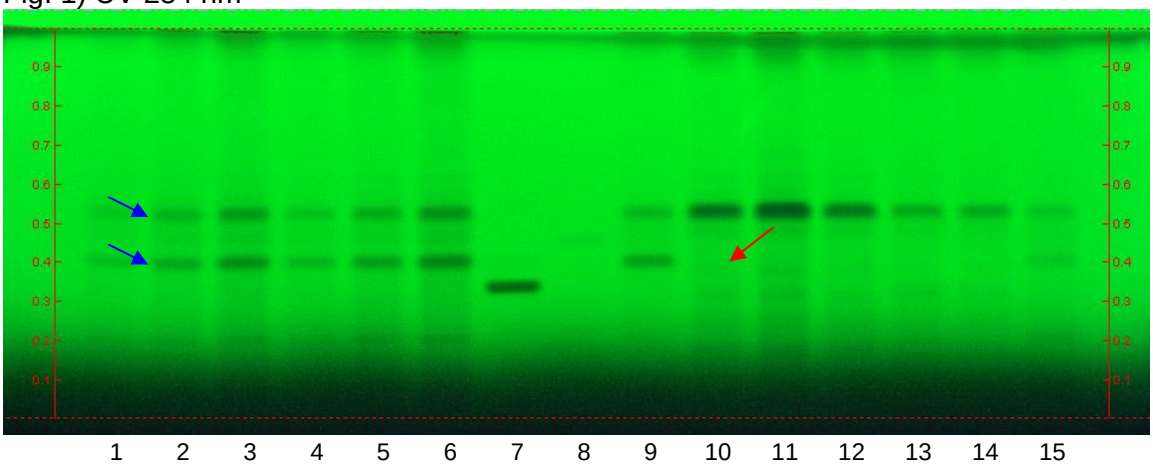
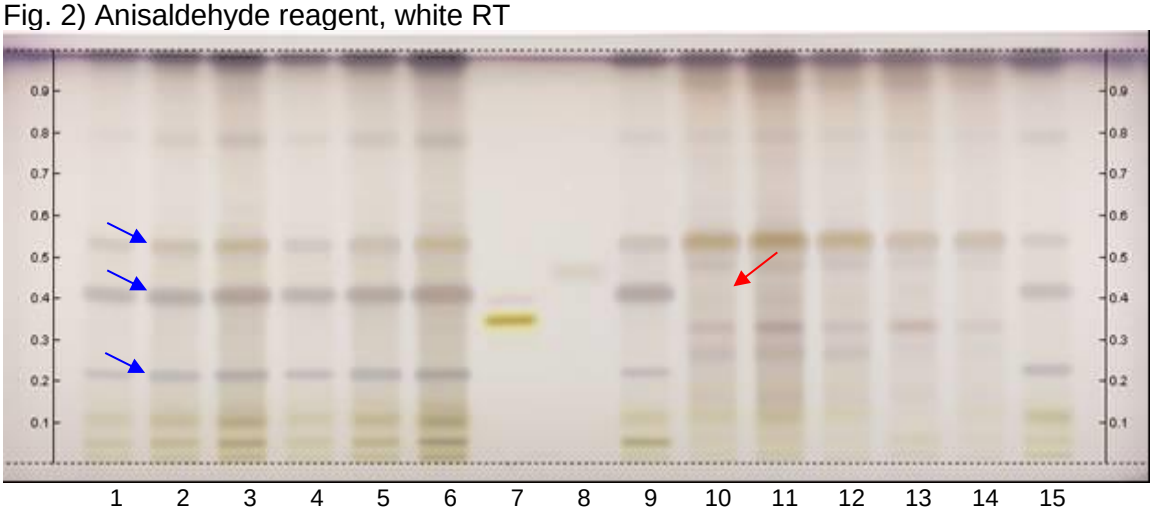


Fig. 2) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	3 µL	European vervain herb 1	9	3 µL	European vervain herb 2
2	**5 µL**	**European vervain herb 1**	10	5 µL	Lemon verbena leaf 1
3	7 µL	European vervain herb 1	11	7 µL	Lemon verbena leaf 1
4	3 µL	European vervain herb 1 (Ph.Eur. extraction)	12	5 µL	Lemon verbena leaf1
5	5 µL	European vervain herb 1 (Ph.Eur. extraction)	13	5 µL	Lemon verbena leaf 2
6	7 µL	European vervain herb 1 (Ph.Eur. extraction)	14	5 µL	Lemon verbena leaf 3
7	5 µL	Rutin	15	5 µL	European vervain herb 3
8	5 µL	Arbutin			

System suitability test
Rutin: light yellow zone at Rf ~ 0.34 (white RT).
Arbutin: light brown zone at Rf ~ 0.46 (white RT).

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
Under UV 254 nm the chromatogram of the test solution shows a quenching zone at Rf ~ 0.40 and at Rf ~ 0.53. Under white RT there is a greyish violet zone at Rf ~ 0.40 right above the position of reference rutin, and another greyish violet zone below rutin at Rf ~ 0.22. Just above the position of reference arbutin there is a grey zone at Rf ~ 0.54 (blue arrows). Around Rf ~ 0.07 there are two yellow zones.

Test for adulteration
The chromatogram of Lemon verbena leaf does not show a zone at Rf ~ 0.40 (red arrow).

Viola arvensis, V. tricolor
(Wild pansy flowering aerial parts)

1. Scope

This method identifies the dried flowering aerial parts of Wild pansy (Viola arvensis Murray and/or Viola tricolor L.) by HPTLC fingerprint.

2. Source of method

Modified from Ph. Eur 7.0 (change in sample preparation).

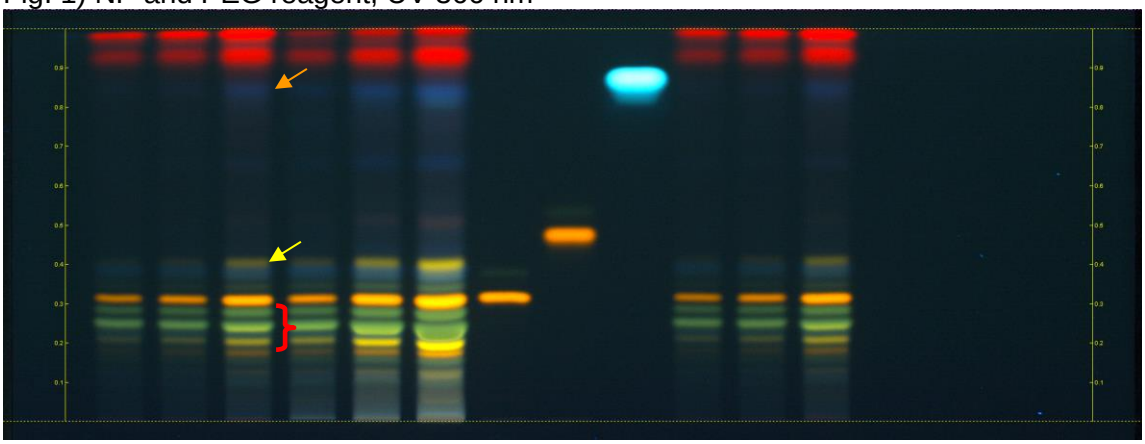
3. Procedure

Sample preparation:	Mix 2 g of powdered sample with 10 mL of methanol and sonicate for 10 minutes, then centrifuge or filter the solutions and use the supernatants / filtrates as test solutions.
Reference substances:	Individually dissolve 2.5 mg of rutin, 1.0 mg of caffeic acid, and 2.5 mg of hyperoside each in 10 mL of methano.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	2 µL of references, 8 µL of test solutions
Mobile phase:	Formic acid, acetic acid, water, ethyl acetate 11:11:27:100 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	1.) NP reagent Preparation: 1 g of natural products reagent in 200 mL of ethyl acetate. 2.) PEG reagent Preparation: 10 g of polyethylene glycol 400 in 200 mL of dichloromethane Use: Heat plate for 3 min at 100°C, dip (time 0, speed 5) in NP reagent, dry and dip (time 0, speed 5) in PEG reagent.
Documentation:	1.) NP and PEG reagent, UV 366nm

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) NP and PEG reagent, UV 366 nm



Track	Volume	Sample	Track	Volume	Sample
1	2 µL	Wild pansy 1	9	2 µL	Caffeic acid
2	4 µL	Wild pansy 1	10	2 µL	Wild pansy 2
3	**8 µL**	**Wild pansy 1**	11	4 µL	Wild pansy 2
4	1 µL	Wild pansy 1 (Extraction Ph. Eur)	12	8 µL	Wild pansy 2
5	2 µL	Wild pansy 1 (Extraction Ph. Eur)	13		Blank
6	3 µL	Wild pansy 1 (Extraction Ph. Eur)	14		Blank
7	2 µL	Rutin	15		Blank
8	2 µL	Hyperoside			

System suitability test
Caffeic acid: blue fluorescent zone at Rf ~ 0.87.
Rutin: orange fluorescent zone at Rf ~ 0.32.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a blue fluorescent zone just below the zone due to reference caffeic acid (orange arrow). A yellow fluorescent zone is seen at Rf ~ 0.40 below the position of reference hyperoside (yellow arrow). Below it there is an intense yellowish orange fluorescent zone corresponding to reference rutin. Below the zone due to rutin there are three yellowish-green fluorescent zones (red arrow).

Ziziphus jujuba var. spinosa (Spine date seed, Suan zao ren)

1. Scope

This method identifies dried Spine date seed (Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou) by HPTLC fingerprint and discriminates dried Indian jujube (Ziziphus mauritiana Lam.) and Japanese raisintree (Hovenia dulcis Thunb.).

2. Source of method

CAMAG, under evaluation by Ph.Eur.

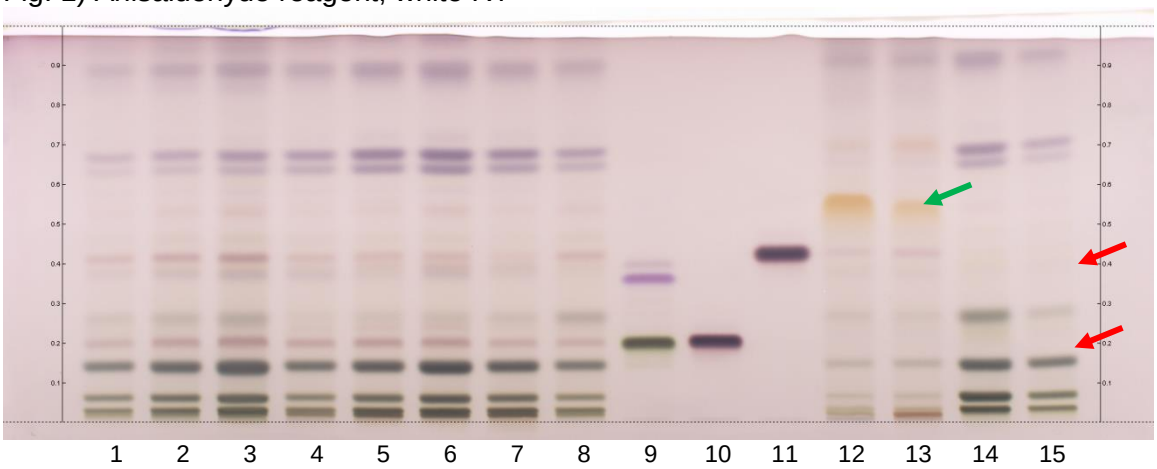
3. Procedure

Sample preparation:	Mix 1 g of powdered sample with 5 mL of methanol and sonicate for 10 minutes on a water bath at 60°C, then centrifuge or filter the solution and use the supernatant / filtrate as test solution.
Reference substances:	Dissolve 1 mg of hederacoside in 1 mL of methanol. Dissolve 1 mg of glycyrrhizic acid in 1 mL of methanol. Optional: dissolve 1 mg of jujuboside A and jujuboside B individually in 1 mL of methanol.
Stationary phase:	HPTLC Si 60 F_{254}
Application:	8 µL of references, 8 µL of test solutions
Mobile phase:	Dichloromethane, acetic acid, methanol, water 64:32:12:8 (v/v/v/v)
Development:	Saturated chamber Developing distance 70 mm from lower edge Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent Preparation: 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid, 10 mL of sulfuric acid and 1 mL of p-anisaldehyde Use: Dip (time 0, speed 5), heat at 100°C for 3 min
Documentation:	1.) Clean plate, white RT 3.) Anisaldehyde reagent, white RT

4. Results

Note: The images presented in this section are examples and are not intended to be used as basis for setting specifications for quality control purposes.

Fig. 1) Anisaldehyde reagent, white RT



Track	Volume	Sample	Track	Volume	Sample
1	5 µL	Spine date seed #1	9	8 µL	Hederacoside, Glycyrrhizic acid (with increasing Rf)
2	**8 µL**	**Spine date seed #1**	10	8 µL	Jujuboside A
3	10 µL	Spine date seed #1	11	8 µL	Jujuboside B
4	8 µL	Spine date seed #2	12	8 µL	Japanese raisintree seed #1
5	8 µL	Spine date seed #3	13	8 µL	Japanese raisintree seed #2
6	8 µL	Spine date seed #4	14	8 µL	Indian jujube seed #1
7	8 µL	Spine date seed #5	15	5 µL	Indian jujube seed #2
8	8 µL	Spine date seed #6			

System suitability test
Hederacoside: green zone at Rf ~ 0.20.
Glycyrrhizic acid: pink zone at Rf ~ 0.36.

Identification
Compare result with reference images. The fingerprint of the test solution is similar to that of the corresponding botanical reference sample. Additional weak zones may be present.
The chromatogram of the test solution shows a violet zone just below the solvent front and a distinct violet double zone in the upper third. There is a pink zone corresponding to jujuboside B just above the position of reference glycyrrhizic acid. At the position of glycyrrhizic acid there is a weak brownish zone. Below it an olive zone is seen. At the position of hederacoside there is a dark pink zone corresponding to jujuboside A. Below this zone a prominent olive zone is detected.

Test for other species
No intense yellow-orange zone is seen at Rf ~ 0.55 (green arrow, Japanese raisin tree seed).
The chromatogram of Indian jujube seed does not show the violet zones corresponding to jujuboside A and B (red arrow).

Alphabetical Table of Content (English)

Agrimony herb 1
Amla fruit 125
Angelica root 13
Anise fruit (flavonoids) 131
Anise oil 133
Aniseed 135
Armand's clematis root 58
Arnebia root 27
Arnica flower 30
Ash leaf 77
Astragalus root 34
Barley grass 83
Belleric myrobalan fruit 181
Bitter Fennel fruit 75
Boldo leaf 122
Calendula flower 38
Caraway fruit 46
Caraway fruit (flavonoids) 44
Caraway oil 49
Cayenne pepper 42
Chamomile flower 101
Chebulic myrobalan fruit 183
Chinese Angelica root 22
Chinese lovage root 96
Chinese Sage 160
Chinese Sage root, Identification 163
Cowslip Primrose Flower 146
Dahurian Angelica root 16
Devil's claw root 79
Doubleteeth Pubescent Angelica root 19
Drynaria rhizome 67
European goldenrod herb 176
European vervain herb 194
False daisy herb 69
Feverfew flower 178
Fleeceflower 144
Giant goldenrod herb 174

Green Tea leaf 40
Iceland moss 52
Isatis root....85
Job's tears seed (syn. Coix seed)....60
Kava rhizome 138
Kudzu root....150
Lady's Mantle herb 3
Lavender flower....87
Lavender oil....89
Lemon verbena leaf.... 5
Lime flower....185
Lovage root 93
Mallow flower 99
Mallow flower 190
Marsh horsetail herb....71
Melissa leaf dry extract....106
Milk thistle fruit....172
Mullein flower 192
Niaouli oil....104
Oat herb 36
Oswego tea 108
Passion flower 120
Red paeony root....114
Ribwort plantain leaf 141
Roman chamomile flower 55
Roselle flower....81
Rosemary leaf 152
Rosemary oil 154
Round cardamom fruit 8
Sage leaf....166
Sage oil 168
Saposhnikovia root....170
Spanish sage oil....158
Spike lavender oil 91
Spine date seed 199
Sweet Fennel fruit 73
Sweet wormwood leaf 32
Thomson's kudzu root 148

Three-lobed sage leaf....156
Tree paeony root bark....117
Triphala powder....128
Turmeric....62
Turmeric (Test for adulteration with nimesulide)....64
Uva ursi leaf....25
Villous amomum fruit....11
Wheat grass....188
White paeony root....111
Wild pansy flowering aerial parts....197