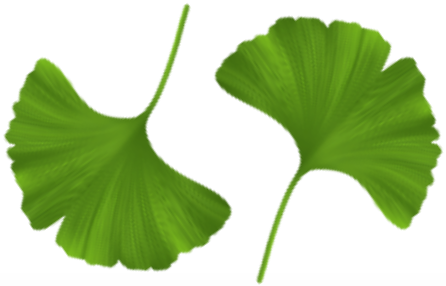
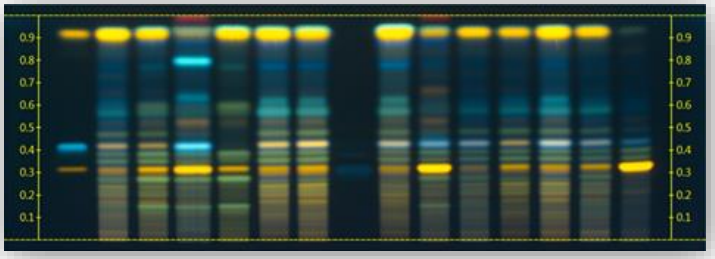
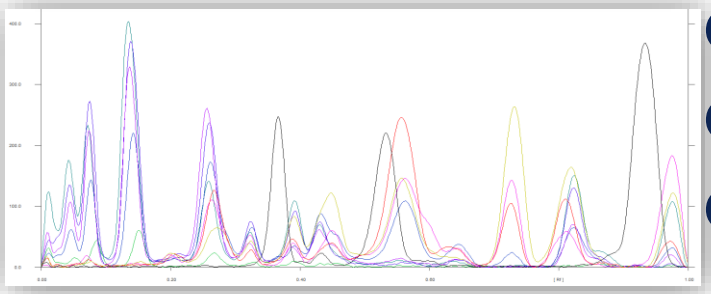


Comprehensive HPTLC fingerprintig pour le contrôle de la qualité des drogues végétales





Comment l'HPTLC peut aider la détection de problèmes de qualité dans les produits à base de plantes médicinales

présenté par Pierre BERNARD-SAVARY

UNIVERSITAT DE BARCELONA

Salvador Cañigueral [1], **Débora Frommenwiler** [1,2], **Roser Vila** [1] et **Eike reich** [2]

[1] Unitat de Farmacologia, Farmacognòsia i Terapèutica, Facultat de Farmàcia i Ciències de l'Alimentació,Université de Barcelone (Espagne)
[2] Camag (Muttenz, Suisse).

HPTLC fingerprint in the quality control of herbal drugs

Content

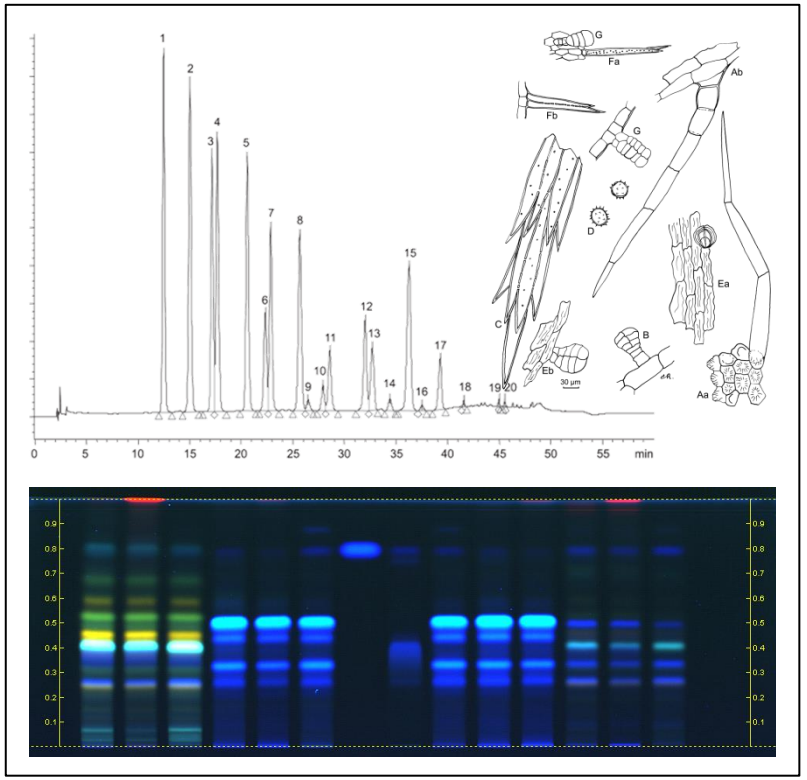
1. Quality control of herbal drugs, preparations and products
2. HPTLC in the quality control of herbal products
3. Comprehensive HPTLC fingerprinting: the concept
4. Comprehensive HPTLC fingerprinting: application examples

Quality of herbal products

What is relevant?

Production process

Quality control



Herbal products

Production

Medicinal plant

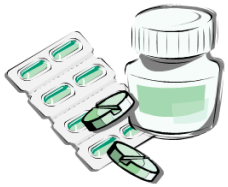
Harvest and post-harvest processing

Herbal drug

Milling / extraction

Herbal preparation
(active ingredient)

Formulation



Herbal product
(medicinal product,
dietary supplement, ...)

Quality control

What is relevant?

1. **Identity**
   Plant species, plant part, chemical profile.
2. **Purity**
   Possible adulterations or falsifications, possible contaminants, etc.
3. **Content**
   Usually assay of compounds of known therapeutic activity or markers.

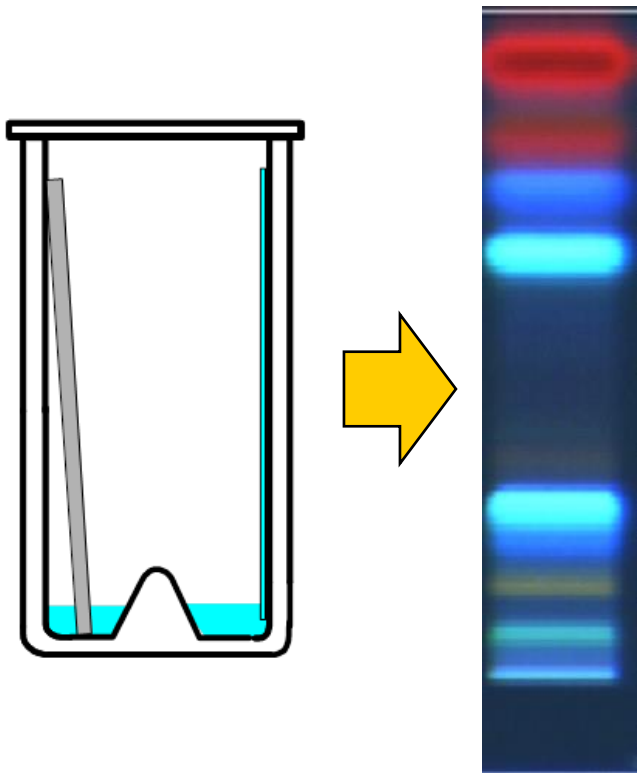
Maintenance along the shelf life time

4. **Stability**

Pharmacopoeia monographs

TLC and quality control of herbals

A classical tool

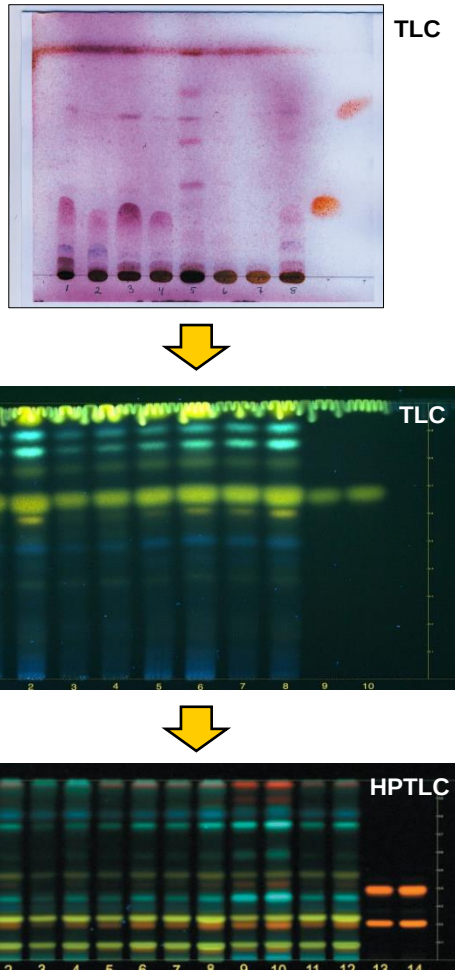


- ✓ Used since years.
- ✓ Included in most Pharmaco-poeias.
- ✓ Identification, detection of adulterations/falsifications
- ✓ Historically with a limited description of the analytical parameters.

The evolution towards HPTLC

The *European Pharmacopoeia* as example

Year	Ph. Eur.	Facts
1969	1st edition	✓ Chapter on TLC ✓ Few parameters described
1989	2th edition	✓ New chapter on TLC (V.6.20.2, renamed as chapter 2.2.27 in Ph. Eur 3.0)
2005	5th edition	✓ Update of the chapter 2.2.27 ✓ Introduction of quantitative TLC ✓ Introduction of HPTLC as an alternative
2017	9th edition	✓ New chapter 2.8.25 on HPTLC for herbal drugs and herbal preparations



HPTLC improvements

Ph. Eur. general chapter 2.8.25

1. Improvement of reproducibility

- ✓ Introduction of **HPTLC**
- ✓ **Standardisation** of methodology (SOPs)
- ✓ Introduction of a **system suitability test** (SST, qualification of the plate)

2. Improvement of the description and interpretation of the chromatograms

- ✓ Sequence of zones (number position, colour)
- ✓ Intensity of zones: **introduction of an intensity marker** and standard wording for its description
- ✓ Publication of **colour pictures of chromatograms**: Knowledge database, not mandatory, given only as information, including several batches to show natural variability.

HPTLC improvements

Ph. Eur. general chapter 2.8.25

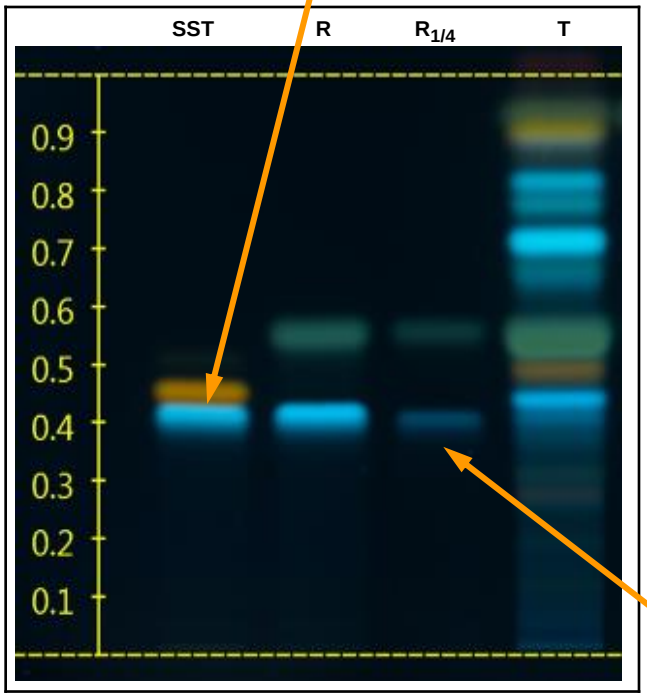
Roman chamomile flower

Description table

Upper edge of plate	
	A greenish-blue fl zone (apigenin) A weak to equivalent brownish-yellow or orange fl zone
	Three light blue fl zones (upper two with a weak to equivalent intensity, the lowest usually intense)
_____	
Apigenin-7-glucoside : A greenish-blue fl zone	A equivalent to intense greenish-blue fl zone (apigenin-7-glucoside) A weak to equivalent brownish-yellow or orange fl zone
Chlorogenic acid: A light blue fl zone	A weak to equivalent light blue fl zone
_____	
Reference solution	**Test solution**

_____: Marks between upper, middle and lower third

System-specific suitability test (SST)



Typical chromatogram

Intensity marker

SST: Reference solution (c)
R: Reference solution (a)
$R_{1/4}$: Reference solution (b). R diluted with factor 4
T: Test solution (T1)

HPTLC improvements

Ph. Eur. general chapter 2.8.25

01/2017:0380

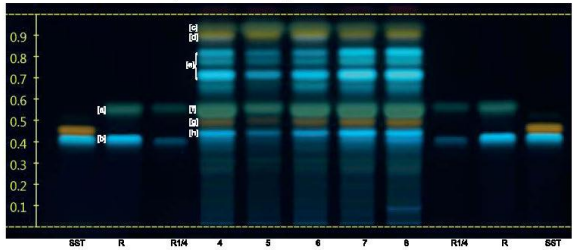
CHAMOMILE FLOWER, ROMAN

Chamomillae romanae flos

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

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

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



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

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

Figure 0380.-2. – *HPTLC chromatogram for identification test C of different batches of Roman chamomile flower*

Publication of chromatograms in the ***Knowledge Data Base*** of the *European Pharmacopoeia*

Comprehensive HPTLC fingerprinting

What it means?

HPTLC fingerprints (images), which are used for identification, are converted into peak profiles and the intensities of selected zones are quantitatively compared to those of the corresponding zones of the reference material

A single analysis → **More information**

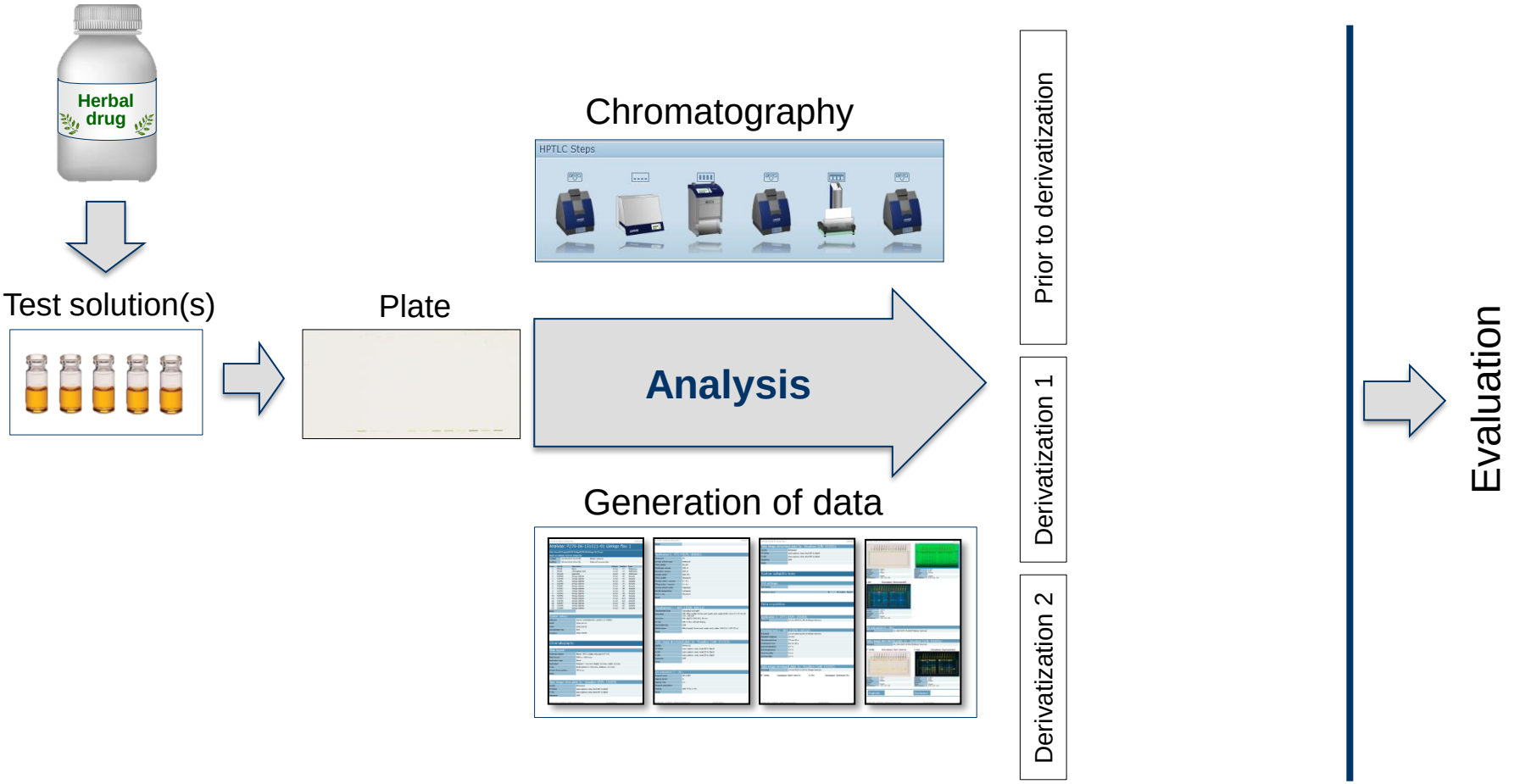
- ✓ Identification
- ✓ Purity
- ✓ Content

HPTLC for identification

Arruda Frommenwiler D, Kim J, Yook CS, Trang Tran TT, Cañigueral S, Reich E (2018) Planta Medica 84 (6-7):465-474.

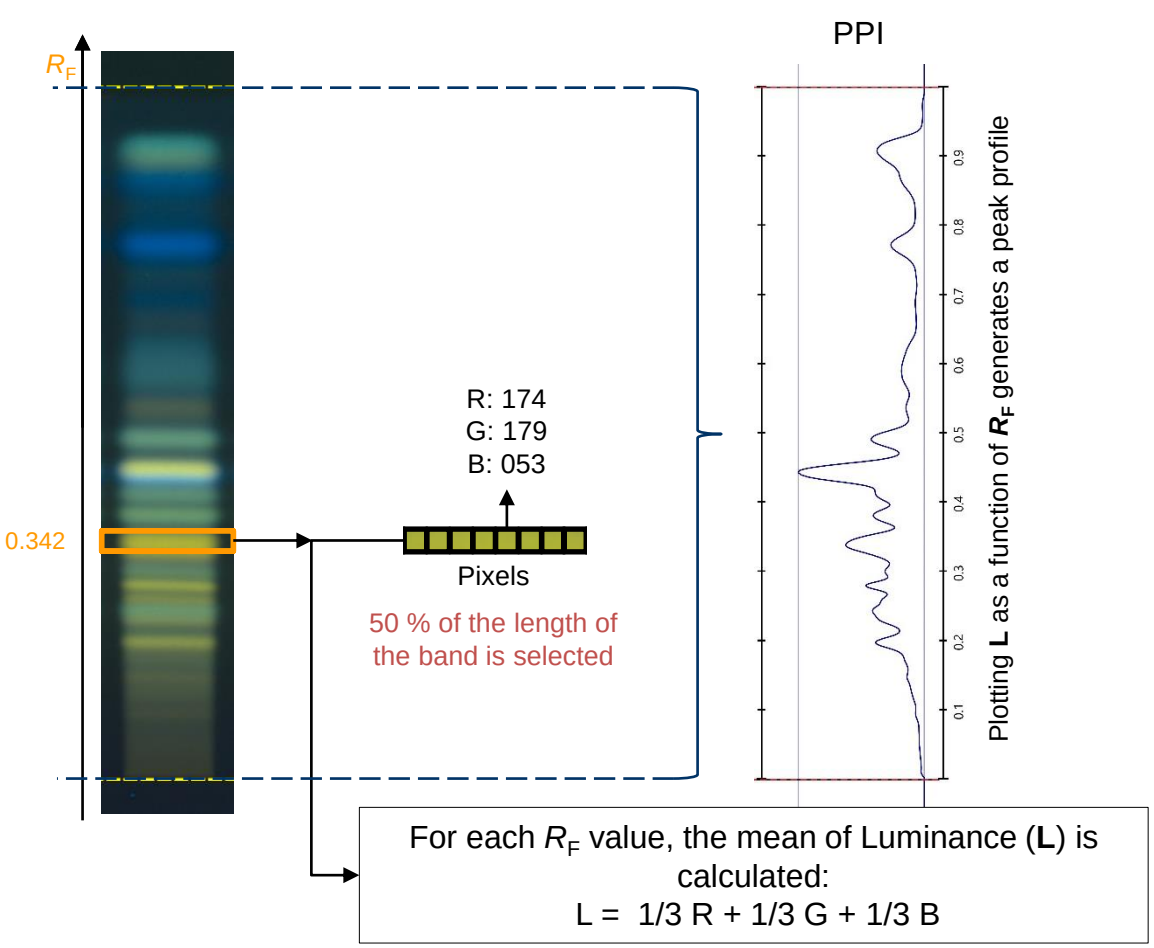
Comprehensive HPTLC fingerprinting

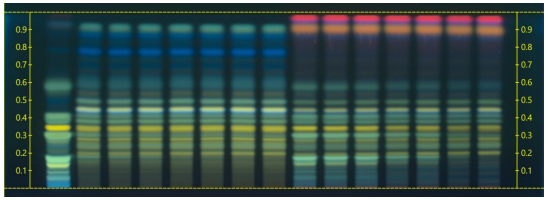
Generation of data



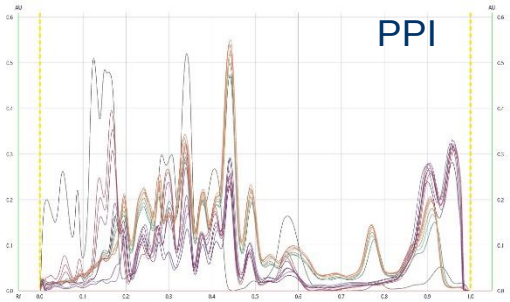
Comprehensive HPTLC fingerprinting

Peak profiles from electronic images (PPI)





Image

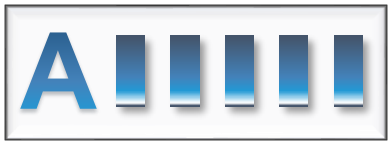


PPI

Comprehensive evaluation of the fingerprint includes image + PPI

Comprehensive HPTLC fingerprinting

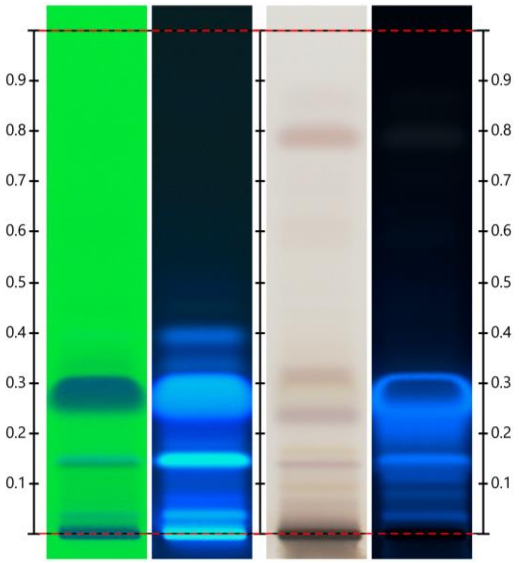
The concept



One analysis

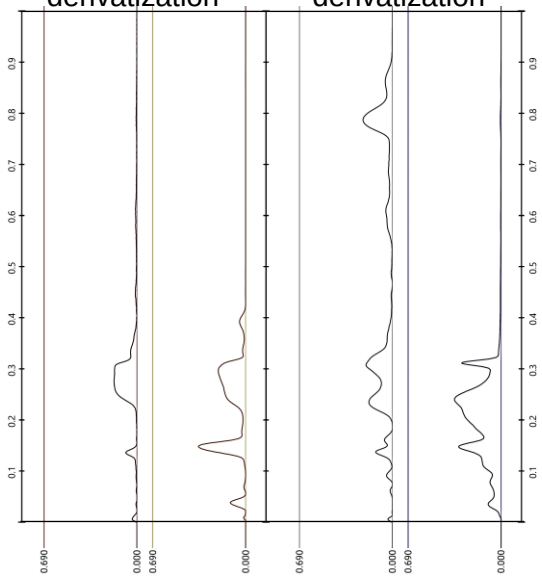
HPTLC images

Prior to derivatization | After derivatization



Peak Profiles from image (PPI)

Prior to derivatization | After derivatization



- Identity
- Purity
- Content

Comprehensive HPTLC fingerprinting

Comprehensive HPTLC fingerprinting

Angelica gigas root

- ✓ The roots of many *Angelica* species have a long use as traditional medicine.
- ✓ Some are traded in East Asian herbal markets under the same common name “Dang gui”:
  - *Angelica sinensis* (Oliv.) Diels, used in China
  - *Angelica acutiloba* (Siebold & Zucc.) Kitag., used in Japan
  - *Angelica gigas* Nakai, used in Republic of Korea

Can ***comprehensive HPTLC fingerprinting*** simplify quality control of *A. gigas* root, giving information on identity, purity and content?

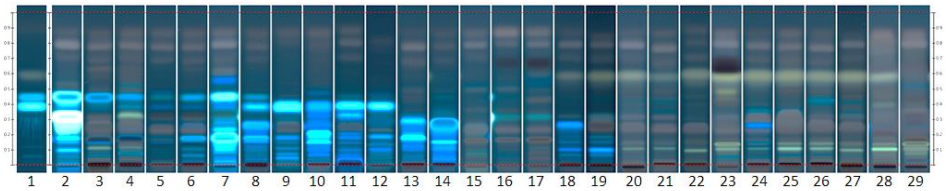
FHH Forum for the Harmonization of Herbal Medicines

Arruda Frommenwiler *et al.* (2018) Planta Medica 84 (6-7):465-474

Comprehensive HPTLC fingerprinting

Angelica gigas root

ID test



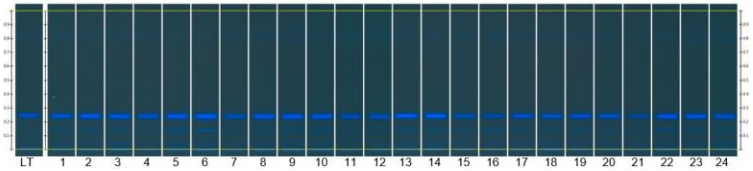
28 *Angelica* and related species can be distinguished with this method

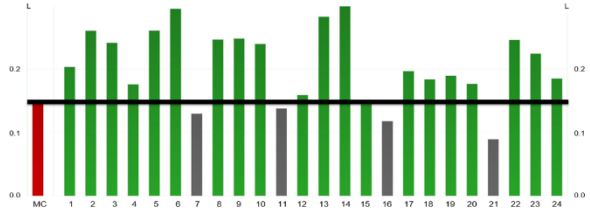
1% of *A. acutiloba in A. gigas*

1% of *A. sinensis in A. gigas*

1% of *A. gigas in A. sinensis*

Can mixtures be detected?
Test performed with the 3 main *Angelica* species





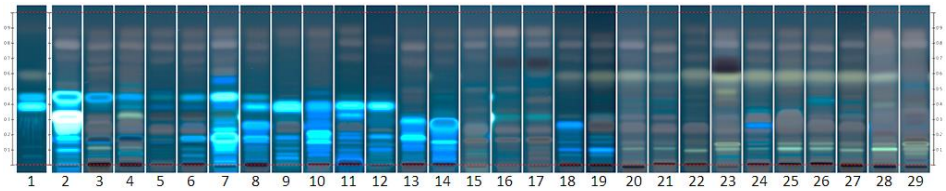
Minimum content of decursin plus decursinol angelate assessed, using a quantified reference standard in a concentration equivalent to the minimum content

Arruda Frommenwiler *et al.* (2018) Planta Medica 84 (6-7):465-474

Comprehensive HPTLC fingerprinting

Angelica gigas root

ID test



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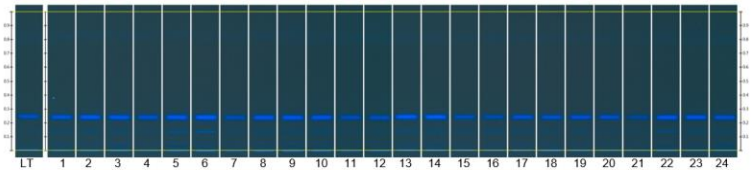

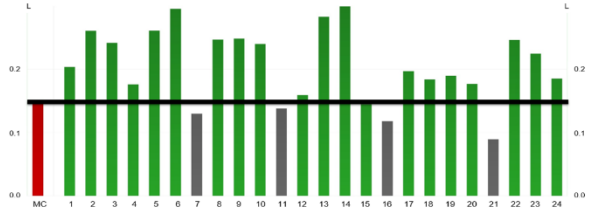

Minimum content of decursin plus decursinol angelate assessed, using a quantified reference standard in a concentration equivalent to the minimum content

Arruda Frommenwiler *et al.* (2018) Planta Medica 84 (6-7):465-474

Ginkgo biloba products

Comprehensive HPTLC fingerprinting for simplified identification and purity tests

Journal of Ethnopharmacology 243 (2019) 112084

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journal homepage: www.elsevier.com/locate/jethpharm

Comprehensive HPTLC fingerprinting as a tool for a simplified analysis of purity of ginkgo products

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ARTICLE INFO

Keywords:
HPTLC
Ginkgo biloba L.
Comprehensive HPTLC fingerprinting
Identity
Adulteration
Dietary supplements

ABSTRACT

Ethnopharmacological relevance: Herbal medicinal products based on ginkgo leaf refined dry extract (GBE) are an European development from the Eastern Asia traditionally used species *Ginkgo biloba* L. Nowadays, ginkgo products have increased the presence in the market, mainly as dietary supplements. Its adulteration with rutin and quercetin or herbal extracts rich in these compounds is a common practice. Tests featuring assays and detection of adulterants need to be performed on top of other existent methods (e.g. identification test). This may increase the costs of evaluating the quality of ginkgo products.

Aim of the study: To prove that comprehensive HPTLC fingerprinting can provide information beyond identification of ginkgo products, avoiding additional chromatographic tests for detection of adulterations.

Materials and methods: The information contained in the fingerprint obtained by HPTLC analysis of flavonoids was used for identification and for detection of adulterants, as well as to verify the limits of rutin and quercetin, which are normally determined by HPLC and used for detection of adulterants. For this purpose, peak profiles were generated from HPTLC chromatogram images. USP-HPLC methods were used for quantification of total flavonoids and testing the limits of rutin and quercetin. HPLC data were used to support the validity of the HPTLC method. An additional reversed phase HPTLC method was developed as a possible confirmatory method for the quercetin limit test.

Results: The proposed HPTLC method uses a particular sequence of detections, resulting in a number of images, which are later interpreted in a certain order. It is able to identify ginkgo products, to detect adulterants (rutin, quercetin, sophora fruit and flower bud, and buckwheat), and, using peak profiles generated from the chromatogram images prior to and after derivatisation, to evaluate the limits of rutin and quercetin. Forty-eight out of fifty-nine ginkgo dietary supplements analysed contained one or more adulterants. Furthermore, results of the HPTLC and HPLC limit tests for rutin and quercetin were in agreement in 98% of the cases. Finally, a decision tree showing the sequence of interpretation of the fingerprints obtained with the different detections after a single HPTLC analysis is included to help the analyst to evaluate whether samples have the correct identity and whether they contain or not adulterants.

Conclusion: A single HPTLC analysis is able to provide information on identity and purity of the products. This simplifies the analytical workflow and reduces the number of analyses prescribed in the USP powdered ginkgo extract monograph.

1. Introduction

Ginkgo (*Ginkgo biloba* L.), considered a sacred tree in the Eastern Asia, is traditionally associated with longevity. The earliest known medicinal use of ginkgo dates back to 2800 BC and it is described for the pseudofruits, which are more frequently used than leaves in Eastern Asia. However, based on some not very well documented uses in traditional Chinese medicine (TCM), a German company developed a medicinal product from leaves for cognitive impairment in dementia. The active ingredient is a 'special' extract – extract *G. biloba* (EGb) 761

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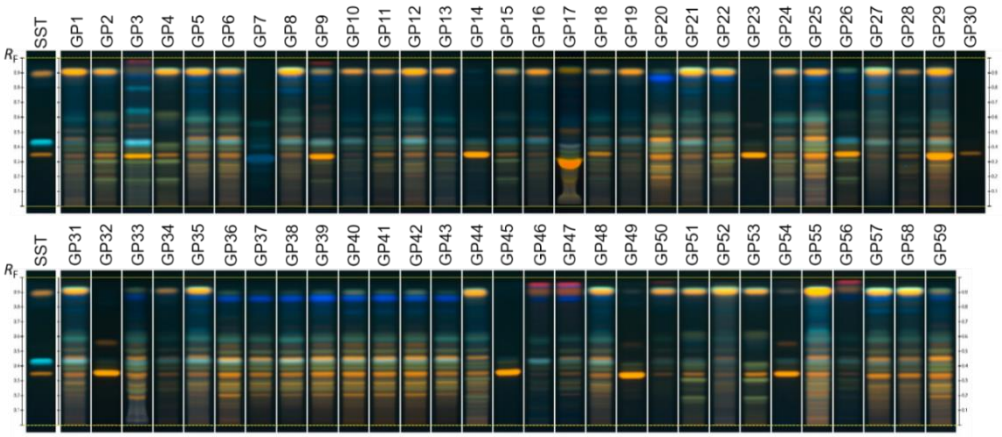
Available online 12 July 2019
0378-8741/

59 Ginkgo dietary supplements



From **11 countries**

- ✓ Colombia
- ✓ Croatia
- ✓ Germany
- ✓ Italy
- ✓ Netherlands
- ✓ New Zeeland
- ✓ Serbia
- ✓ Spain
- ✓ Switzerland
- ✓ UK
- ✓ USA

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E and Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo dry extract purified

USP

Chemical identification

- HPTLC of flavonoids
- HPLC of flavonoids (from assay)

Assay

- Flavonoid glycosides by HPLC
- Terpene lactones by HPLC

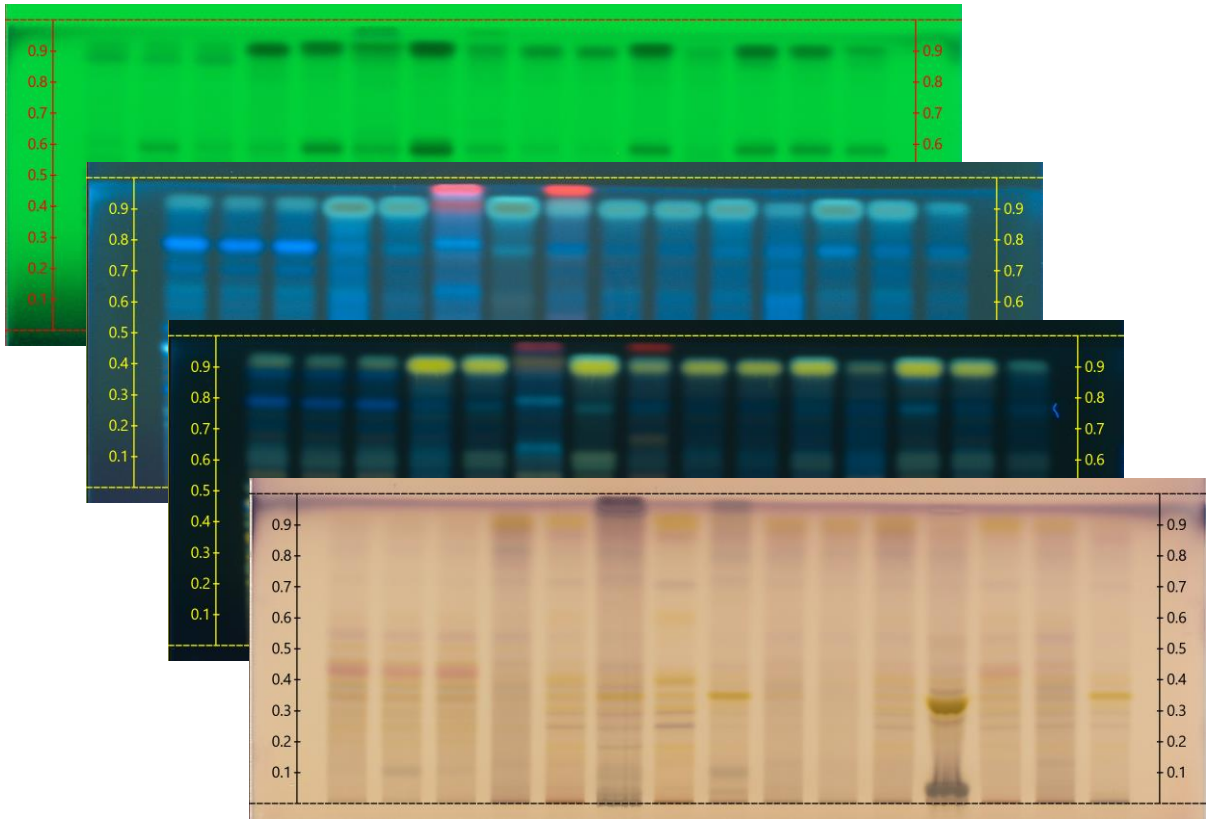
Limit tests

- Quercetin (≤ 0.5%) and rutin (≤ 4%) by HPLC
- Ginkgolic acids by HPLC

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo biloba products

Comprehensive HPTLC fingerprinting for simplified identification and purity tests



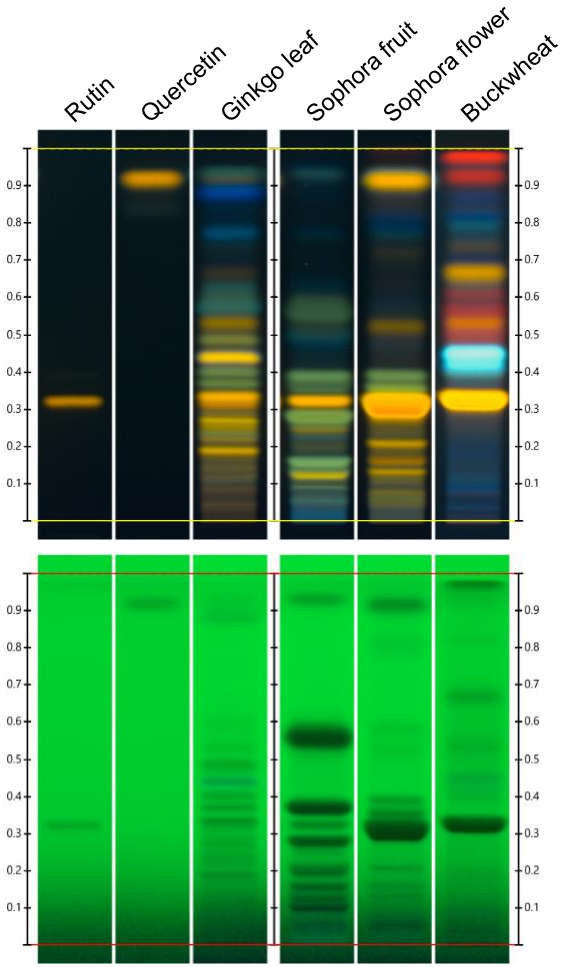
One single analysis, but 4 detections on the same plate:

- ✓ UV 254 nm (visual and PPI)
- ✓ UV 366 nm
- ✓ NP + UV 366 nm (visual and PPI)
- ✓ NP + anysaldehide reagent + daylight

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo products

Possible adulterants



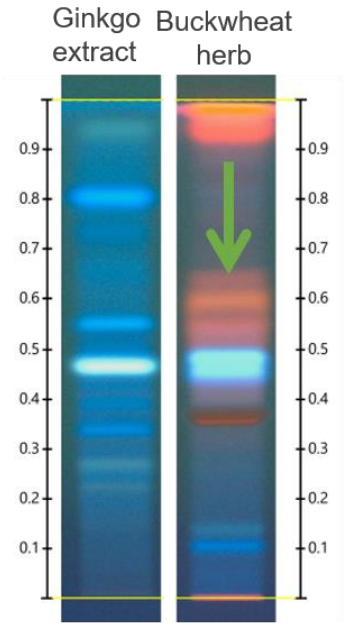
- **Rutin**
- **Quercetin**
- Extracts from ***Sophora japonica*** **flower**
- Extracts from ***Sophora japonica fruit***
- Extracts from **buckwheat** **leaf**

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo products

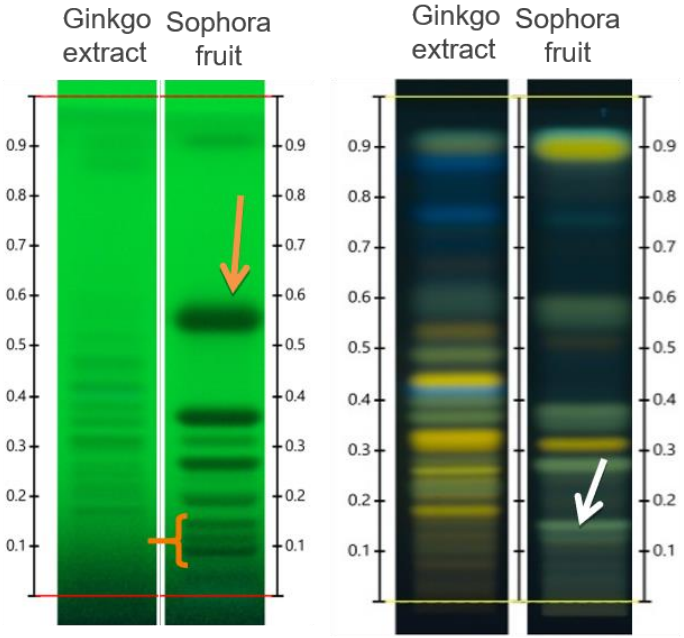
Detection of adulterants

Detection of buckwheat



UV 366 nm

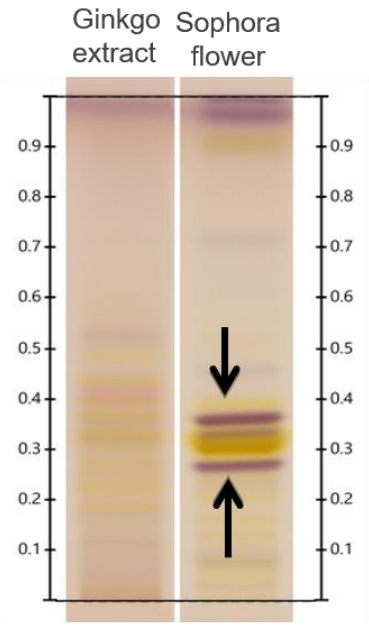
Detection of sophora fruit



UV 254 nm

UV 366 nm, derivatized with NP reagent

Detection of sophora flower



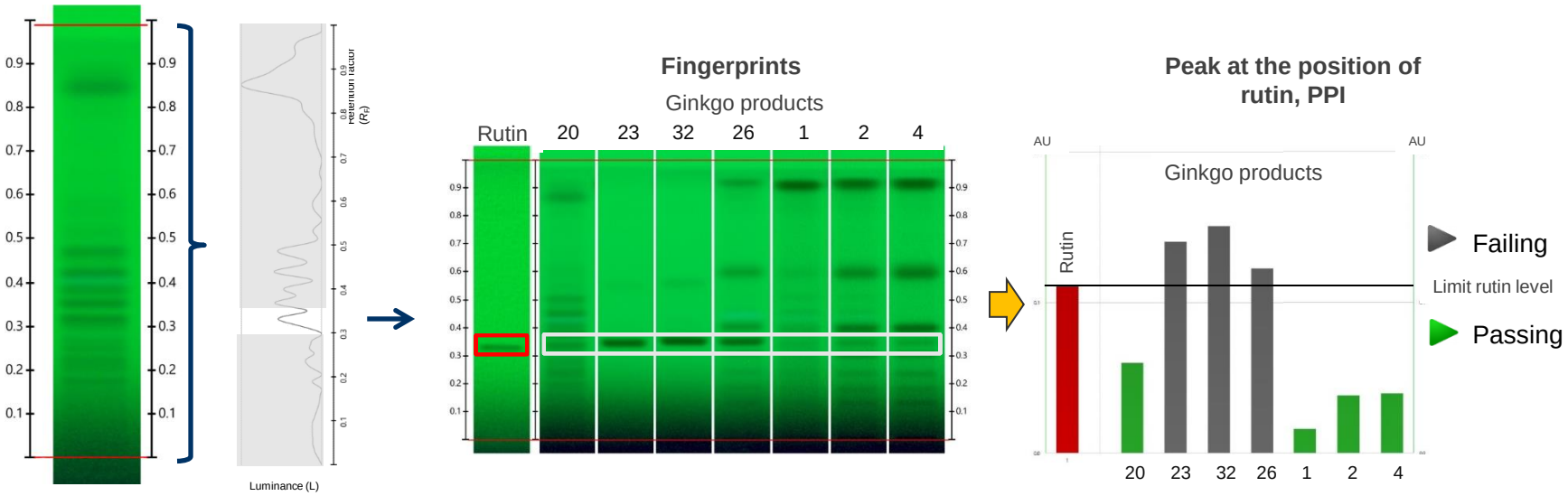
White light, derivatized with NP plus anisaldehyde reagents

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo biloba products

Comprehensive HPTLC fingerprinting for the limit test of rutin

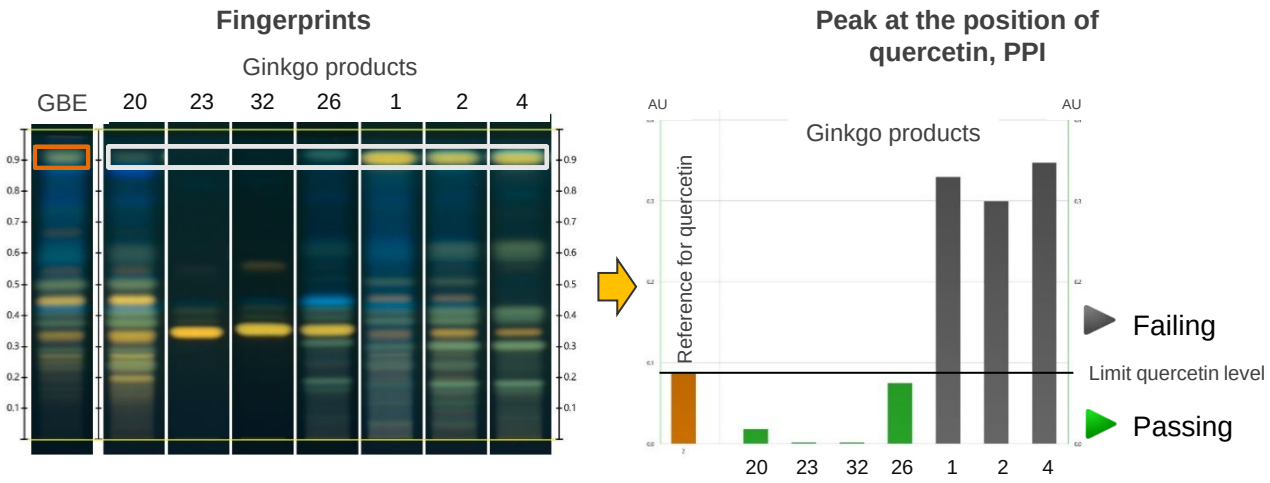
Images into peak profiles



Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo biloba products

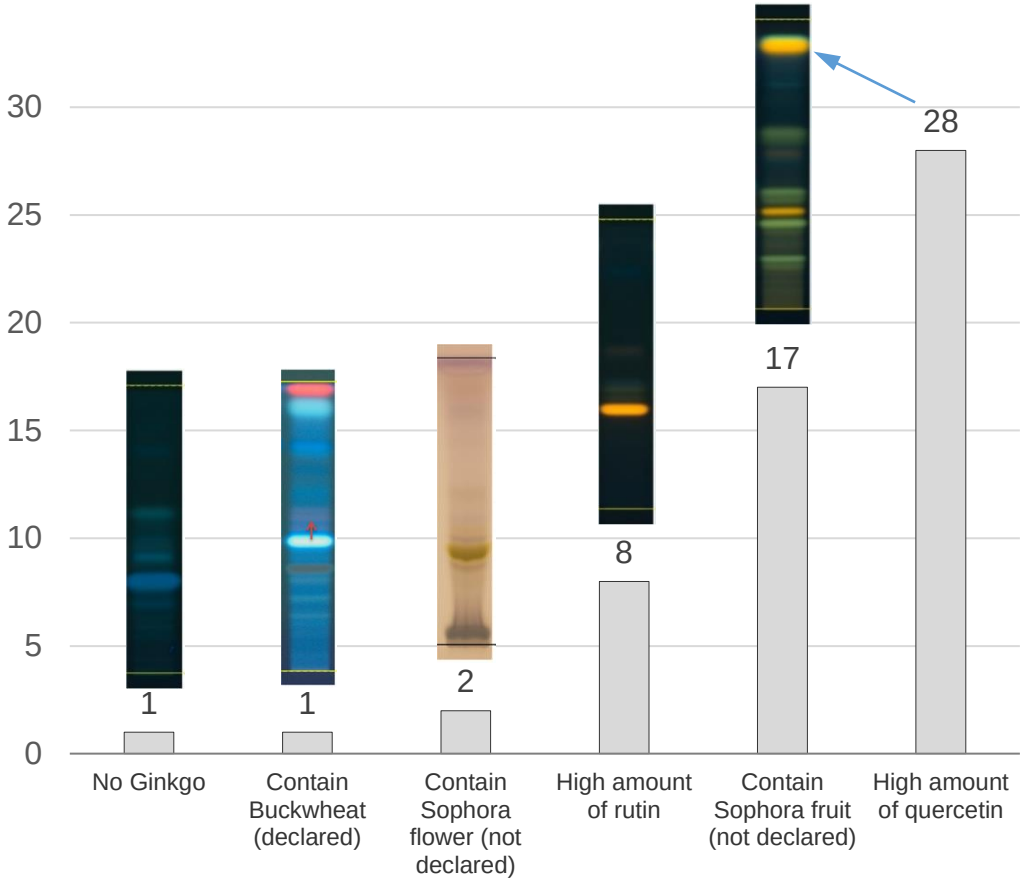
Comprehensive HPTLC fingerprinting for the limit test of quercetin



Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo biloba products

Comprehensive HPTLC fingerprinting for simplified identification and purity tests



59 ginkgo products
from **11 countries**

Only **11 products** were considered compliant after the analytical results.

Samples failing one of the tests during HPTLC evaluation will also fail the HPLC limit test

HPLC limit tests become redundant

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

Ginkgo biloba products

Comprehensive HPTLC fingerprinting for simplified identification and purity tests

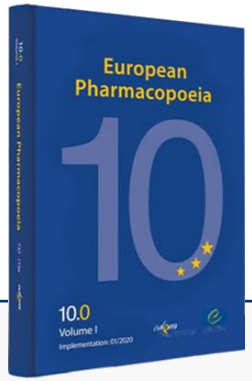
A single identification analysis (HPTLC of flavonoids)

- ✓ Allows identification
- ✓ Allows the detection of adulterations and identify the adulterant.
- ✓ Avoids one HPLC analysis prescribed by the USP for limits of quercetin and rutin

Frommenwiler DA, Booker A, Vila R, Heinrich M, Reich E, Cañigueral S (2019) J Ethnopharmacol, 243: 112084

European Pharmacopoeia

Application of comprehensive HPTLC fingerprinting to TCM herbal drugs



- Includes 73 monographs on TCM
- TCM monographs use the analytical marker approach to evaluate the content of the herbal drug.

Alternative to assays

Project

⇨ Simplify the determination of content of TCM herbal drugs

⇨ Reduce the n° of tests to be performed during quality control

⇨ HPTLC was considered during pilot study

Ph. Eur. TCM working party

Comprehensive HPTLC fingerprinting for **identification** and **minimum content**

Fritillaria thunbergii bulb

Corydalis rhizome

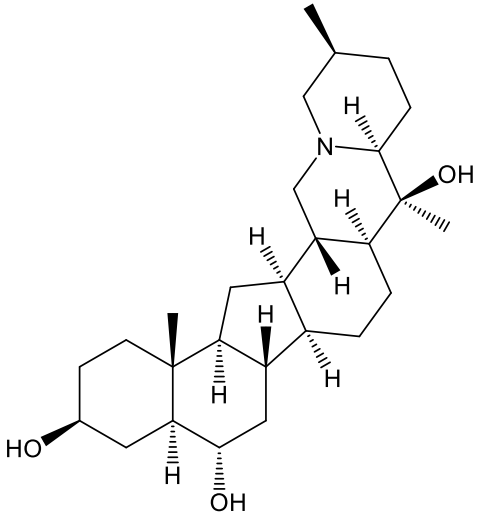
Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

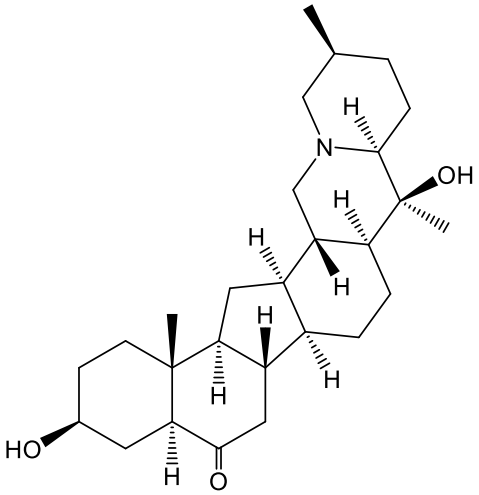
Fritillaria thunbergii Miq.

Objective

Use HPTLC for identification and to assess compliance of the herbal drug to a minimum content of peimine and peiminine, as alternative to an HPLC assay.



Peimine



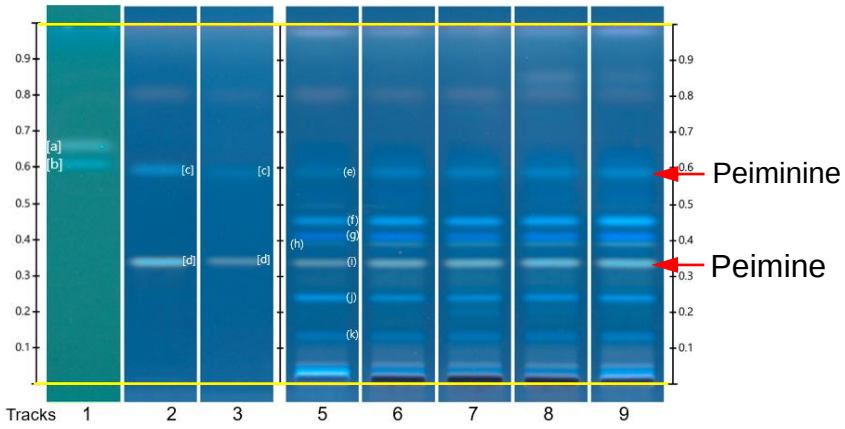
Peiminine

Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

1. Adaptation of the HPTLC ID method to quantification

- ✓ Mobile phase optimization for baseline separation of markers.
- ✓ Repeatability of the separation ($\Delta R_F \leq 0.02$).
- ✓ Optimisation of the extraction method.
- ✓ Optimisation of the SST.
- ✓ Optimisation of the detection.
- ✓ Setting acceptance criteria for identification



Top of the plate		
---	[c] Peiminine: a blue fluorescent zone ---	(e) a faint to equivalent blue zone (peiminine) ---
		(f) A blue fluorescent zone
		(g) A blue fluorescent zone
		(h) A very faint to faint greenish zone
[a] Theobromine: A quenching zone	[d] Peimine: a greenish fluorescent zone	(i) A greenish fluorescent zone (peimine)
[b] Brucine: A quenching zone ---	---	---
		(j) A blue zone
		(h) A faint to equivalent blue zone
Bottom of the plate		
SST (UV 254 nm)	*Reference solutions (a) and (b)*, UV 366 nm after derivatization	*Test solution A*, UV 366 nm after derivatization

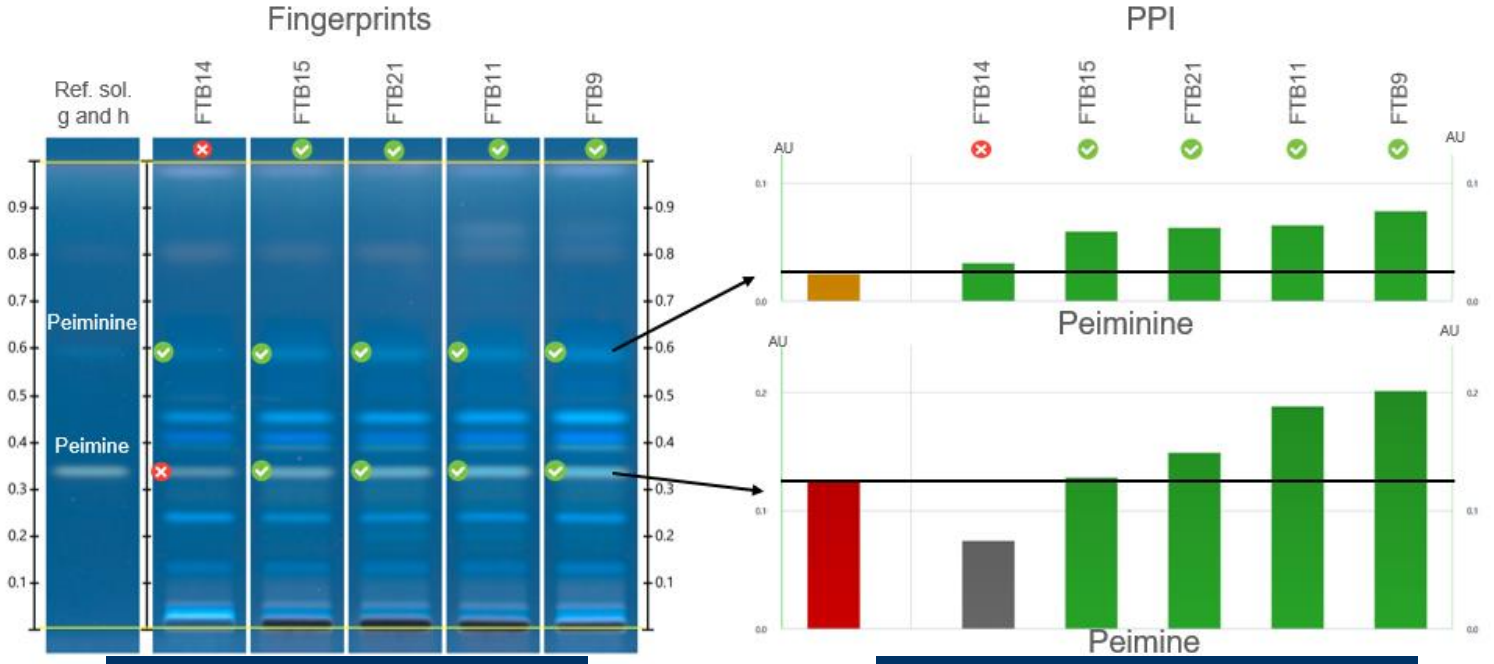
Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

2. Development of the test for minimum content

- ✓ Linearity for each marker.
- ✓ Establishment of a minimum content for each marker

Results are expressed as pass/fail rather than a content value

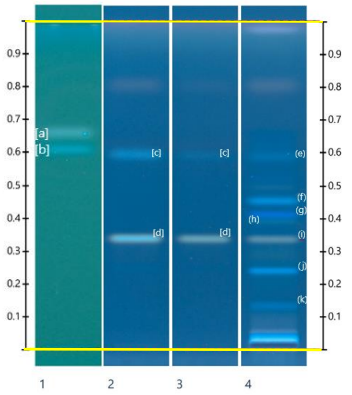


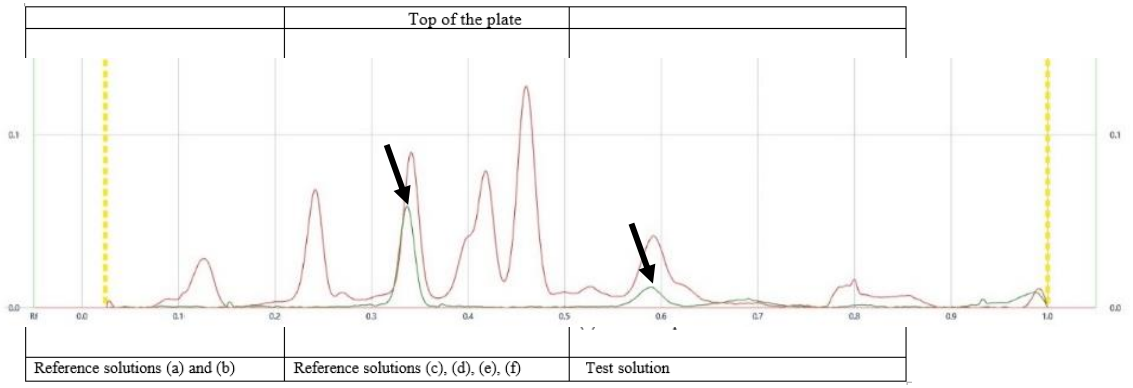
Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

2. Inter-laboratory trial: organisation

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





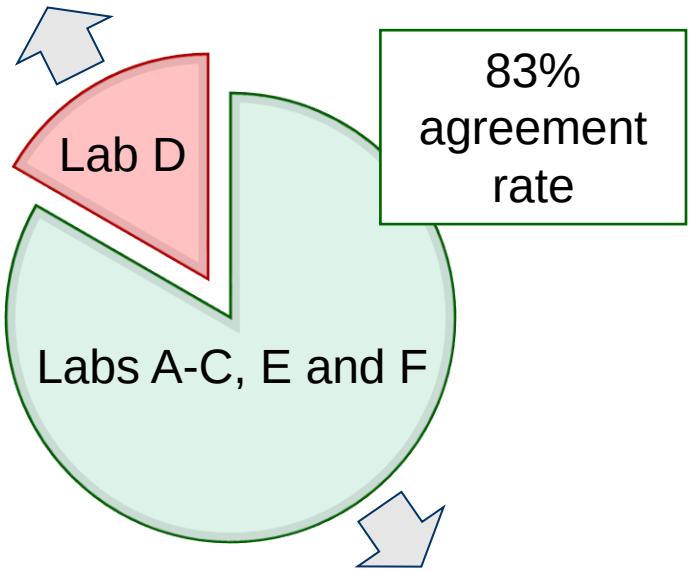
Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

3. Inter-laboratory trial: Results

Identity of the samples

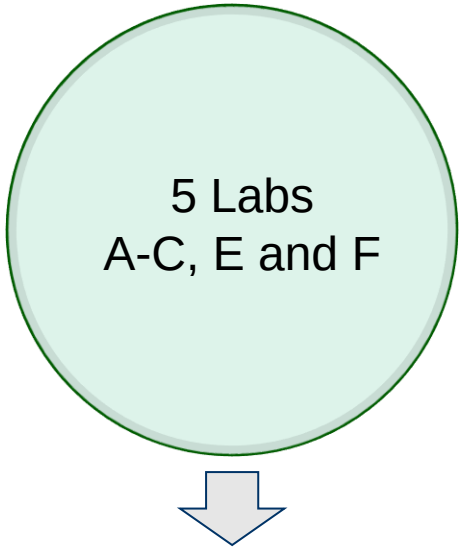
This lab was excluded from other tests



83% agreement rate

Pass all samples: all zones described in the SOP were detected

Minimum content test based on visual evaluation



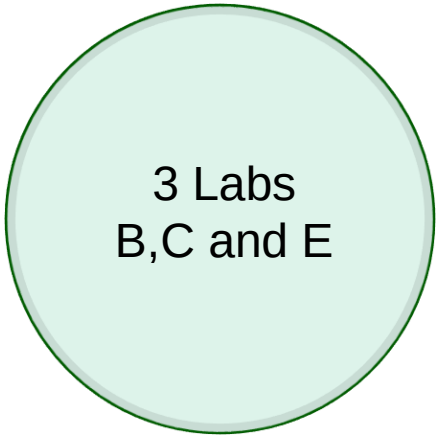
All labs pass samples 1,3-5 and **fail samples 2 and 6**: the failing samples had lower content of peimine

Ph. Eur.: TCM herbal drugs

Fritillaria thunbergii bulb case study

3. Inter-laboratory trial: Results

Minimum content test based on peak profile from image (PPI)



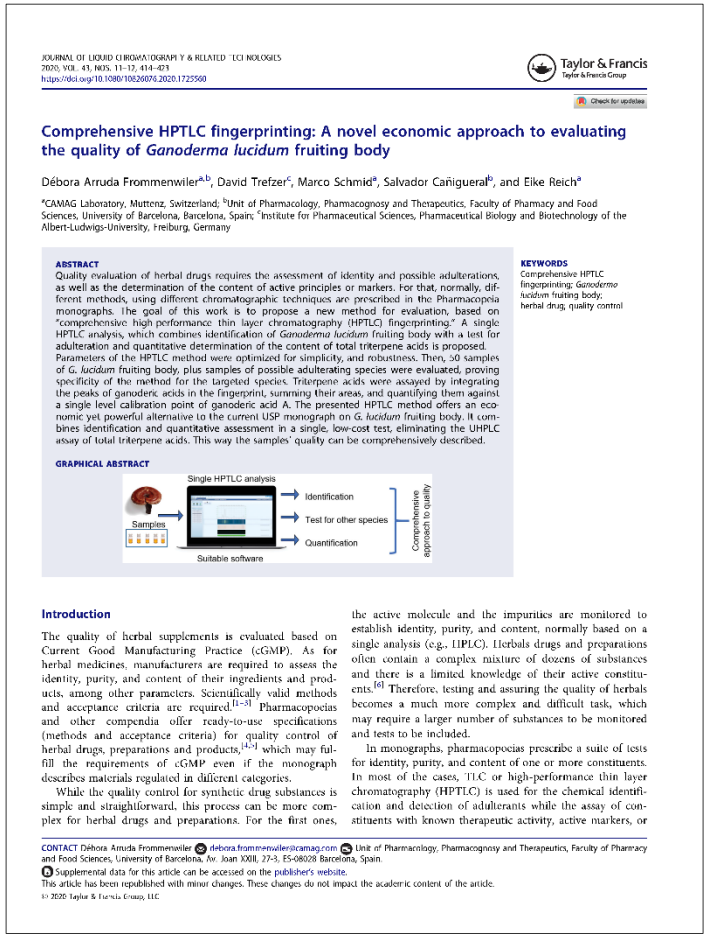
All labs pass samples 1,3-5 and **fail samples 2 and 6**: the failing samples had lower content of peimine.

Minimum content tests (visual / PPI)

Participants in the tests agreed on the results for the same sample independently of the method used.
One lab excluded (problems with the intensity of the zones).

Ganoderma lucidum (reishi)

Comprehensive HPTLC fingerprinting for quality control



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2020, VOL. 43, NOS. 11–12, 414–423
https://doi.org/10.1080/10826076.2020.1725560

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Comprehensive HPTLC fingerprinting: A novel economic approach to evaluating the quality of *Ganoderma lucidum* fruiting body

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ABSTRACT

Quality evaluation of herbal drugs requires the assessment of identity and possible adulterations, as well as the determination of the content of active principles or markers. For that, normally, different methods, using different chromatographic techniques are prescribed in the Pharmacopeia monographs. The goal of this work is to propose a new method for evaluation, based on "comprehensive high performance thin layer chromatography (HPTLC) fingerprinting." A single HPTLC analysis, which combines identification of *Ganoderma lucidum* fruiting body with a test for adulteration and quantitative determination of the content of total triterpene acids is proposed. Parameters of the HPTLC method were optimized for simplicity, and robustness. Then, 50 samples of *G. lucidum* fruiting body, plus samples of possible adulterating species were evaluated, proving specificity of the method for the targeted species. Triterpene acids were assayed by integrating the peaks of ganoderic acids in the fingerprint, summing their areas, and quantifying them against a single level calibration point of ganoderic acid A. The presented HPTLC method offers an economic yet powerful alternative to the current USP monograph on *G. lucidum* fruiting body. It combines identification and quantitative assessment in a single, low-cost test, eliminating the UHPLC assay of total triterpene acids. This way the samples' quality can be comprehensively described.

KEYWORDS

Comprehensive HPTLC fingerprinting; *Ganoderma lucidum* fruiting body; herbal drug; quality control

GRAPHICAL ABSTRACT

Introduction

The quality of herbal supplements is evaluated based on Current Good Manufacturing Practice (cGMP). As for herbal medicines, manufacturers are required to assess the identity, purity, and content of their ingredients and products, among other parameters. Scientifically valid methods and acceptance criteria are required.[1–3] Pharmacopoeias and other compendia offer ready-to-use specifications (methods and acceptance criteria) for quality control of herbal drugs, preparations and products,[4,5] which may fulfill the requirements of cGMP even if the monograph describes materials regulated in different categories.

While the quality control for synthetic drug substances is simple and straightforward, this process can be more complex for herbal drugs and preparations. For the first ones, the active molecule and the impurities are monitored to establish identity, purity, and content, normally based on a single analysis (e.g., HPLC). Herbals drugs and preparations often contain a complex mixture of dozens of substances and there is a limited knowledge of their active constituents.[6] Therefore, testing and assuring the quality of herbals becomes a much more complex and difficult task, which may require a larger number of substances to be monitored and tests to be included.

In monographs, pharmacopoeias prescribe a suite of tests for identity, purity, and content of one or more constituents. In most of the cases, TLC or high-performance thin layer chromatography (HPTLC) is used for the chemical identification and detection of adulterants while the assay of constituents with known therapeutic activity, active markers, or

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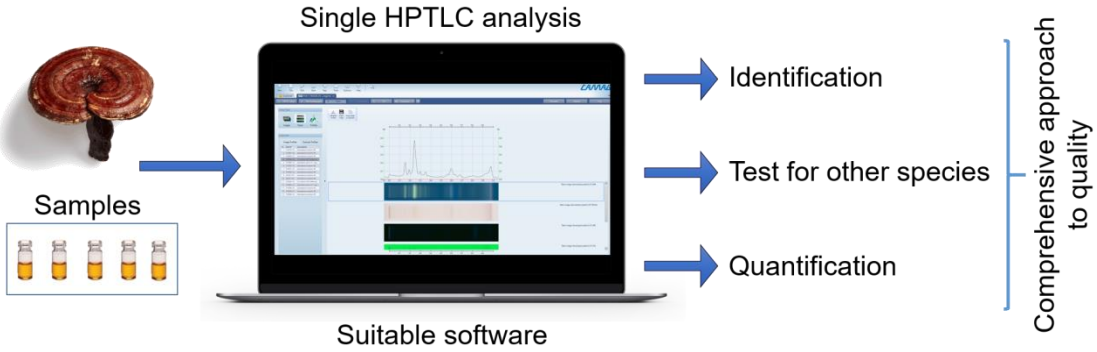
Supplemental data for this article can be accessed on the publisher's website.



- ✓ Identification
- ✓ Differentiation from other species
- ✓ **Quantification of triterpene acids as ganoderic acid A**

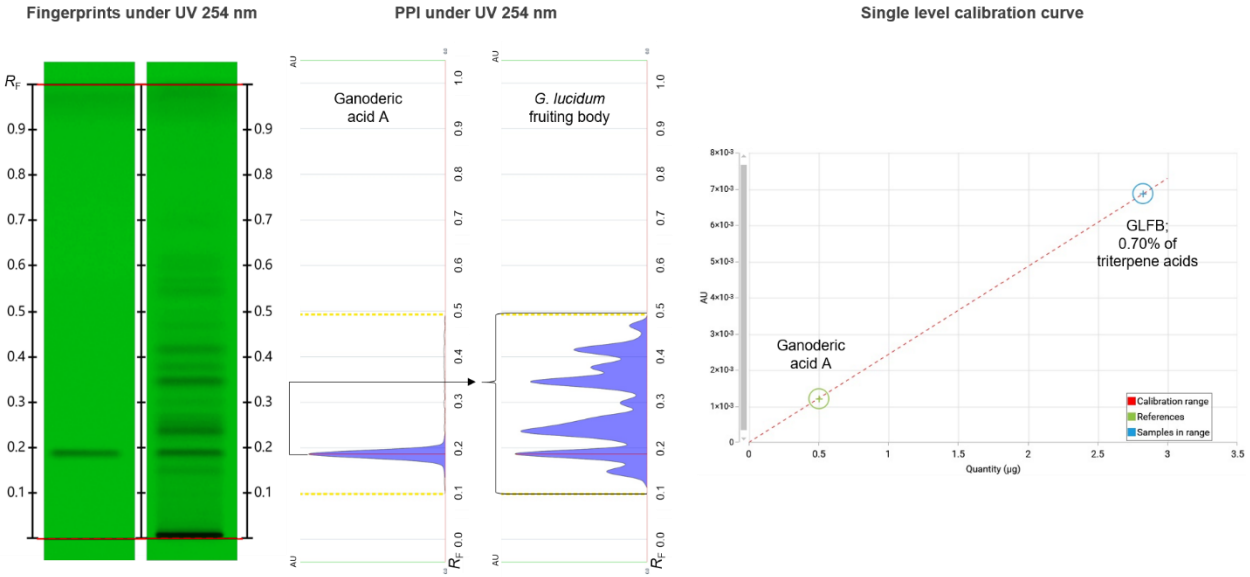
Frommenwiler DA, Trefzer D, Schmid M, Cañigueral S, Reich E, (2020) J Liq Chromatogr Rel Technol, 43 (11-12): 414-423

Ganoderma lucidum (reishi)

Quantification of triterpene acids by HPTLC

Quantification of triterpene acids as ganoderic acid A, using a single reference substance and a single point calibration

- ✓ Optimization of HPTLC parameters
- ✓ Optimization of the extraction method
- ✓ Selection of detection modes for identification and quantification
- ✓ Specificity
- ✓ Linearity
- ✓ Repeatability of the quantification



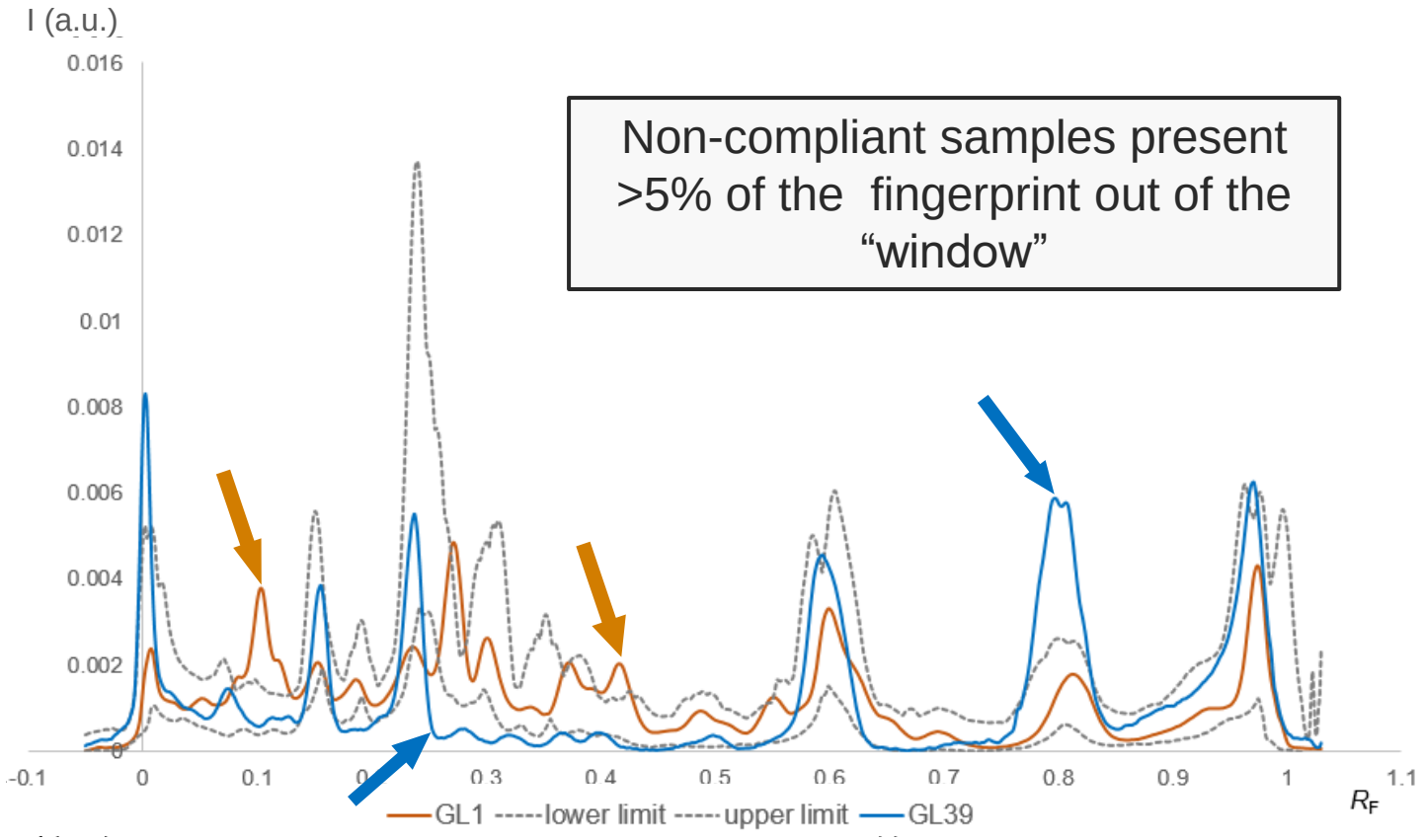
Minimum content of **total triterpene acids**: 0.25% expressed **as ganoderic acid A**, based on acceptable quality samples.

Frommenwiler DA, Trefzer D, Schmid M, Cañigueral S, Reich E, (2020) J Liq Chromatogr Rel Technol, 43 (11-12): 414-423

Ganoderma lucidum (reishi)

Automatic identification by HPTLC

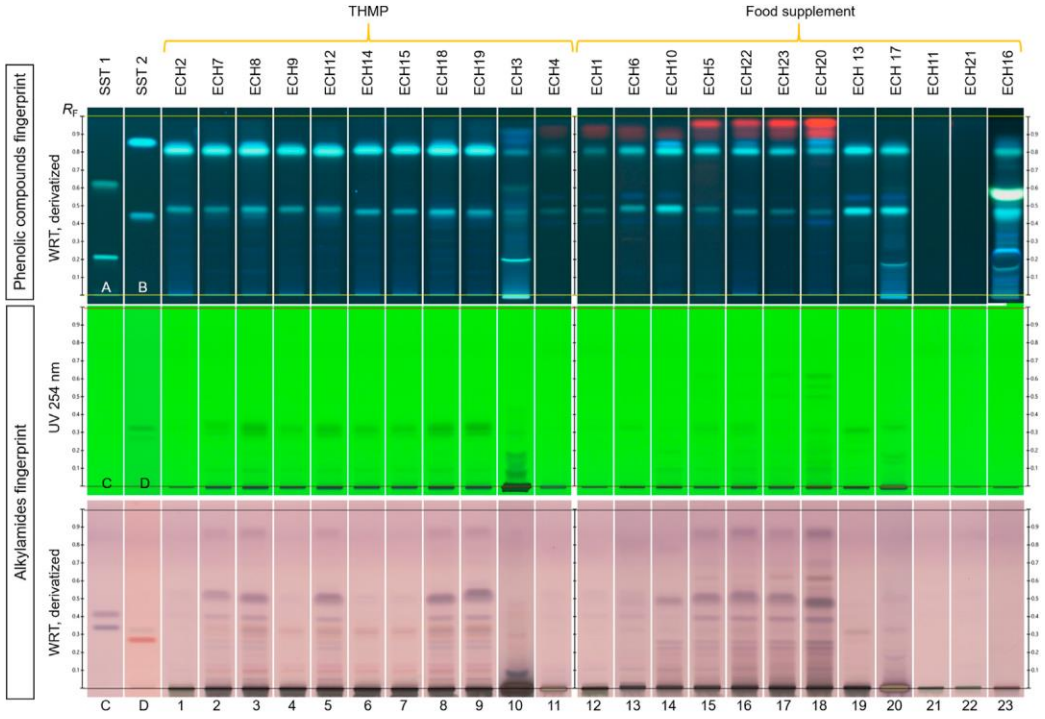
Automatic identification based on pattern recognition



Non-compliant samples present >5% of the fingerprint out of the "window"

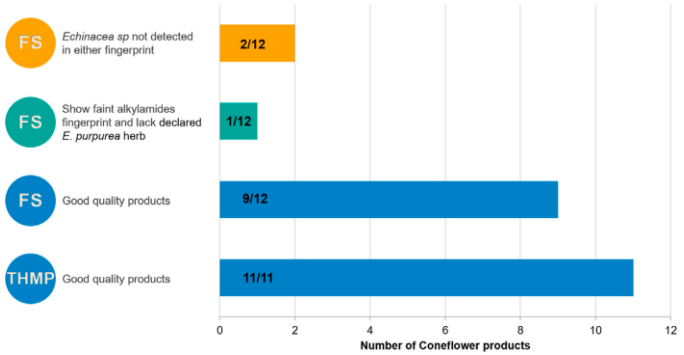
Frommenwiler DA, Trefzer D, Schmid M, Cañigueral S, Reich E, (2020) J Liq Chromatogr Rel Technol, 43 (11-12): 414-423

Comparison between label and content 3 cases studies



Echinacea purpurea comparison THMP/Food Supplement

Results of the HPTLC evaluation, self explananing

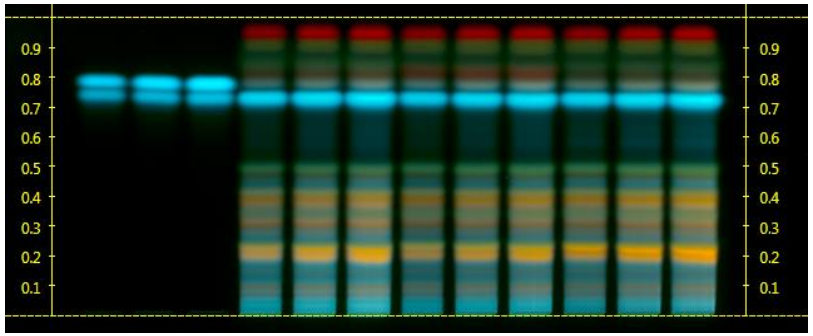


Frommenwiler DA, Reich E,Sharaf MHM, Cañigueral S and Etheridge CJ (2022), Investigation of market herbal products regulated under different categories: How can HPTLC help to detect quality problems? Front. Pharmacol. 13:925298.doi: 10.3389/fphar.2022.925298

Quality of herbal drugs and herbal preparations

Comprehensive HPTLC fingerprinting

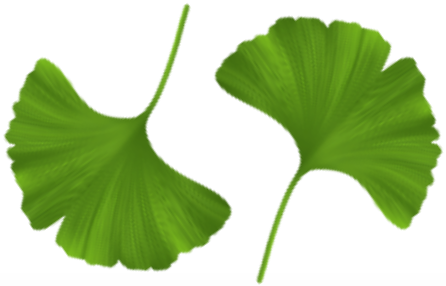
HPTLC

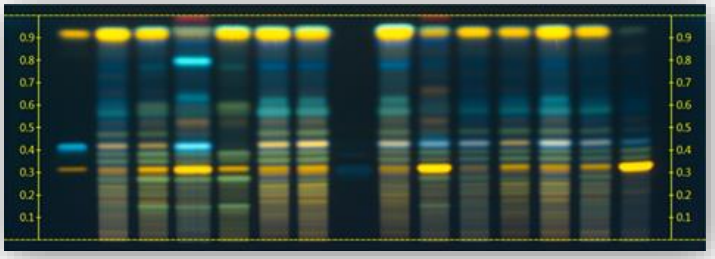


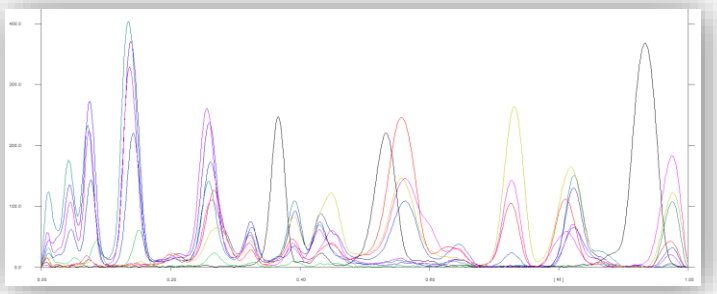
One single analysis

Quality control

- Identity
- Purity
- Content of active principles and markers







Merci beaucoup pour votre attention