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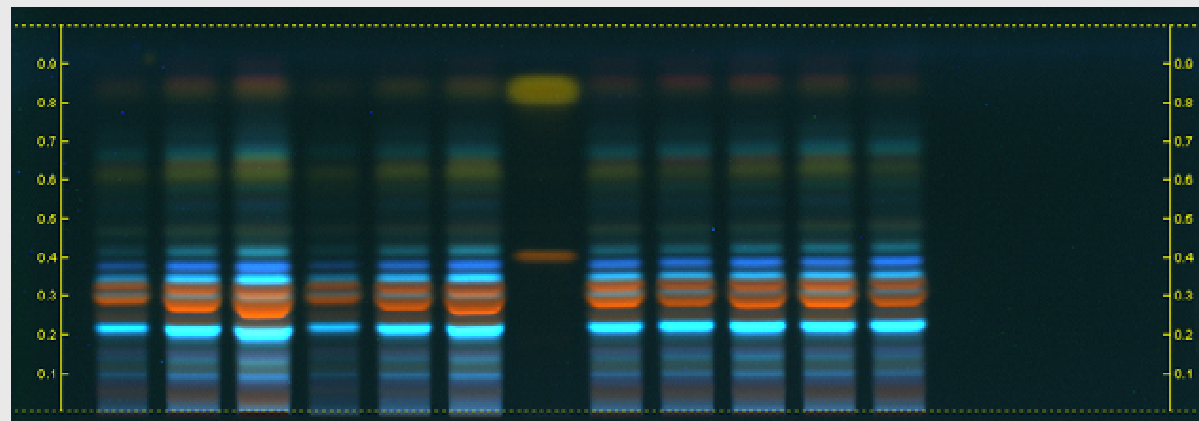
HPTLC

Thoughts from the laboratory

Eike Reich



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Our mission (since 2002)

- We strive to make HPTLC an accepted and standardized analytical method well known for its unique properties throughout the world.
- We intend to be an accredited laboratory well known for its applied research skills and its analytical competence.
- We develop, propagate and sell know-how with reference to HPTLC to academic institutions, governmental and regulatory bodies and industrial customers.
- In so doing, we extent the level of awareness of HPTLC as well as the knowledge about its advantages and special feature of performance as a qualitative and quantitative analytical methodology.
- We promote the scientific basis and practical recommendations for a standardization of the HPTLC methodology.
- We are a center of competence for education/training, consulting and laboratory services.

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Vision 2012

By 2012 we will have established worldwide HPTLC as primary tool for sophisticated qualitative analysis and quantitative screening, which is complementing other analytical techniques, particularly HPLC.

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What is TLC?

- Chromatography for the poor (cheap)
- Simple manual chromatography for everyone (students?)
- Rapid
- Flexible
- Reference and test solution side by side
- “Just” qualitative, preliminary estimation at best

- Unpredictable
- Unreliable

→ Manual technique, simple instruments, TLC plates

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What is HPTLC?

High Performance Thin-Layer Chromatography

TLC for the 21st century

- Instrumental TLC
  - Application
  - Development
  - Documentation
  - Densitometry
- Truly „plug and play“
- Fully cGMP compliant

A new concept

- Instruments
- Scientific basis
- Standardized methodology
- Validated methods

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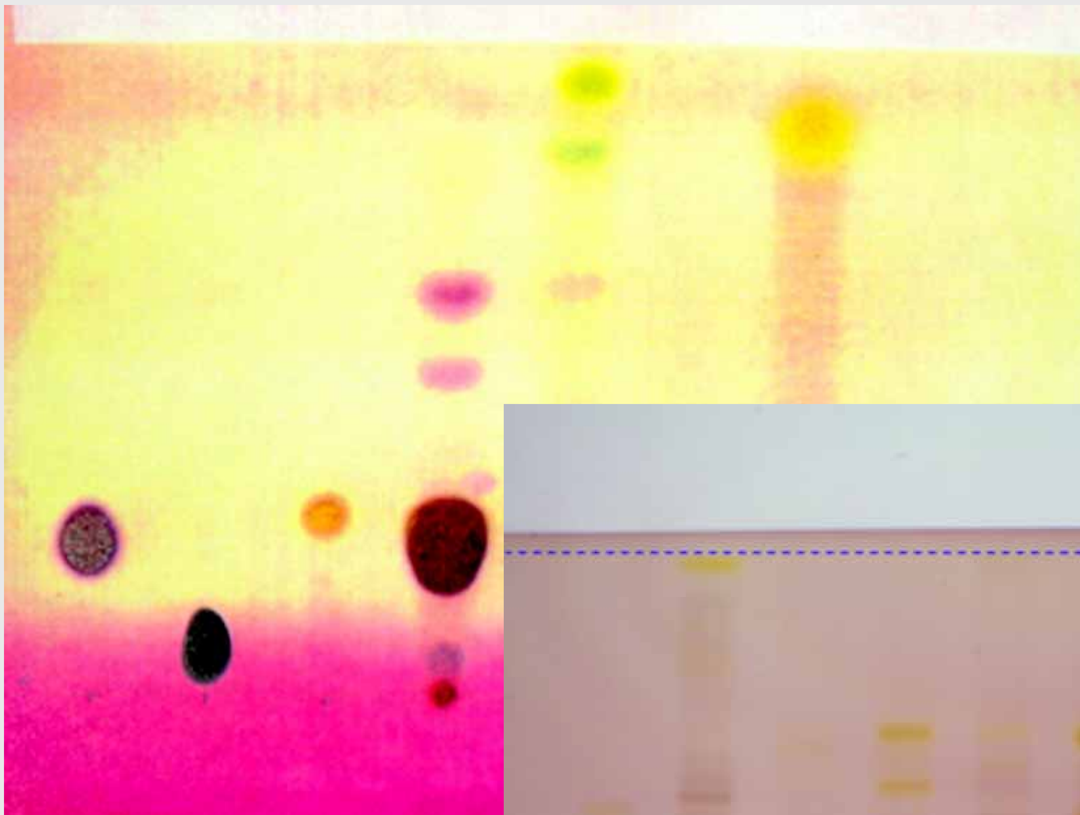
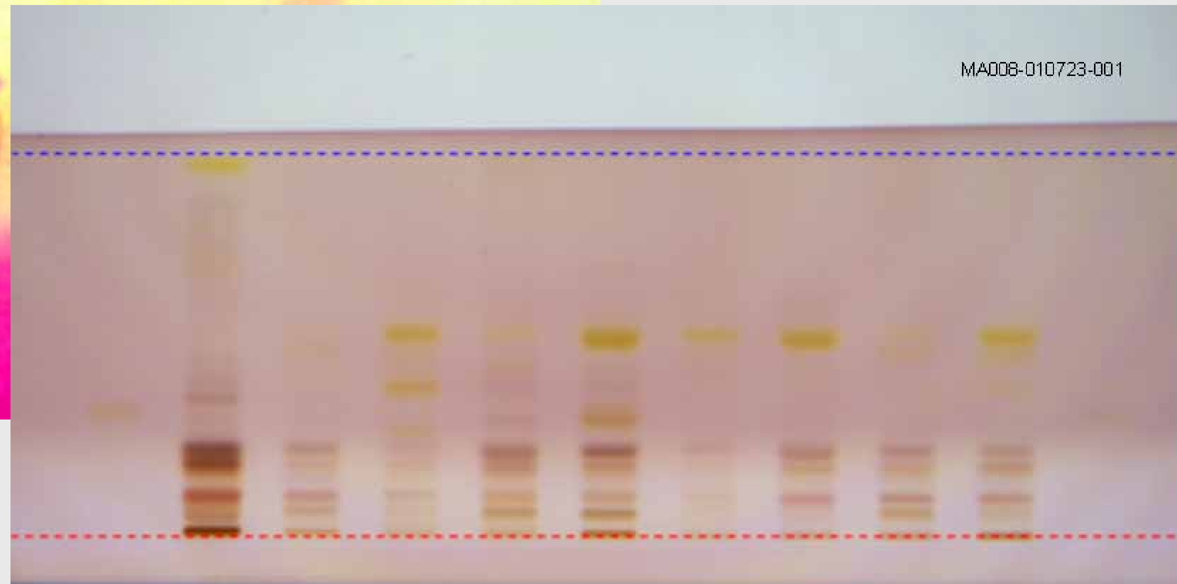
TLC or HPTLC?





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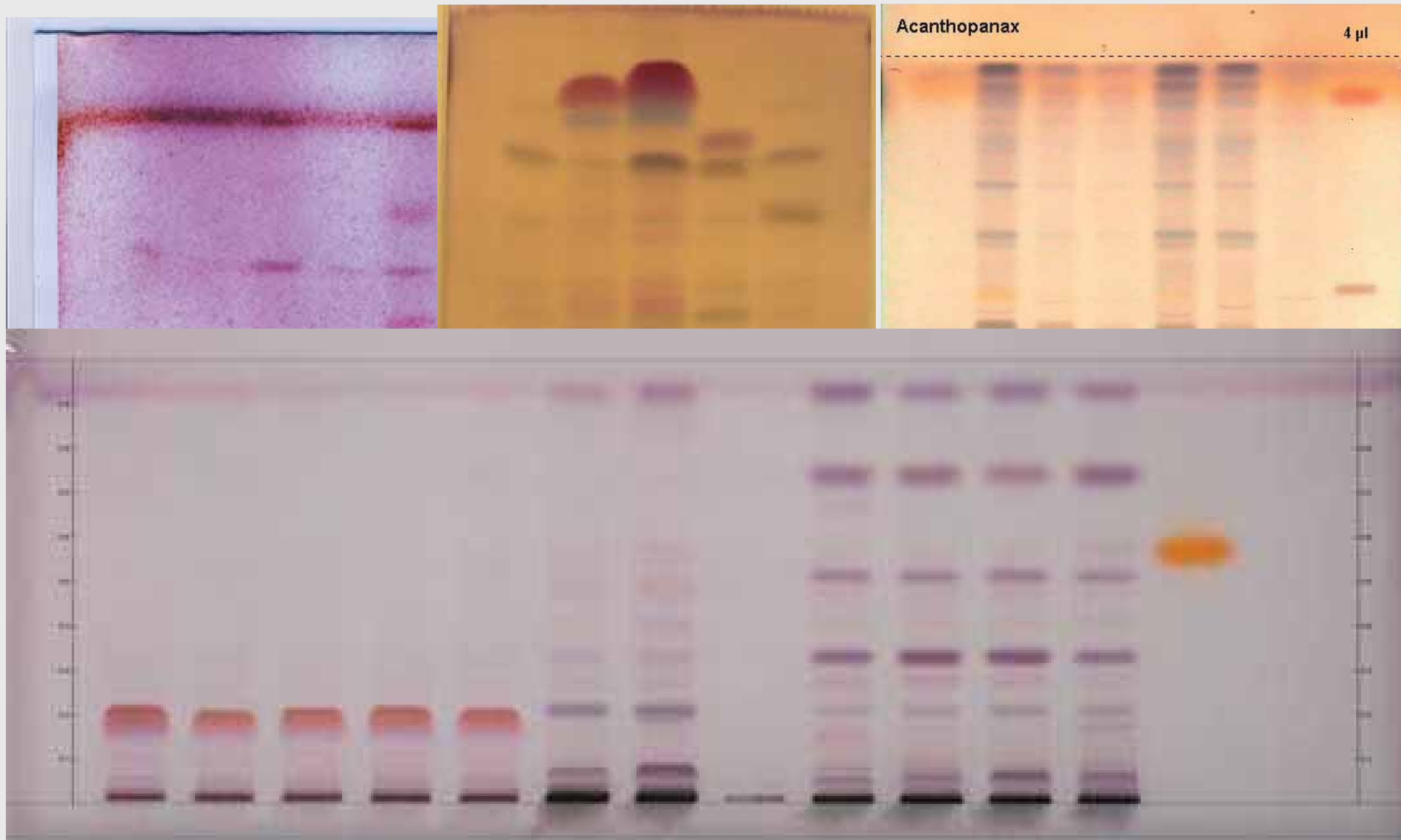
Identification of *Acanthopanax*



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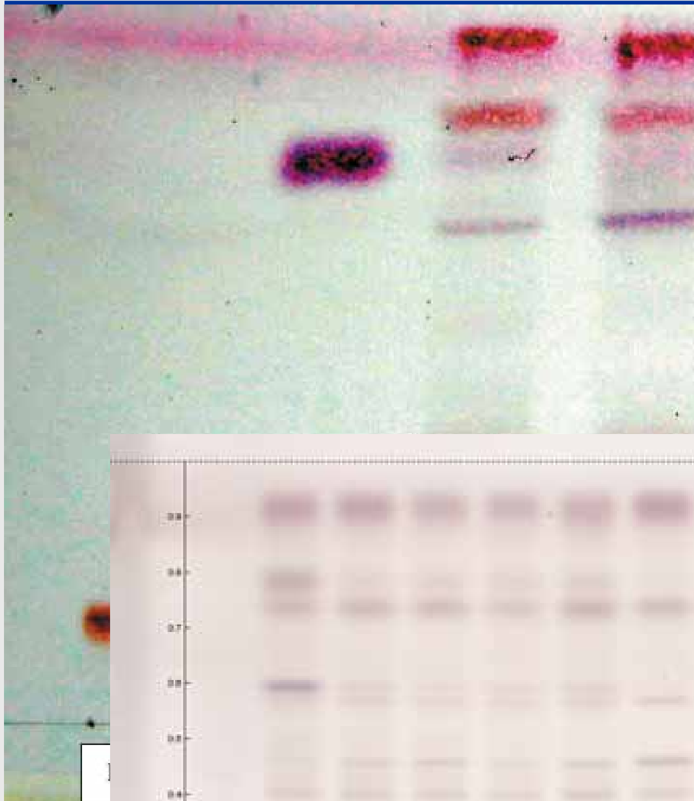
Identification of Peonies



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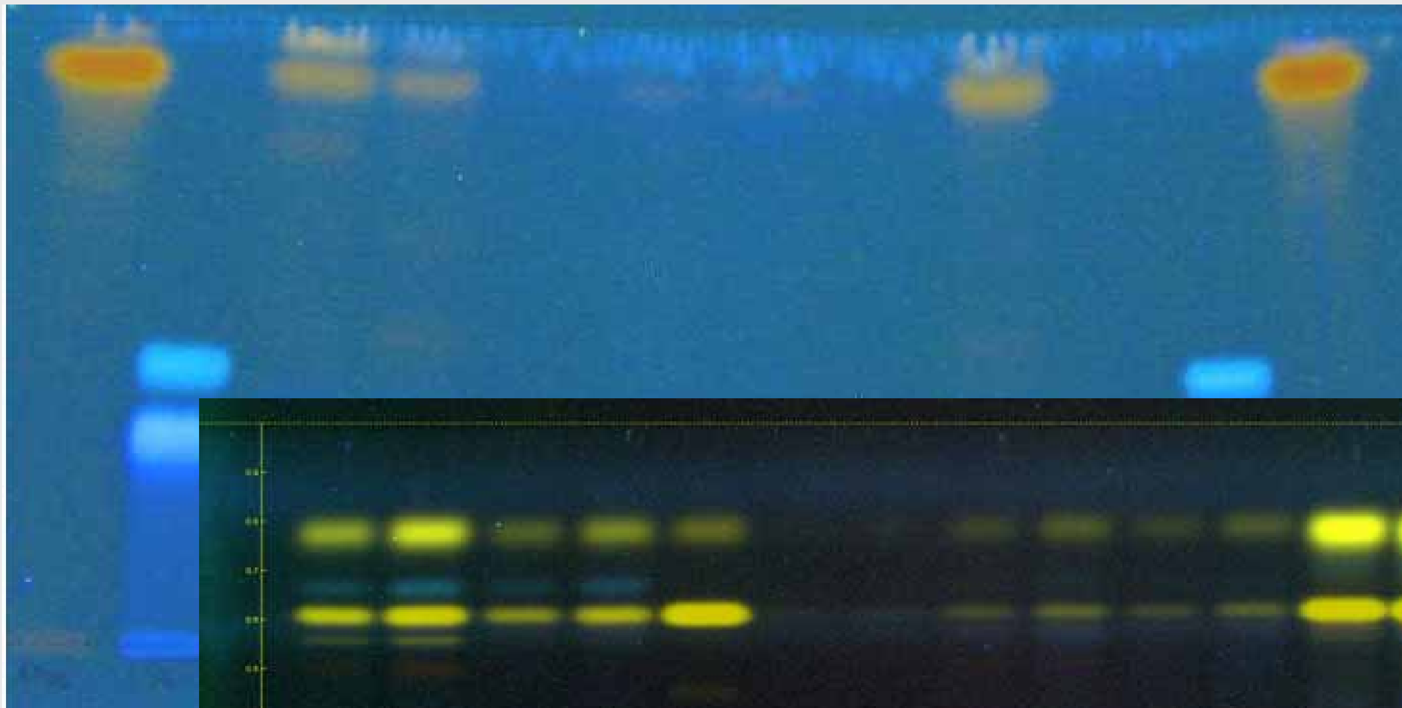
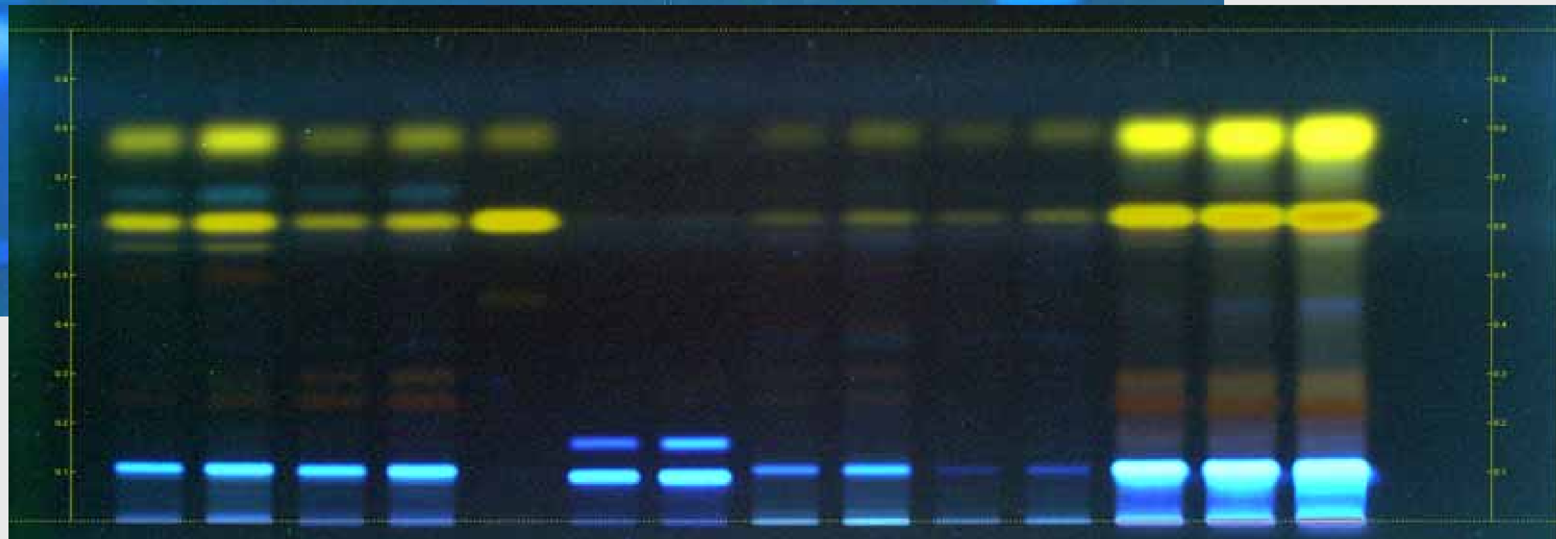
Identification of Fleece flower





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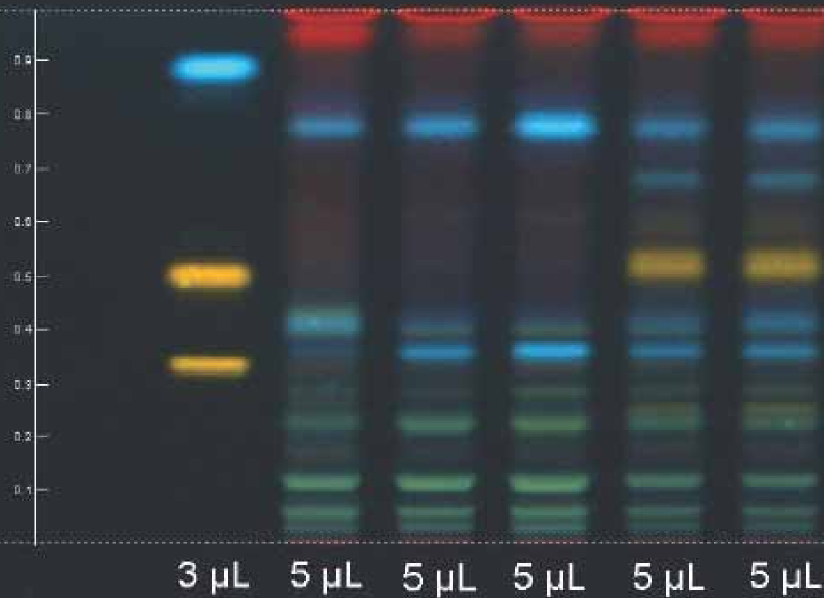
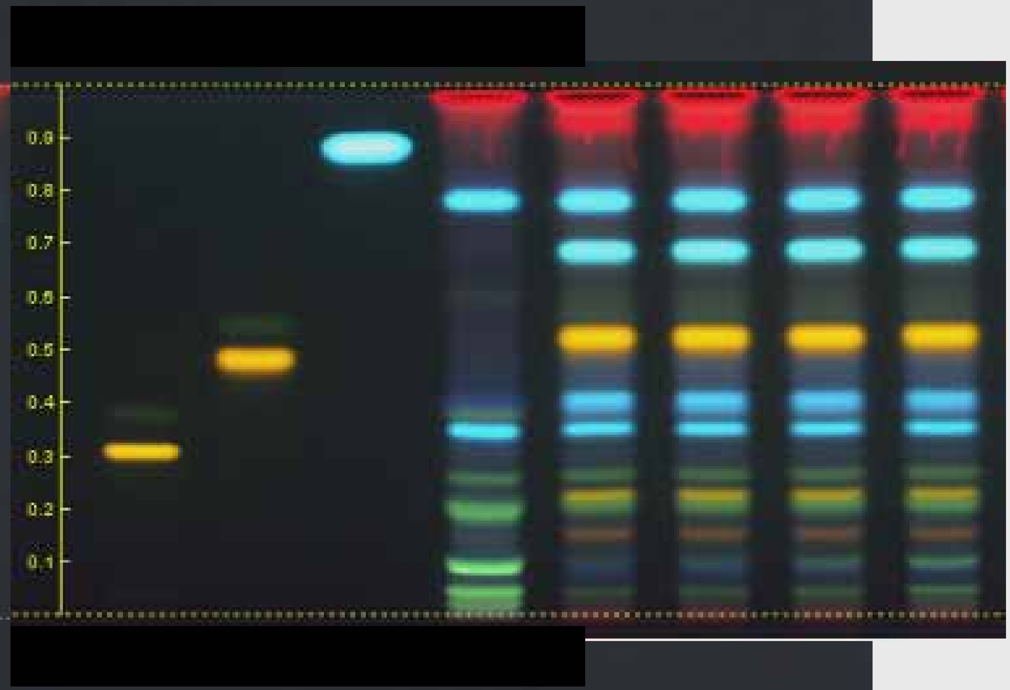
ID and adulteration of common horse tail





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TLC in chapter 2.2.27

- **Chromatographic tank** with a flat bottom or twin trough, of inert, transparent material, of a size suitable for the plates used and provided with a tightly fitting lid. For horizontal development the tank is provided with a trough for the mobile phase and it additionally contains a device for directing the mobile phase to the stationary phase.
- **Micropipettes, microsyringes, calibrated disposable capillaries** or other application devices suitable for the proper application of the solutions.
- **Fluorescence detection device** to measure direct fluorescence or the inhibition of fluorescence.
- **Visualization devices and reagents**. Suitable devices are used for derivatization to transfer to the plate reagents by spraying, immersion or exposure to vapor and, where applicable, to facilitate heating for visualization of separated components.
- **Documentation**. A device may be used to provide documentation of the visualized chromatogram, for example a photograph or a computer file.

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Sample application

- **Sample application**. Apply the prescribed volume of the solutions at a suitable distance from the lower edge and from the sides of the plate and on a line parallel to the lower edge; allow an interval of at least 10 mm (5 mm on high-performance plates) between the centers of circular spots and 5 mm (2 mm on high-performance plates) between the edges of bands. Apply the solutions in sufficiently small portions to obtain circular spots 2-5 mm in diameter (1-2 mm on high-performance plates) or bands 10-20 mm (5-10 mm on high-performance plates) by 1-2 mm.
- In a monograph, where both normal and high-performance plates may be used, the working conditions for high-performance plates are given in the brackets [ ] after those for normal plates.

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Development

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

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Possible choices in methodology

- TLC layer
- Manual application
- Transparent container
- (Pickle jar?)
- UV-Lampe (λ?)
- Manual spraying / immersion

- HPTLC layer
- Automatic application
- Automatic Developing Chamber
- Scanner
- Automatic immersion / spraying

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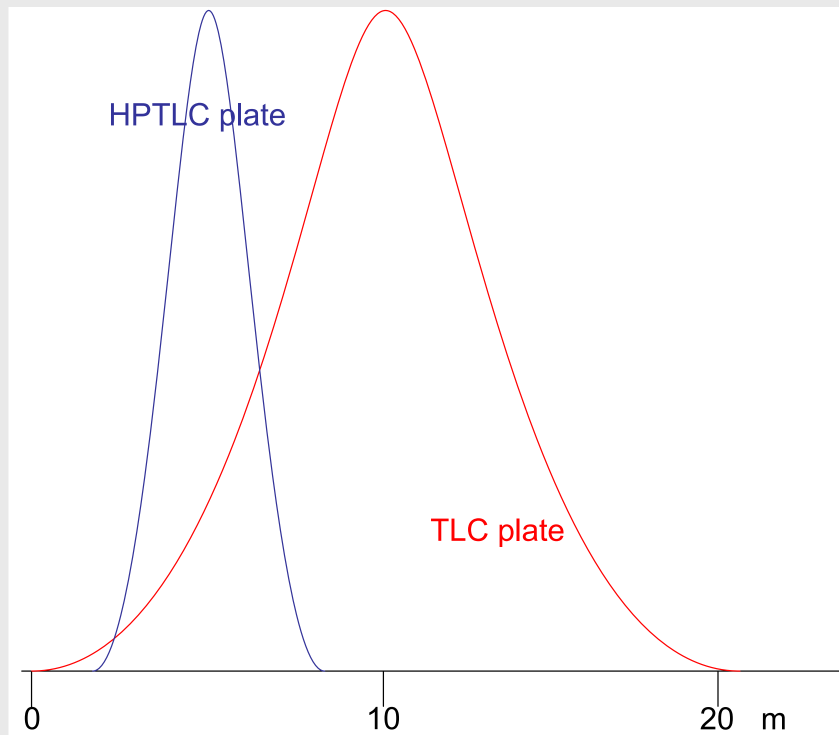
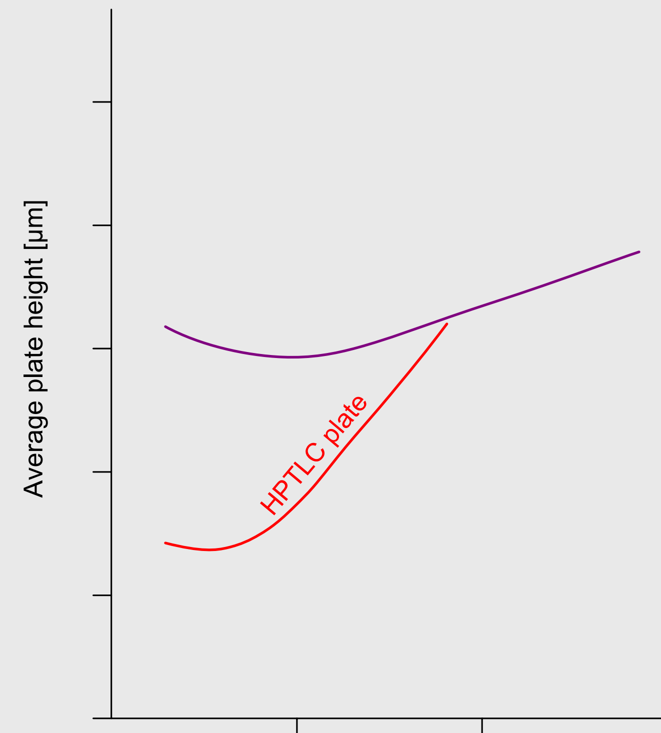
TLC or HPTLC

- Pharmacopoeias see difference primarily in the plate yet assume similar results





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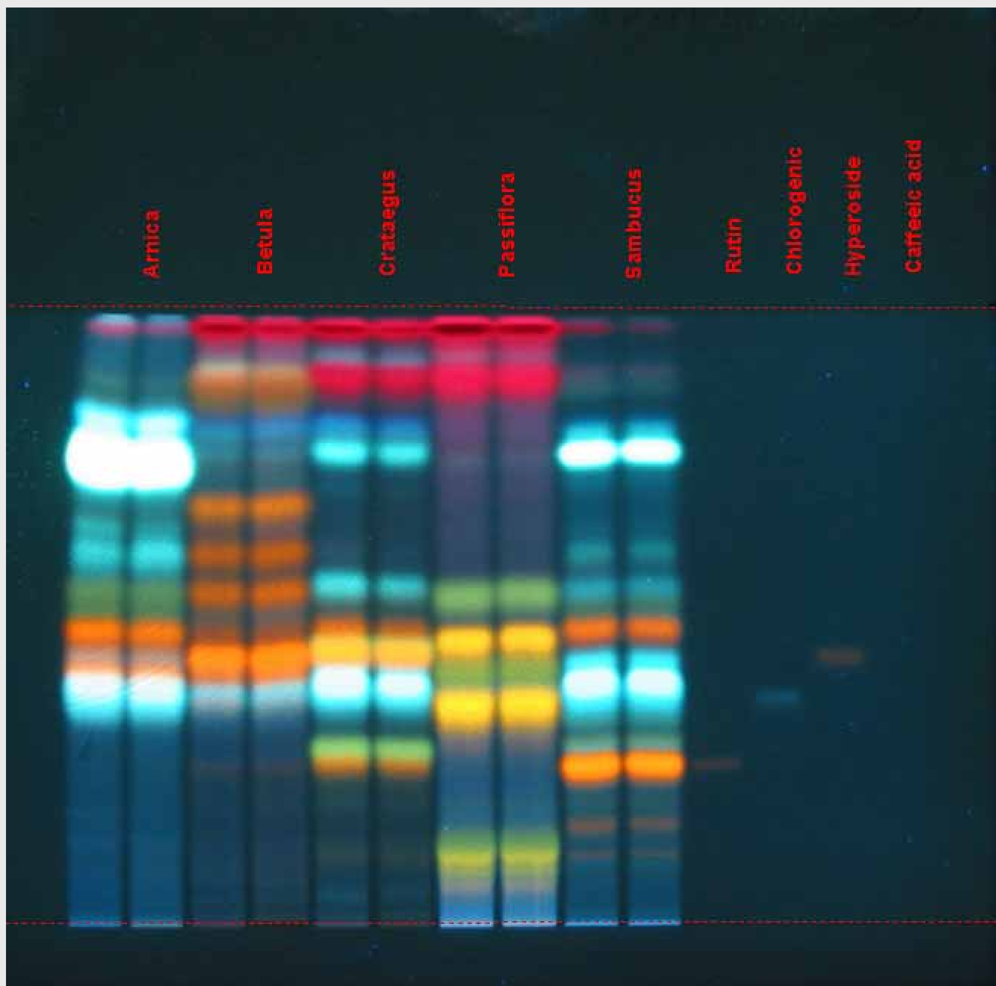
Comparison TLC-HPTLC of flavonoids

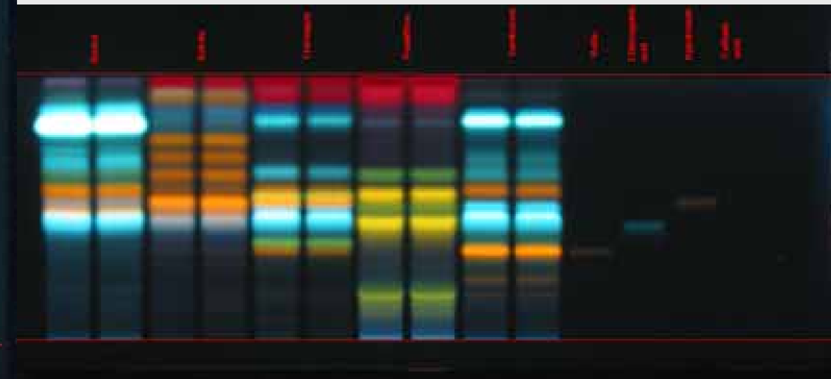


TLC plate 20 x 20 cm

(135 mm)

HPTLC plate 20 x 10 cm

(60 mm)



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The goal: optimization and standardization

- Plate setup and handling
- Sample application (as band)
- Chamber geometry and saturation
- Humidity control
- Developing distance
- Derivatization procedure
- Documentation (electronic images)
- Evaluation

→ SOP

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SOP for HPTLC

- Should be the basis for all work
- Applies to all methods
- All deviation need to be recorded
- Our SOP is in full compliance with PhEur, USP, ChP

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The future

- SOP is in accordance with Chapter 2.2.27
- SOP is basis for an HPTLC method template

  → winCATS template
- PhEur monograph is basis for “written” HPTLC method

  →Method for identification of Birch leaf
- Result table is replaced by reference image
- Corresponding instrument methods are derived from template

  → winCATS method
- Comparison of results with reference images

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A vision becomes reality…

1994 DSHEA

1995 San Diego: HPTLC for DS > 150 attendees

1996 join AHP, first ID method: Valerian

1998 join AHPA: Introduction of HPTLC for identification

1998 join AOAC: Introduce the concept of HPTLC for ID

2001: Echinacea, first validated method

2003: Scientific Note: Pharmeuropa TLC vs HPTLC

2003: Visit to FDA: Screening for Aristolochic acids

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A vision becomes reality…

2004: Change of PhEur Chapter 2.2.27

2005: Change of USP Chapter <621>

2005: PhEur new style guide for elaboration of monographs

2005: FDA published draft of cGMP for industry comments

2006: Join UNPA: Introduce the HPTLC fingerprint concept

2007: Publication of the Book

2007: cGMP become effective for large companies

2008: Validation paper JAOAC

2008: Development of a USP training course

2009: cGMP become effective for medium companies

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A vision becomes reality…

2009: USP DS compendium is published

2009: USP screening for Aflatoxins by HPTLC

2009: PhEur screening for Aristolochic acids by HPTLC

2009: PhEur test for adulteration of Black cohosh

2009: Method collection completed (100 released)

2010: PhHelv decides to publish electronic images for reference

2010: PhEur accept request for revision: Distinction HPTLC / TLC

2010: cGMP in effect for all companies in the US

2010: EU begins discussion about regulations of DS

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What also happened…

- Chinese TCM Atlas
- Indian Standards
- Indian TLC of Ayurvedic Drugs
- Malaysian Herbal Pharmacopoeia
- Thai HP
- Philippines HP
- Chinese Pharmacopoeia German ed.
- ???

→ We NEED harmonization

→ We NEED help

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Some assumptions

- Analytical work is a burden and therefore avoided whenever possible (academia is the other way around)
- People do NOT care about TLC (or HPTLC)
- People care even less about “wonderful” instruments
- If there is enough pressure most companies will comply by readily embracing solutions, particularly if those make their life easier.

Federal Register

Monday,
June 25, 2007

Part II

Department of Health and Human Services

Food and Drug Administration

21 CFR Part 111

Current Good Manufacturing Practice in Manufacturing, Packaging, Labeling, or Holding Operations for Dietary Supplements; Final Rule

Petition To Request an Exemption From 100 Percent Identity Testing of Dietary Ingredients; Interim Final Rule

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- 100% ID
- Suitable and scientifically valid methods
- HPTLC as a method is mentioned

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CAMAG HPTLC Method Collection

- Based on standardized methodology → SOP
- Same template for all methods
- Illustration of existing pharmacopoeial or validated methods
- SST for simple transfer – validation
- Reference images for identification

And: QMS documents
Instructions for generating new methods

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The product

- Web based application
  - Easy maintenance
  - Complete control and info about customer activities
- Customer buys an access key for controlled method download
- New methods can be uploaded for exchange
- Promotion by AHPA
- 100 methods released, data for 50 more prepared

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CAMAG Laboratory Network

- To help us grow the method collection
- To train customers in their language
- To peer validate new methods
- To standardize HPTLC globally

CAMAG Laboratory

CSI Laboratory	USP
TCM University of Shanghai / Nikyang	ChPh
University of Barcelona (Spain)	PhEur
Technical College Wädenswil (Switzerland)	PhEur
University of Graz (Austria)	PhEur
Kew Gardens (UK)	
Korea	
Malaysia	
Thailand	
INDIA?	

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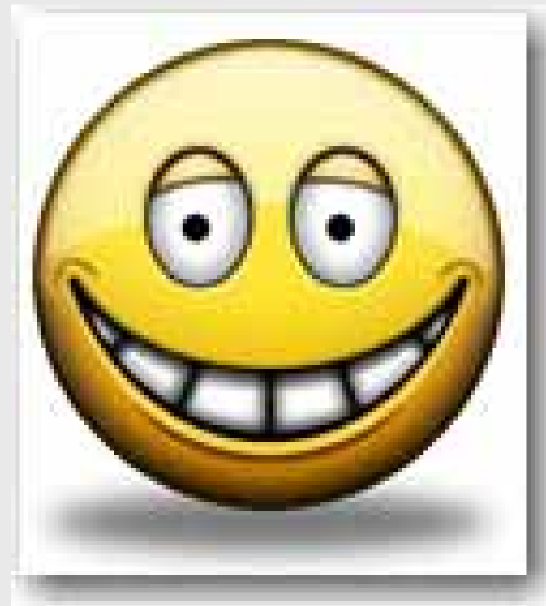
What else is going on?



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Special issue of JAOAC on HPTLC (2009/10)

- Co-editor with Joe Sherma
- Invited contributions (accepted)
  - Uni Freiburg (diploma thesis) Zhi Li, Germany
  - FH Wädenswil, Prof. Meier, Switzerland
  - AHP, Roy Upton, USA
  - Phytotechnologies, Al Leung, USA
  - Alchemists, Sidney Sudberg, USA
  - Prof. Xie, China
  - Prof. Yue, China

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Publications (1):

KAALE ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 93, NO. 6, 2010 1

DRUG FORMULATIONS AND CLINICAL METHODS

An Interlaboratory Investigation on the Use of High-Performance Thin Layer Chromatography to Perform Assays of Lamivudine-Zidovudine, Metronidazole, Nevirapine, and Quinine Composite Samples

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Muhimbili University of Health and Allied Sciences, Laboratory for Pharmaceutical Analysis, School of Pharmacy, PO Box 65013, Dar es Salaam, Tanzania
EIKE REICH
CAMAG-Laboratory, Muttenz, Switzerland
THOMAS P. LAYLOFF
Management Sciences for Health, Arlington, VA

Two laboratories extensively investigated the use of HPTLC to perform assays on lamivudine-zidovudine, metronidazole, nevirapine, and quinine composite samples. To minimize the effects of differences in analysts' technique, the laboratories conducted the study with automatic sample application devices in conjunction with variable wavelength scanning densitometers to evaluate the plates. The HPTLC procedures used relatively innocuous, inexpensive, and readily available chromatography solvents used in the Kenyon or the Global Pharma Health Fund Minilabs® TLC methods. The use of automatic

spotting techniques and in individual visual acuity in assessing the amounts contained in the developed sample spots. (6)

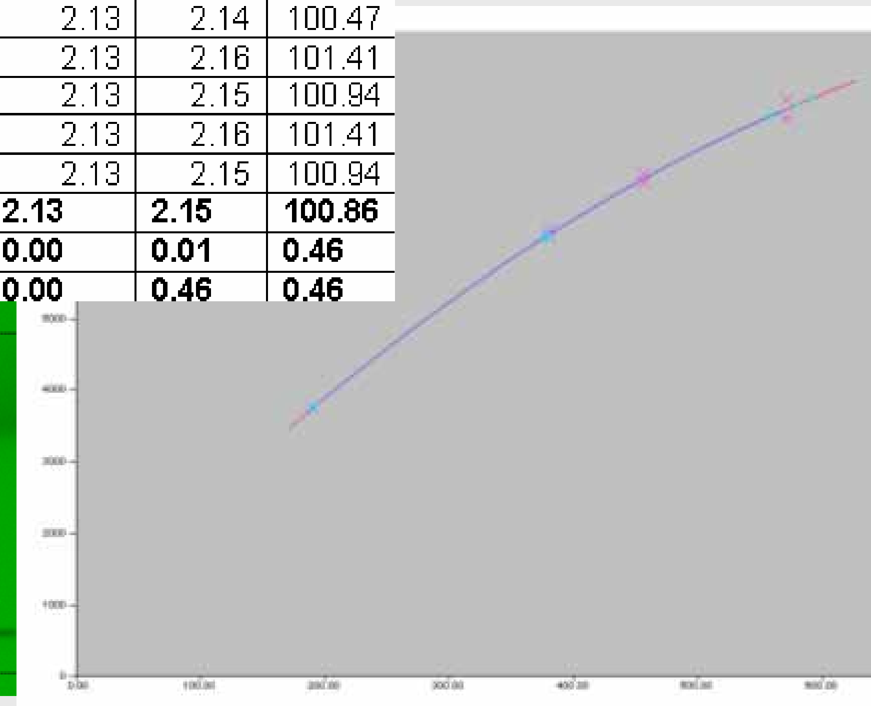
In 1997, the Global Pharma Health Fund introduced the Minilab®, which incorporates this TLC technology platform into a very facile, robust, inexpensive, convenient, and secure system that can be used to detect substandard and counterfeit pharmaceutical products (7). In addition, the Minilab features excellent manuals and presentations, which make it convenient for users to identify needed supplies and to refresh their training. The Minilab platform currently includes assessment methods for over 40 products; the majority of the methods are for products in the World Health Organization

DO NOT DISTRIBUTE

Quantitation of drugs by TLC

Table 0-3. Intra-day accuracy data at three concentration levels (n= 6)

Run #	Responses at concentration levels (µg/µL)								
	50% (plate 1)			100% (plate 2)			150% (plate 3)		
	Expected	Observed	% Accuracy	Expected	Observed	% Accuracy	Expected	Observed	%Accuracy
1	0.99	0.98	98.99	1.63	1.65	101.23	2.13	2.13	100
2	0.99	1	101.01	1.63	1.65	101.23	2.13	2.14	100.47
3	0.99	1	101.01	1.63	1.65	101.23	2.13	2.16	101.41
4	0.99	1	101.01	1.63	1.66	101.84	2.13	2.15	100.94
5	0.99	0.99	100.00	1.63	1.67	102.45	2.13	2.16	101.41
6	0.99	1	101.01	1.63	1.65	101.23	2.13	2.15	100.94
Mean	**0.99**	**1.00**	**100.51**	**1.63**	**1.66**	**101.53**	**2.13**	**2.15**	**100.86**
SD	**0.00**	**0.01**	**0.71**	**0.00**	**0.01**	**0.43**	**0.00**	**0.01**	**0.46**
%rsd	**0.00**	**0.71**	**0.71**	**0.00**	**0.43**	**0.43**	**0.00**	**0.46**	**0.46**



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Significance / Targets

- Collaboration with a Lab in Afrika
- Results are in line with guidance of WHO
- Approaching WHO for funding future work

- Customs and FDA labs in African countries

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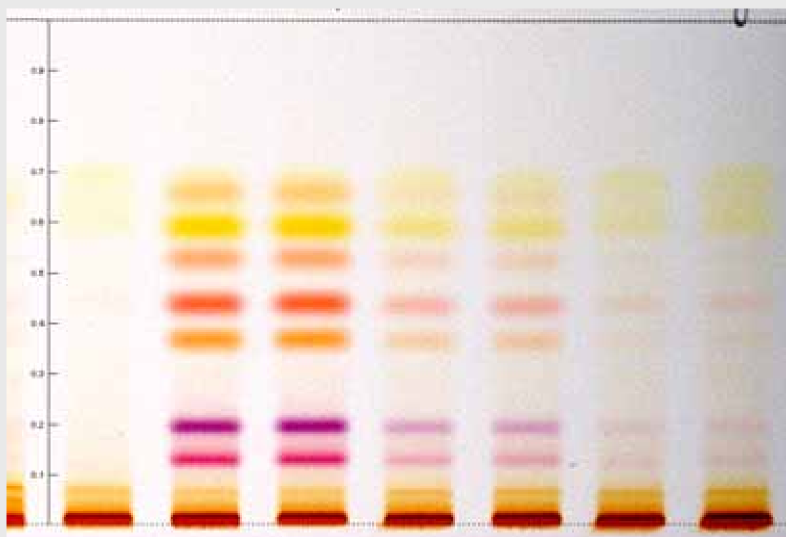
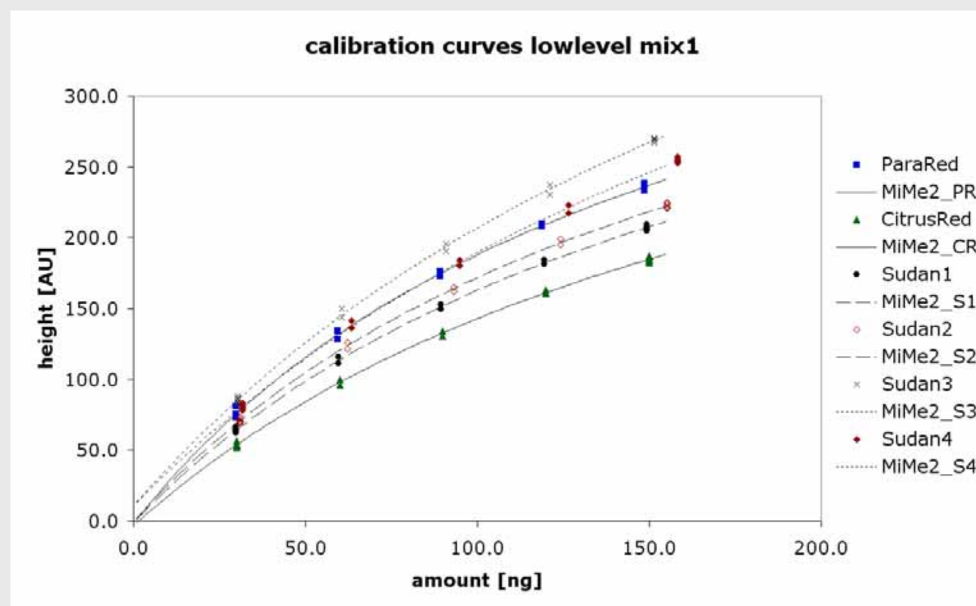
Adulterated Spices (2009)

- A Validated HPTLC Method for the Determination of Illegal Dyes in Spices and Spice Mixtures
- **Journal of Liquid Chromatography & Related Technologies**, 32: 1273–1288,





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Significance / Target

- Collaboration with State Food Authority
- Very practical and inexpensive method:
  Screening of spices vs. trace analysis of food
- Solution to a real problem!

- Food industry
- Authorities
- Control labs

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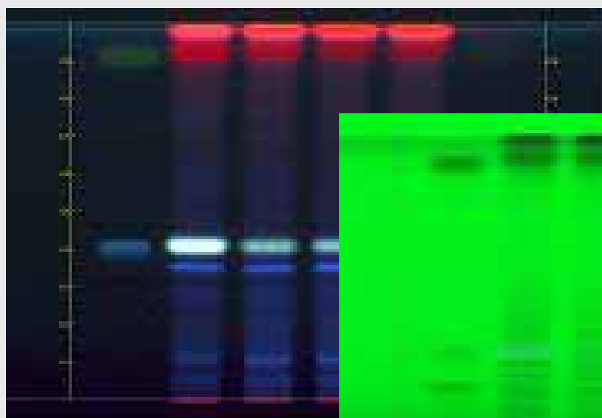
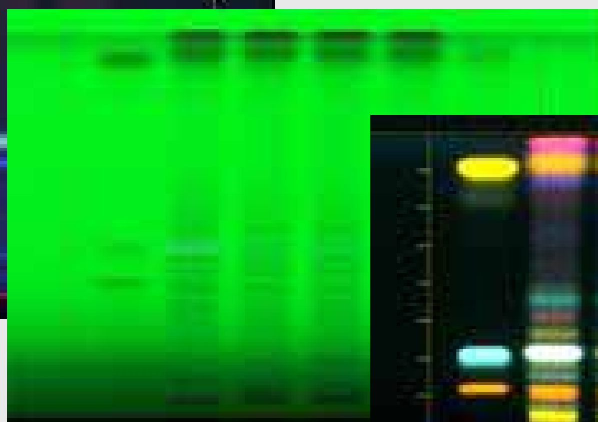
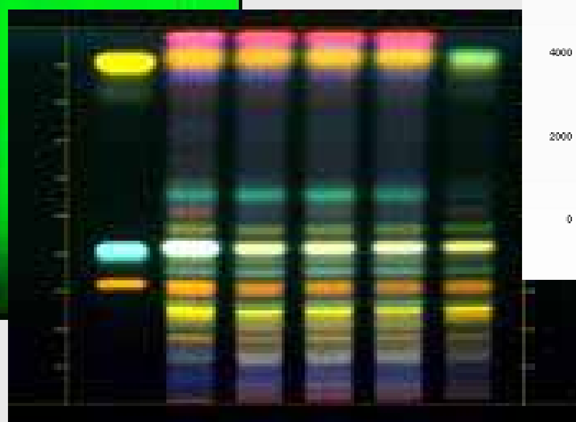
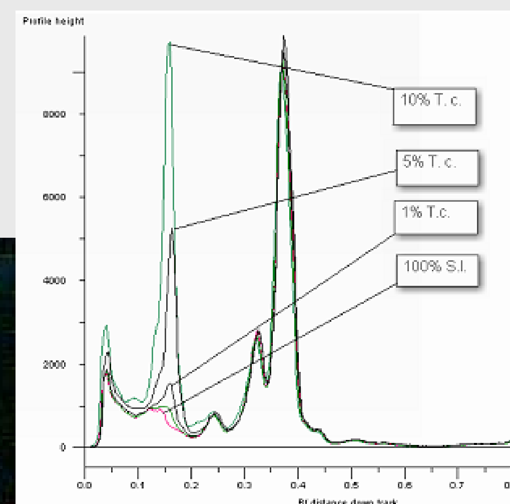
Plant Analysis 2008 – Planar Chromatography

- Mini review on the identification of medicinal plants and advances in methodology/instrumentation
- **Planta Medica**, 2009 eFirst, *DOI:10.1055/s-0028-1088389*.

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Significance

- Invited contribution.
- Definition of HPTLC as
  - A form of, not a synonym for „Planar Chromatography“
  - Orthogonal to HPLC
  - Significantly different from TLC
  - High-tech and to be considered seriously
- This special issue is avalaible for free download
- Advertised by Planta Medica by email

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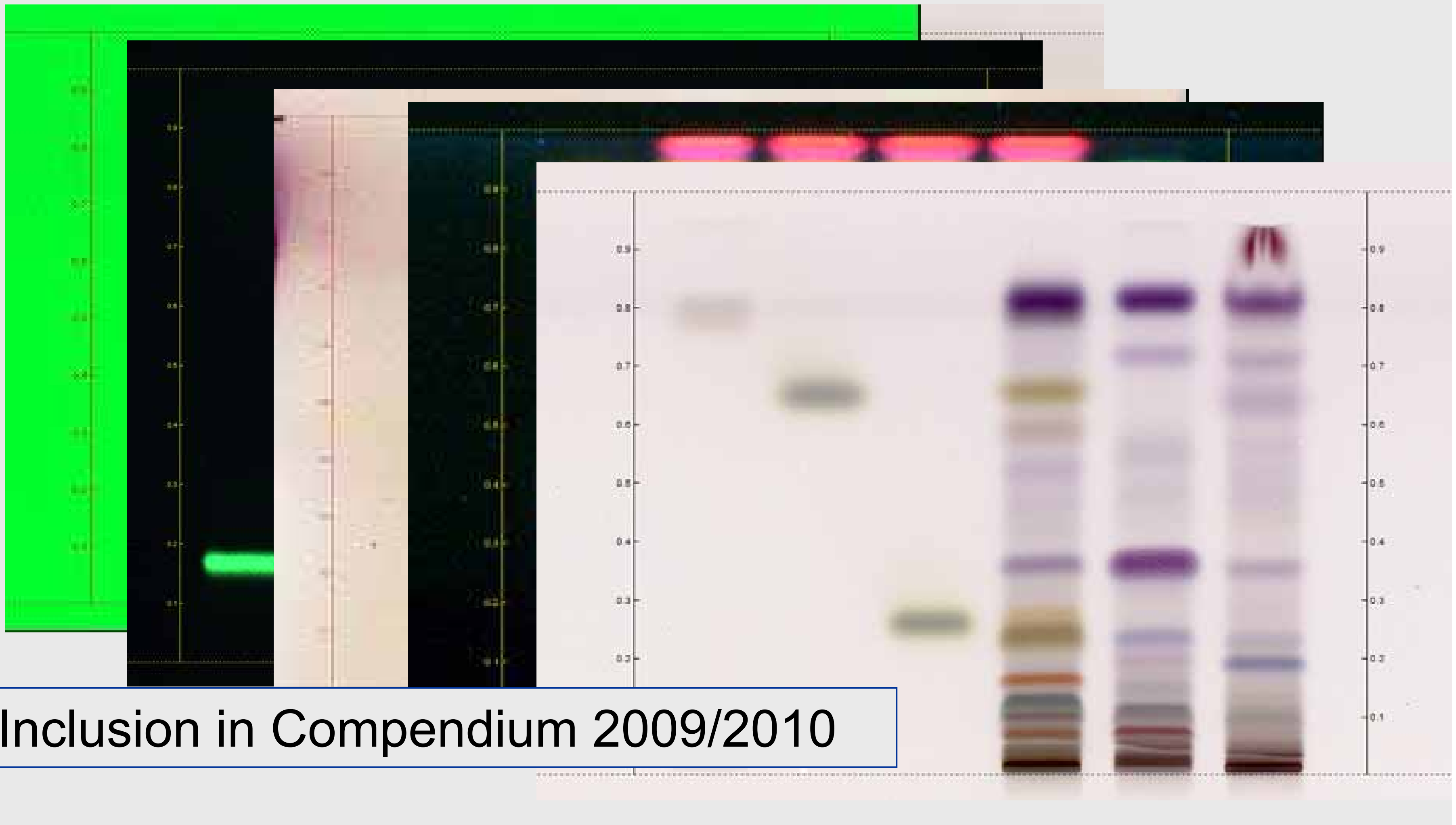
„Illustration“ of USP methods for ID



Inclusion in Compendium 2009/2010

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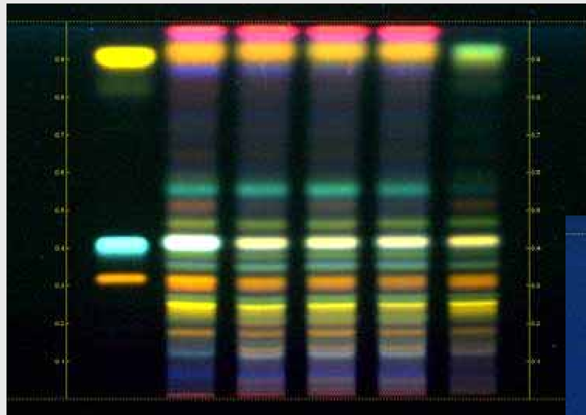
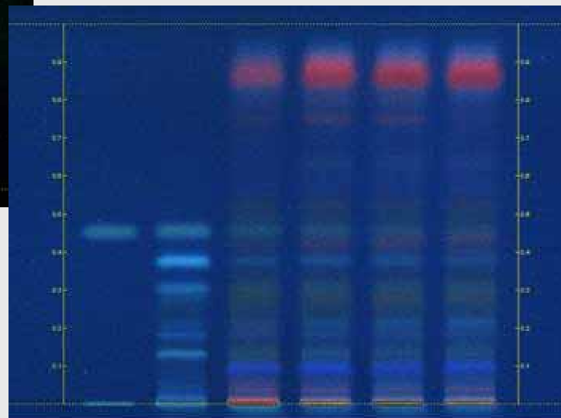
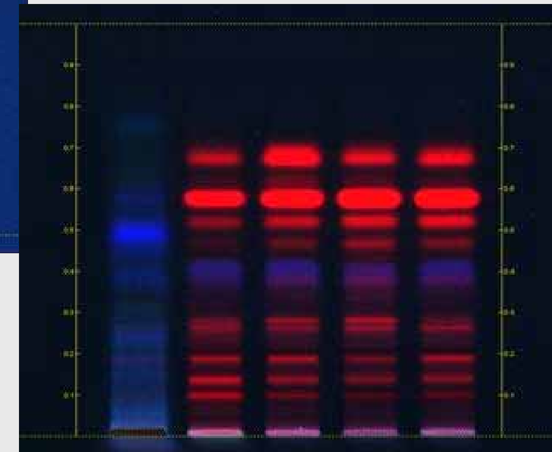
USP Compendium: Ginkgo







Flavonoids **Terpene lactones** **Ginkgolic acids**

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Limit test for Aflatoxins (B2, 2ppb) in plants

- Existing test (TLC) is not sensitive enough 2ppm
- Matrix interference likely
- *Proposal: affinity columns, HPLC, post column derivatization, fluorescence detection*
- Alternative: Affinity columns (if necessary), HPTLC, visual evaluation / scanning densitometry

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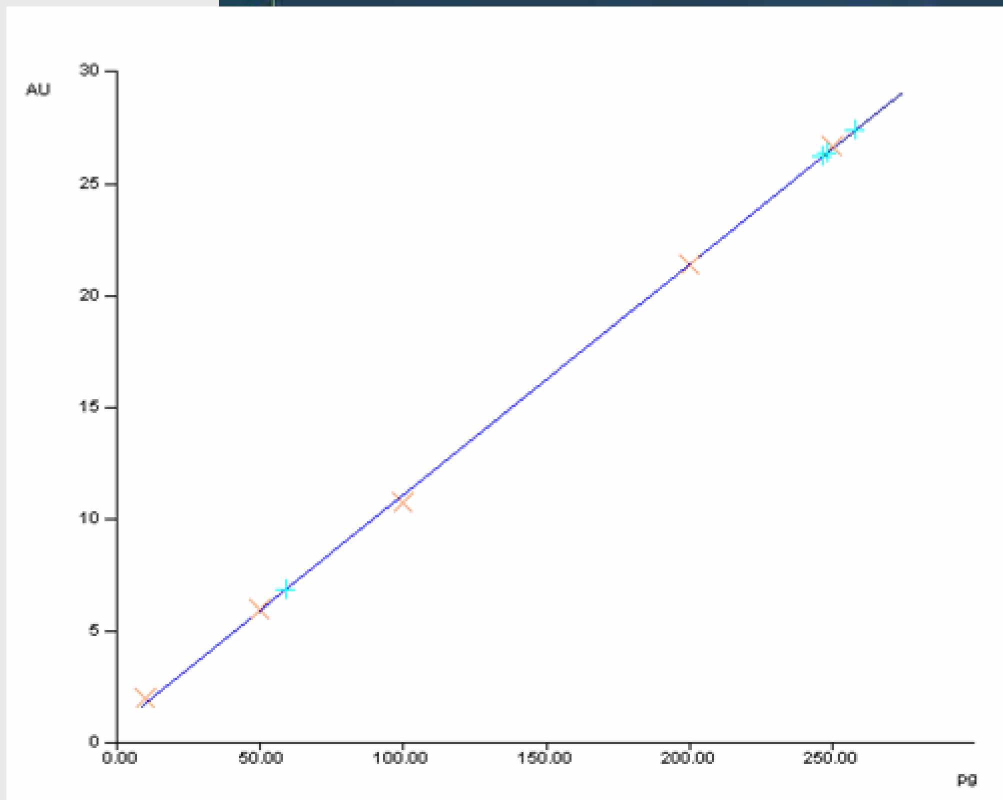
Aflatoxin B2: USP limit test 2 ppb in plants



Asian ginseng

Black cohosh

Rf: 0.39

Tomato extract

Rf: 0.39

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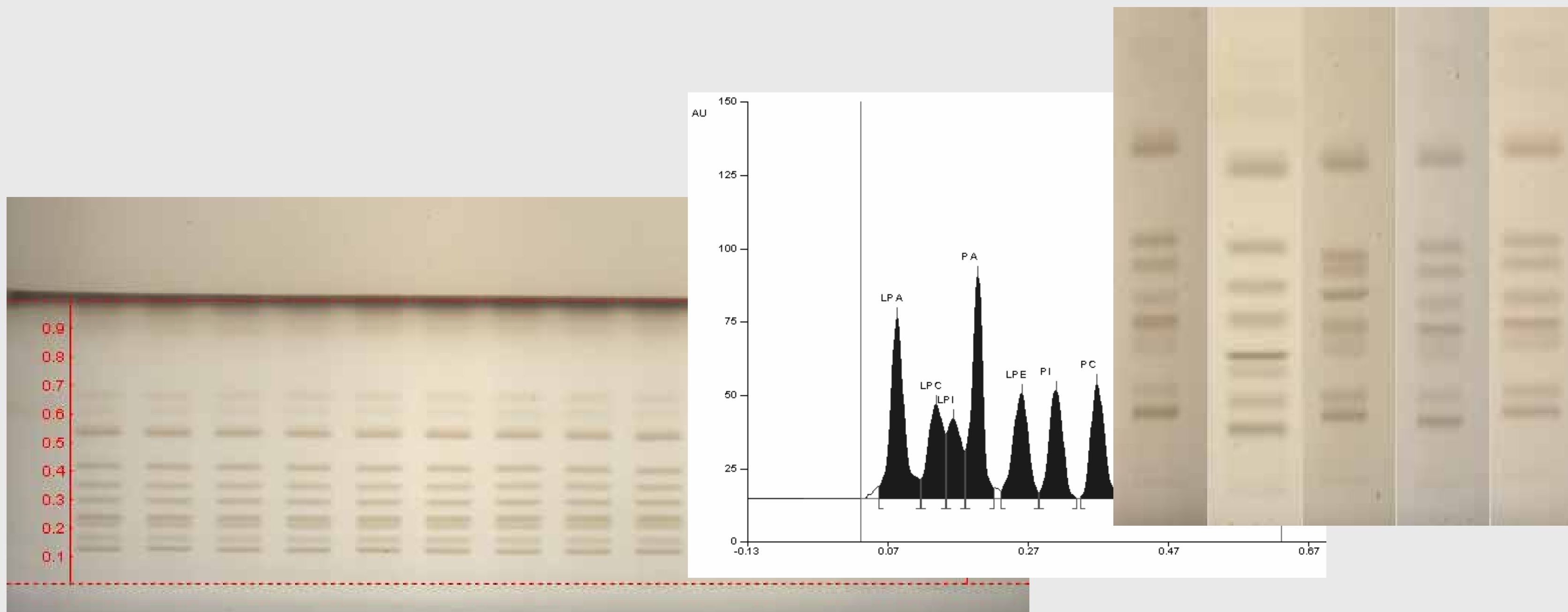
Phospholipids (2008)

- Separation of Phospholipids by HPTLC – an Investigation of Important Parameters
- **J. Liq. Chromatogr. Related Technol** 31, 1857–1870



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Parameters

- Relative humidity
- Temperature and duration of derivatization
- Pre-washing / activation of plate
- Plate batches
- Content / concentration of ammonia

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Humidity and pre-treatment of plate

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Different batches of plates

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Significance

- Systematic approach to the analysis of PL
- Consequent focus on instrumentation, standardization, and reproducibility.
- Basis for individual method development / research

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Other publications:

- **2008 The Column:** LC GC 10/08 „Do wrinkle creams really work? HPTLC for the analysis of lipids“
- **2009 Cover Story:** Modernizing TLC. New instrumentation, materials, and analysis techniques take lab staple into high-performance arena. Kemsley, J. (2009) C&EN News 87(20), 11-18.

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CBS

CAMAG Laboratory: Method Development in Practice

Validated HPTLC method for skin lipids

Karin Rothenbühler

In cooperation with the University of Basel and under supervision of Prof. Matthias Hamburger, Ms. Karin Rothenbühler has worked on her master thesis [1] in Pharmaceutical Biology at the CAMAG laboratory.

Introduction

Even though numerous literature references indicate that TLC/HPTLC is the method of choice for skin lipid analysis, no validated quantitative HPTLC method was found. Following the recent publication on the HPTLC analysis of phospholipids [2], Ms. Rothenbühler developed and validated a HPTLC method for the most important markers of non-polar lipid classes of the human stratum corneum: squalene, triolein, palmitic acid, 1,2-dipalmitoyl-sn-glycerol, stearyl palmitate, cholesteryl palmitate, and cholesterol.

In combination with appropriate sampling procedures the method was rapid, robust and reliable for use by the R&D laboratories of cosmetic industry. The advantages of HPTLC, in comparison to RP-HPLC, are its simple derivatization as a prerequisite for lipid detection and the separation based on functional groups.

Sample preparation

Skin samples were obtained from the inner forearm of volunteers either by direct extraction with ethanol, abrasion of a defined area with a razor blade or with Epiglu, a tissue adhesive for medicinal use.

Standard solutions

2 mg each of squalene, triolein, palmitic acid, 1,2-dipalmitoyl-sn-glycerol, stearyl palmitate, cholesteryl palmitate, and cholesterol were dissolved in 10 mL of chloroform – methanol 1:1. For quantification 1 mL of this solution was diluted with 4 mL of chloroform – methanol 1:1.

Chromatogram layer

HPTLC plates LiChrospher silica gel 60 F_{254} (Merck), 20 × 10 cm, pre-washed by developing with methanol followed by drying in an oven at 120°C for 20 min.

Sample application

Bandwise with ATS4, band length 8 mm, track distance min. 10 mm, distance from lower edge 8 mm, distance from left edge min. 15 mm, application volumes 2–30 µL of samples and 2.5–10 µL of standard solutions.

Chromatography

In the ADC2 with chamber saturation for 20 min first with toluene to a migration distance of 80 mm followed by drying for 5 min, then with n-hexane, t-butyl methyl ether, acetic acid 80:20:1 to 45 mm. Separation was affected by the plate activity, therefore plates were conditioned prior to both developments at 33 % relative humidity for 10 min using a saturated solution of magnesium chloride.

Post-chromatographic derivatization

Copper(II) sulfate reagent was prepared by dissolving 20 g Copper(II) sulfate pentahydrate in 200 mL of cooled methanol and then adding 8 mL of sulphuric acid 98 % and 8 mL of orthophosphoric acid 85 %. With the Immersion Device III the plate was dipped for 6 s into the reagent, then dried for 30 s with cold air and finally heated on a plate heater for 30 min at 140 °C.

Densitometry

TLC Scanner 3 with winCATS software, absorption measurement at 350 nm.

Results and discussion

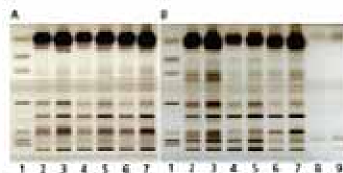
Since there was no standardized methodology for sampling of human skin samples, various techniques were evaluated. Skin samples from the inner forearm of volunteers were obtained by use of various commercial tapes, medicinal tissue adhesive,

direct abrasion with a razor blade, cold wax, aluminium foil, cling film or by direct application of organic solvents.

Standards (track 1: cholesterol, 2: triolein, 3: stearyl palmitate, 4: ceramid VI, 5: squalene, 6: cholesteryl palmitate*, 7: palmitic acid, 8: 1,2-dipalmitoyl-sn-glycerol) and various extracts of skin lipids (track 9: using ethanol, 10: cyclohexane – ethanol 1:9, 11: cyclohexane – ethanol 1:4, 12: n-hexane – methanol 2:3)*

**encircled, other zones are impurities*

The major challenge involved with the use of tapes and glues was to selectively extract the skin lipids from the carrier material, whereas the direct application of organic solvents was limited to ethanol to avoid skin irritations. Three sampling methods (direct extraction with ethanol, abrasion, Epiglu) were suitable for quantitative investigations, as neither skin nor glue matrix interfered with the lipid zones. However, standardization of the sampling procedure was still difficult because the depth of skin removed (number of stratum corneum layers) was unclear and the area of treated skin was difficult to define; further investigations are under way.



Sampling A) by direct abrasion and B) with Epiglu; track 1: lipid standard mix, 2–7: skin lipid samples, 8–9: Epiglu blanks

The method was validated regarding stability, robustness, precision of R_f values and standard determinations as well as linear range. All lipid standards were stable for at least 3 h in chloroform – methanol 1:1 and on the plate prior to development. Standards as well as extracted skin samples were stable during chromatography. The derivatized chromatogram was stable for at least 2 h. The influence of relative humidity on the separation was evaluated at 5 %, 33 %, 47 %, and 75 % using the ADC 2. For reproducible selectivity, the relative humidity must be maintained between 33 % and 47 %. The precision of the R_f values of seven compounds was determined between plates (3 plates developed on the same day, followed by one plate each on two different days). Differences of R_f values were <0.03 and thus well below the acceptance criterion of <0.05. Precision of measurement ($n = 9$) was compared on various HPTLC silica gel 60 plates of different layer thickness (100 and 200 µm) and particle shapes (irregular and spherical particles LiChrospher). The HPTLC LiChrospher Si 60 F_{254s} plates with a thickness of 200 µm showed the lowest relative standard deviations (%RSD ≤ 5 %) for all investigated compounds. The linear range for all but two of the selected markers was between 100 and 350 ng/band (40–160 ng for cholesterol, 100–280 ng for 1,2 dipalmitoyl-sn-glycerol). Calibration curves showed correlation coefficients of $r > 0.9975$.

Further information is available on request from the authors.

[1] K. Rothenbühler, Master thesis, Institute for Pharmaceutical Biology, University of Basel, 2009

[2] D. Handloser, V. Widmer, E. Reich, J. Liq. Chromatogr. Rel. Technol. 31 (2008) 1857

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Masters Thesis: Kathrin Rothenbühler

Focus:

- Development and validation of an HPTLC method for qualitative and quantitative analysis of skin lipids
- Development and validation of an adequate sampling technique for lipids from human skin

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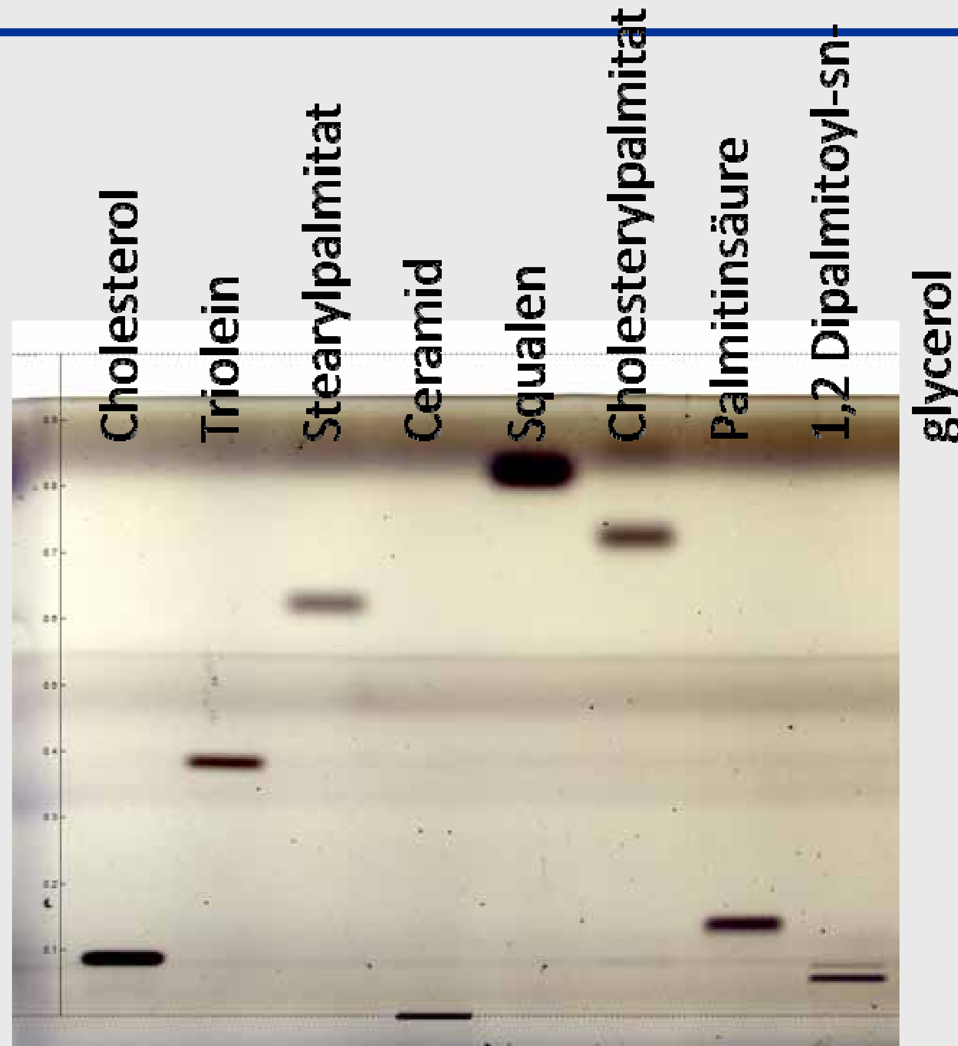
Results

- HPTLC method for separation of skin lipids:
  - Squalen
  - Cholesterol and cholesterol esters
  - Triglycerides, Diglycerides
  - Wax esters
  - Free fatty acids
- Quantitative, validated method for cholesterol, cholesterol ester, free fatty acids, and triglycerides in human skin

Results: Optimized HPTLC method



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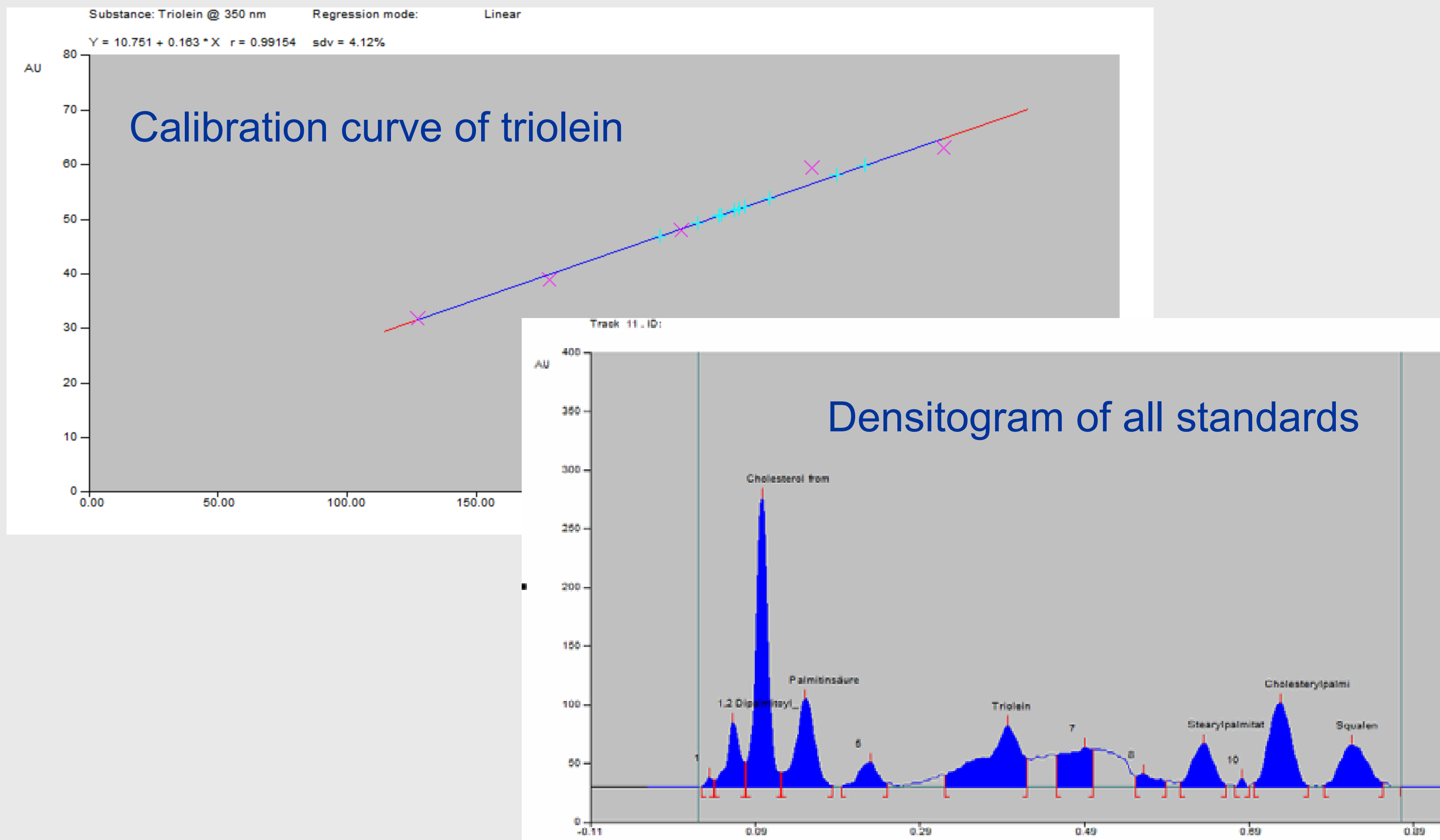
Results



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Results

- Evaluation of different sampling techniques (tapes, wax, solvents)
- Comparison of results from lipid assay with protein assay (Pentapharm) showed no correlation
- New HPTLC method (ADC2) for ceramides, comparison with existing AMD2 method

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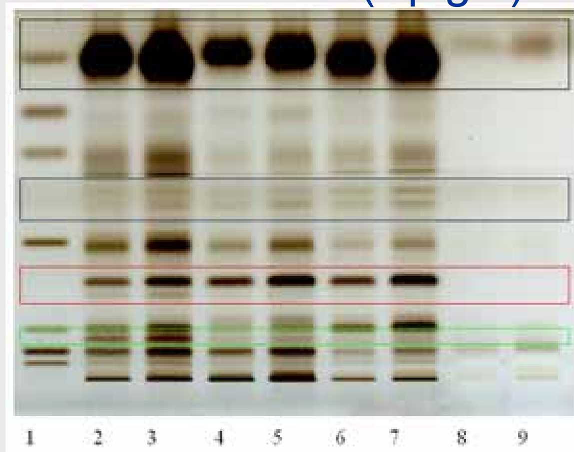
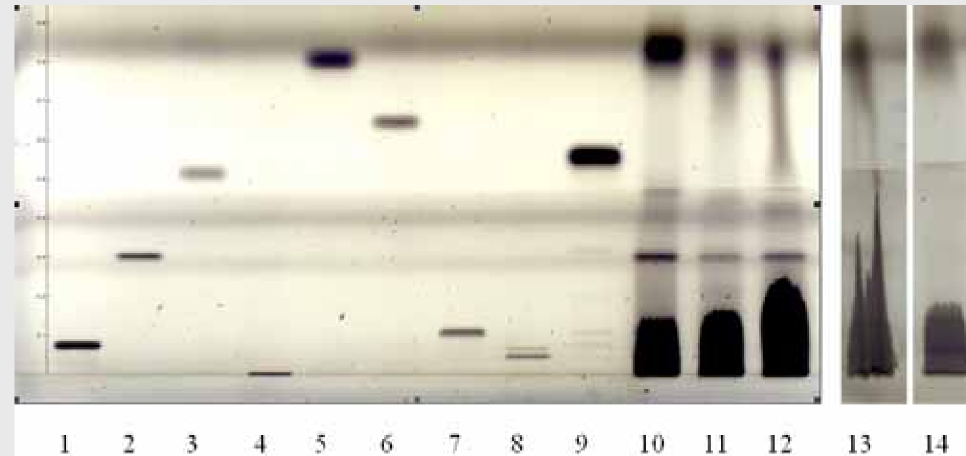
Evaluation of different sampling techniques

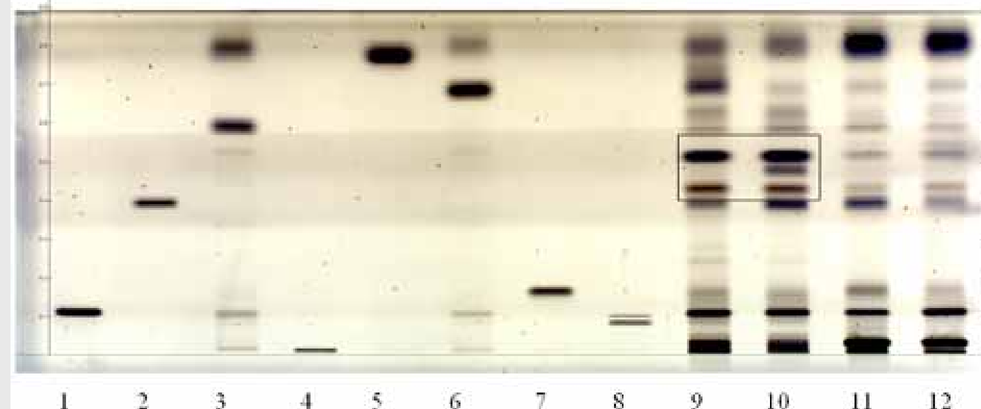
Tissue adhesive (Epiglu)



3M tape



Different solvents



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Masters Thesis: Deana Nikolic

Focus:

- Development and validation of an HPTLC method for quantitative analysis of ceramides
- Development and validation of an adequate sampling technique for lipids from human skin
- 4 week cosmetic study

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Results: CBS 105

1: Cholesterol, 2: Squalen, 3: Standard mix, 4: Skin mimic (Ceramide I, II, III, VI, IX, Cholesterol, Behenic acid and Sphingokine); skin samples taken with 5: Filterpaper A, 6: Filterpapier B, 7: Filterpapier C, 8: Filterpaper C +lactate, 9: Filterpaper D, 10: Filterpaper D + sampling tool

Sampling device

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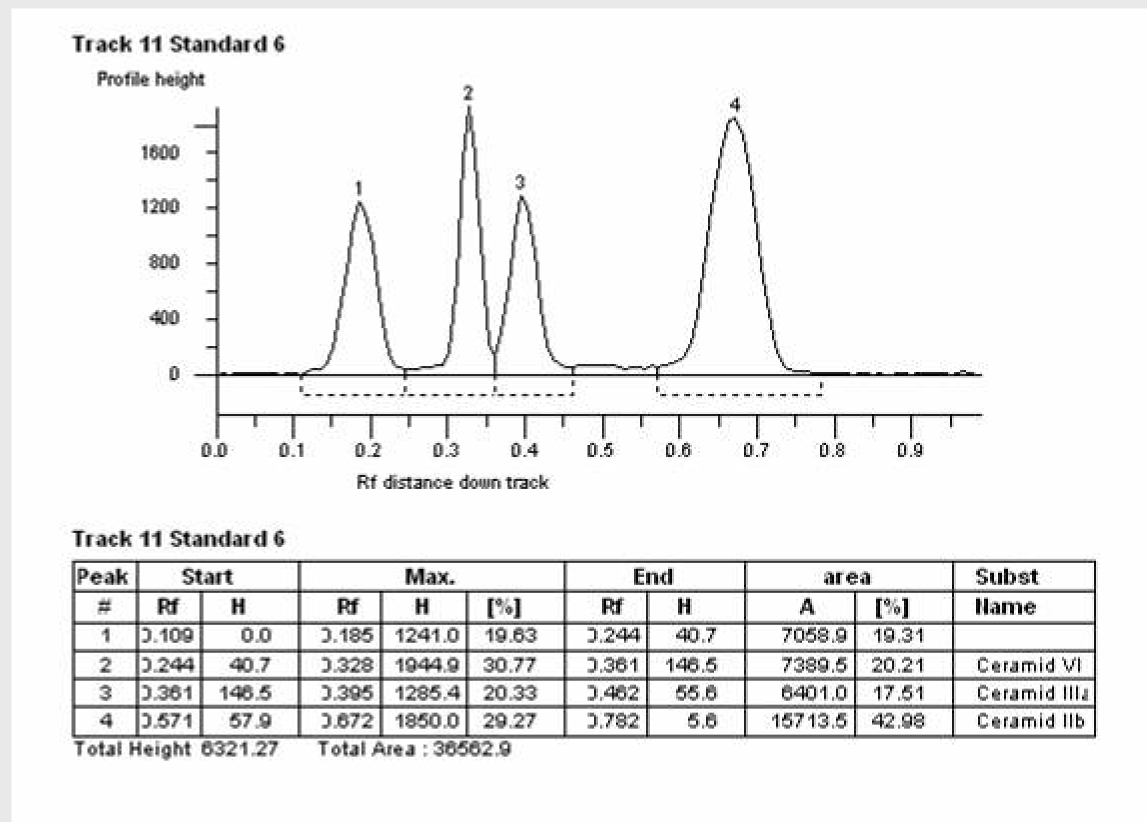
Good separation in one step



Track 11 Standard 6

Peak	Start		Max.			End		area		Subst
#	Rf	H	Rf	H	[%]	Rf	H	A	[%]	Name
1	0.109	0.0	0.185	1241.0	19.63	0.244	40.7	7058.9	19.31	
2	0.244	40.7	0.328	1944.9	30.77	0.361	146.5	7389.5	20.21	Ceramid VI
3	0.361	146.5	0.395	1285.4	20.33	0.462	55.6	6401.0	17.51	Ceramid IIIa
4	0.571	57.9	0.672	1850.0	29.27	0.782	5.6	15713.5	42.98	Ceramid IIb

Total Height 6321.27 Total Area : 36562.9

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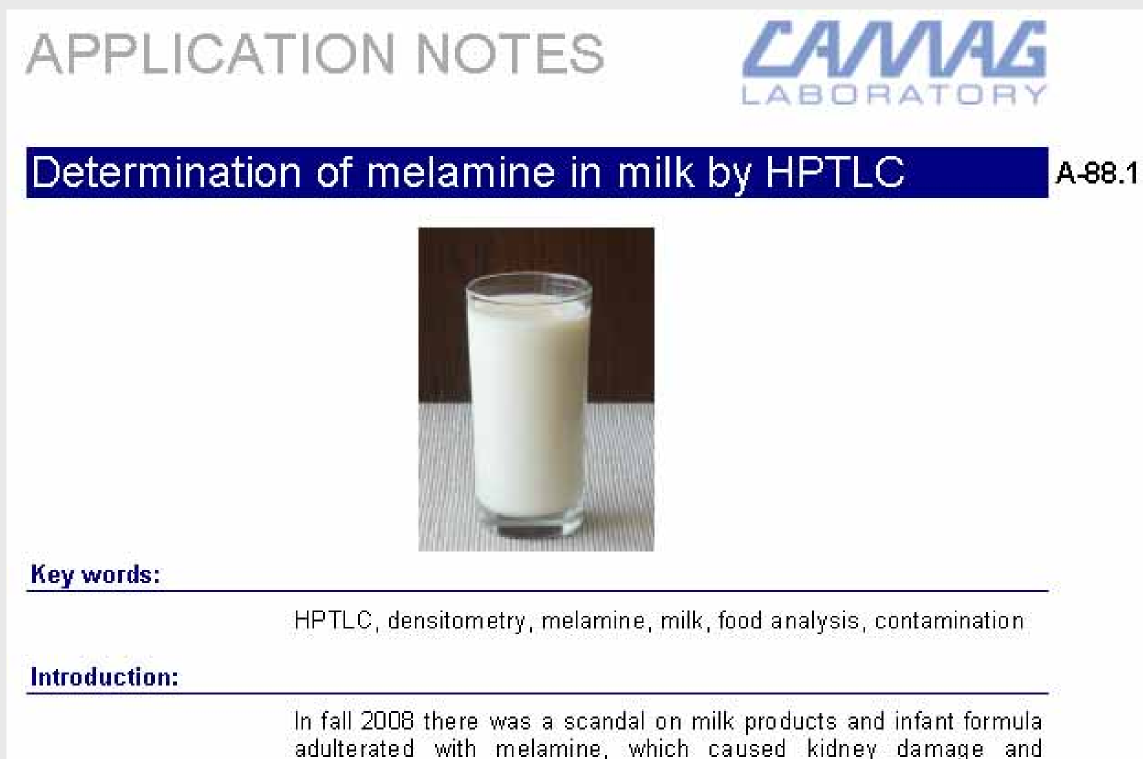
A-88.1 Melamine in milk

- HPTLC method for screening and quantification of melamine in milk (limit of detection 20 mg/L)



APPLICATION NOTES

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Determination of melamine in milk by HPTLC A-88.1

Key words:

HPTLC, densitometry, melamine, milk, food analysis, contamination

Introduction:

In fall 2008 there was a scandal on milk products and infant formula adulterated with melamine, which caused kidney damage and

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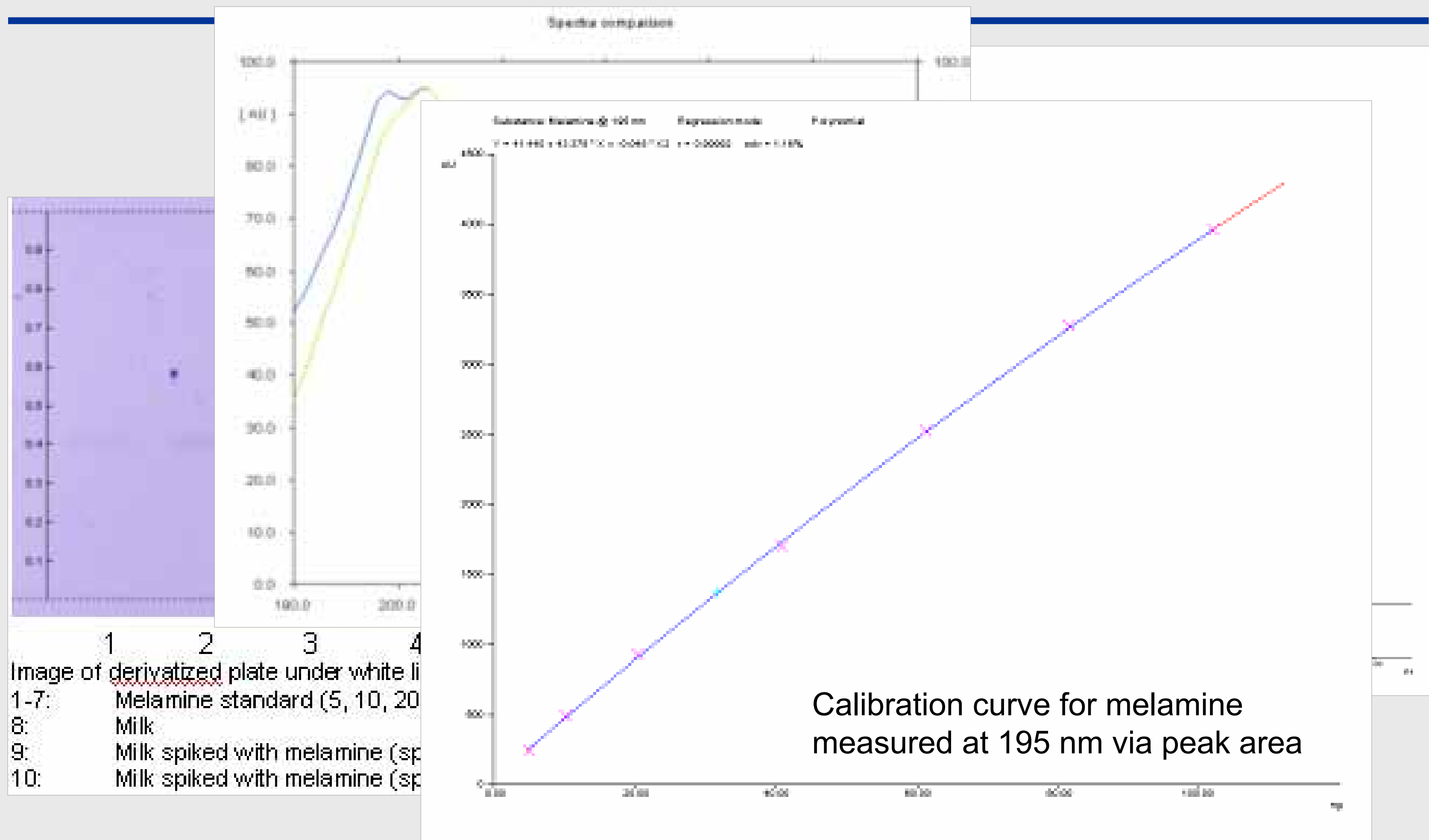
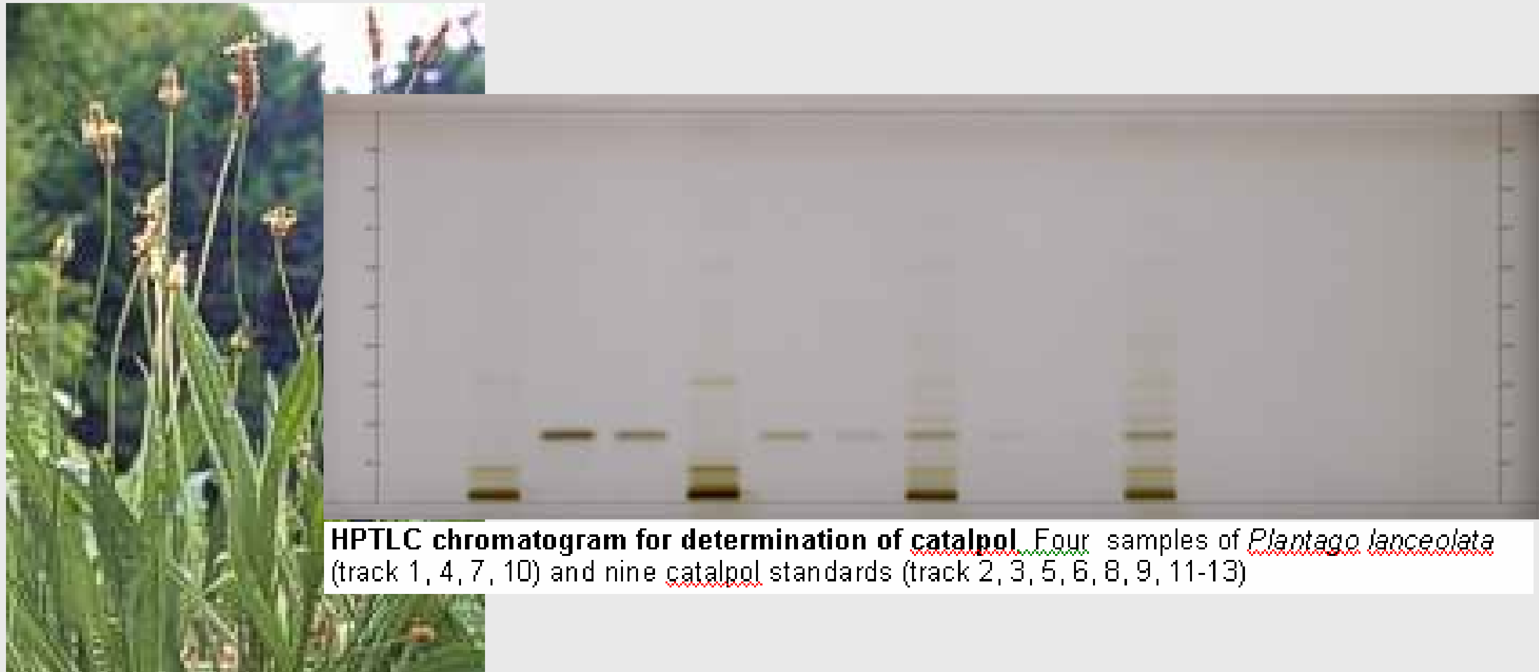
A-88.1 Melamine in milk



A-87.1 Aucubin and catalpol in Plantain

- HPTLC quantification of aucubin and catalpol in leaves of Ribwort Plantain (Plantago lanceolata)



HPTLC chromatogram for determination of catalpol. Four samples of *Plantago lanceolata* (track 1, 4, 7, 10) and nine catalpol standards (track 2, 3, 5, 6, 8, 9, 11-13)

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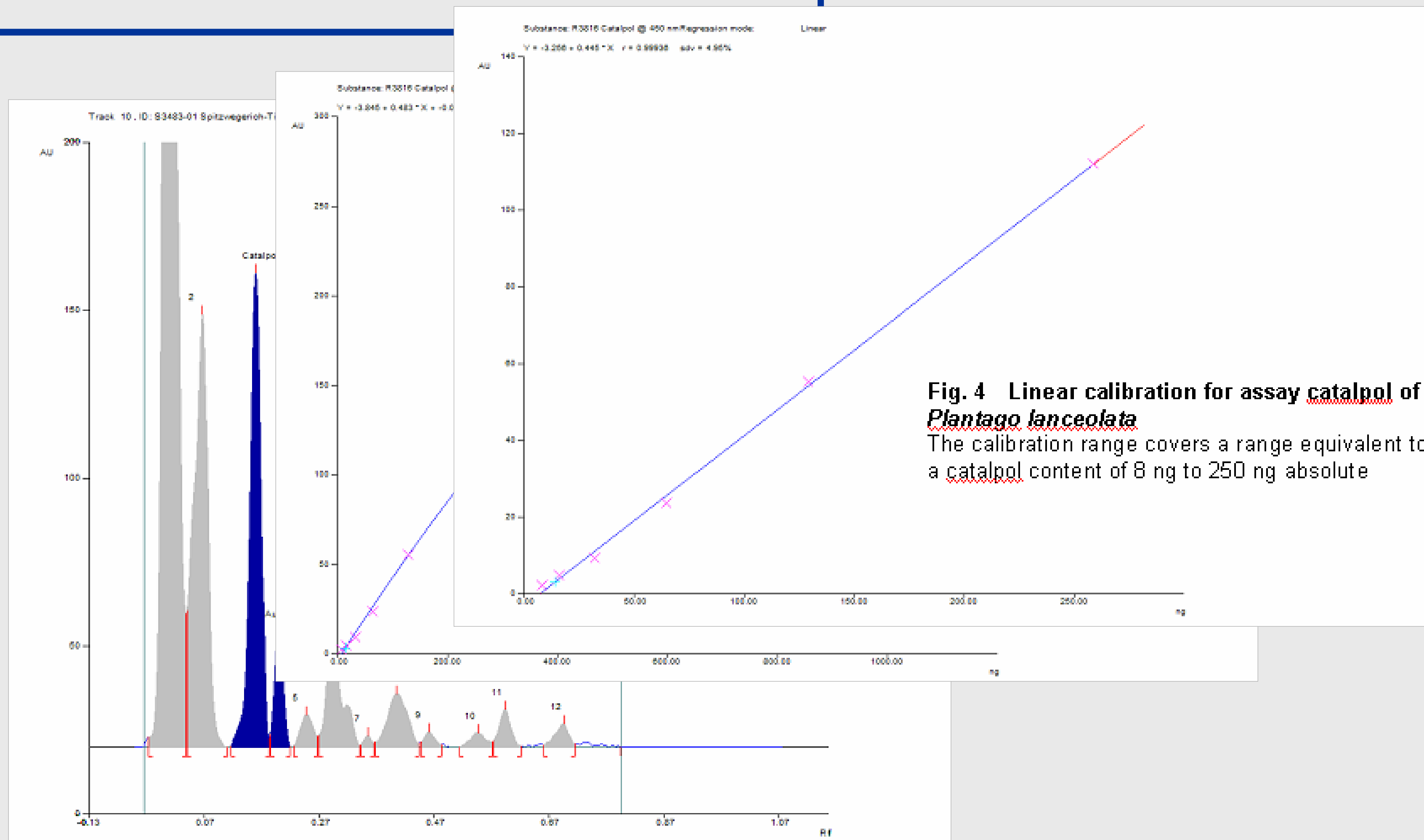
A-87.1 Aucubin and catalpol in Plantain



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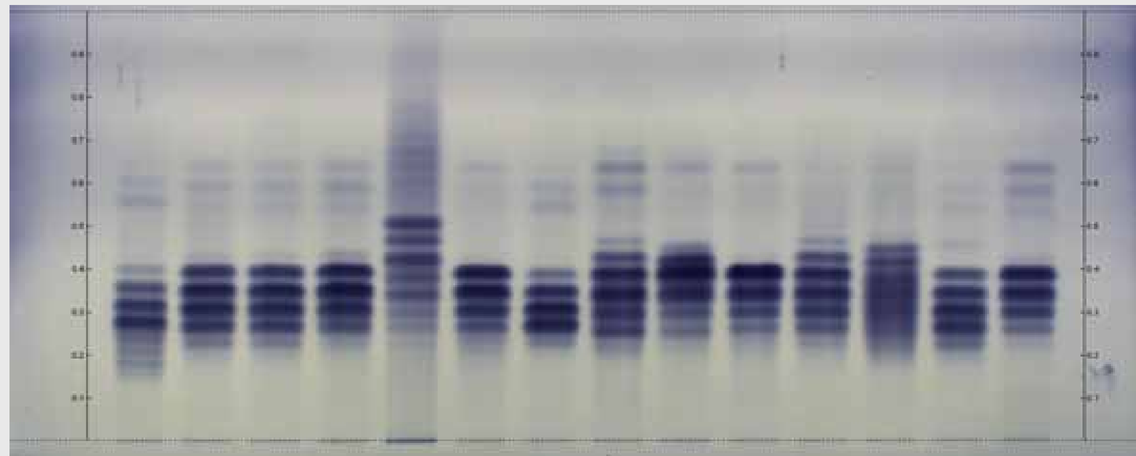
F37 Identification of fatty oils

Basis for request for revision of PhEur Chapter 2.3.2



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

1: Arachis oil
2: Sesame oil
3: Sesame oil roasted
4: Maize oil
5: Linseed oil
6: Sunflower oil
7: Almond oil
8: Wheat germ oil
9: Evening primrose oil
10: Safflower oil type 1
11: Soy bean oil
12: Borage oil
13: Argan oil
14: Grape seed oil

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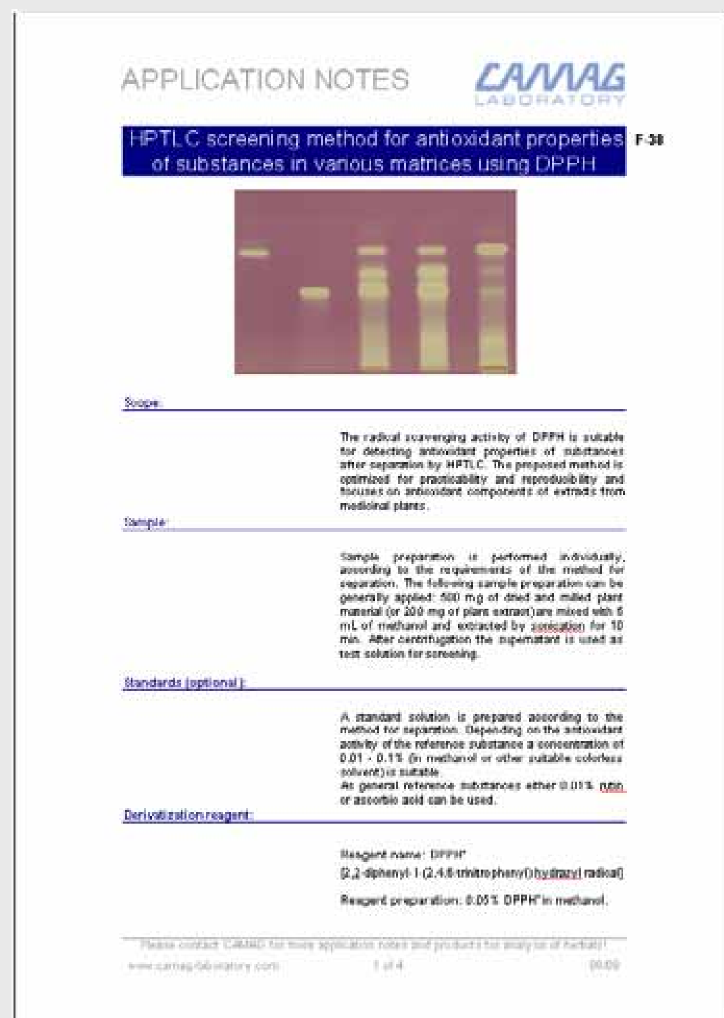
F 38: Activity screening with DPPH



APPLICATION NOTES — CAMAG LABORATORY

HPTLC screening method for antioxidant properties of substances in various matrices using DPPH F-38

Scope:

The radical scavenging activity of DPPH is suitable for detecting antioxidant properties of substances after separation by HPTLC. The proposed method is optimized for practicability and reproducibility and focuses on antioxidant components of extracts from medicinal plants.

Sample:

Sample preparation is performed individually, according to the requirements of the method for separation. The following sample preparation can be generally applied: 500 mg of dried and milled plant material (or 200 mg of plant extract) are mixed with 5 mL of methanol and extracted by sonication for 10 min. After centrifugation the supernatant is used as test solution for screening.

Standards (optional):

A standard solution is prepared according to the method for separation. Depending on the antioxidant activity of the reference substance a concentration of 0.01 - 0.1% (in methanol or other suitable colorless solvent) is suitable.
As general reference substances either 0.01% rutin or ascorbic acid can be used.

Derivatization reagent:

Reagent name: DPPH*
(2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical)

Reagent preparation: 0.05% DPPH* in methanol.

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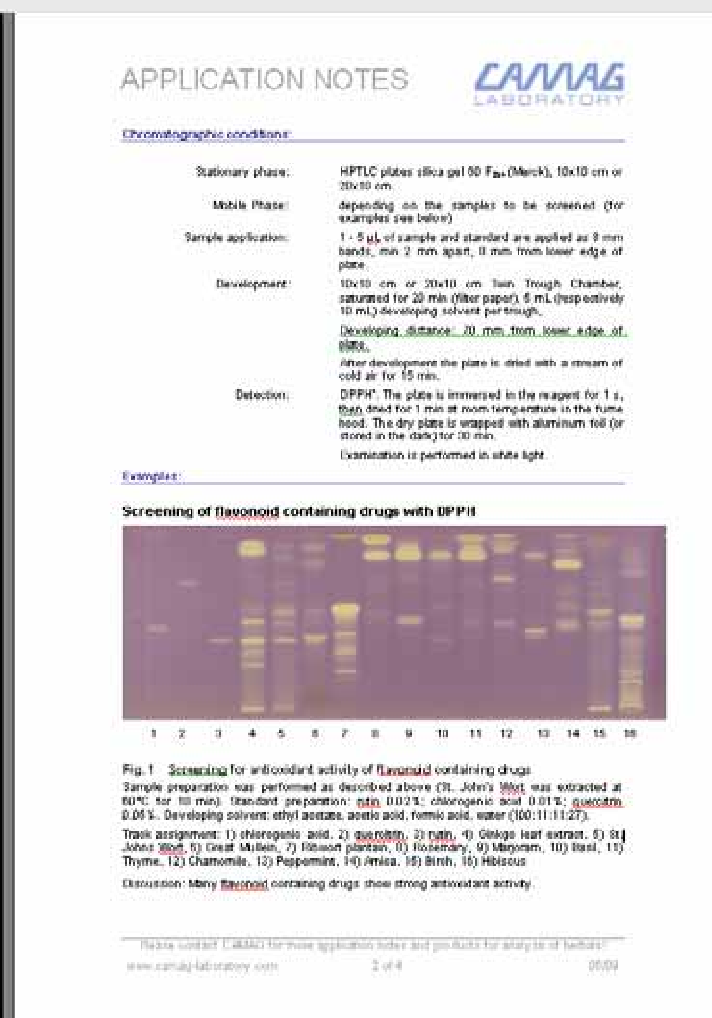

APPLICATION NOTES — CAMAG LABORATORY

Chromatographic conditions:

Stationary phase:	HPTLC plates silica gel 60 F_{254} (Merck), 10x10 cm or 20x10 cm.
Mobile Phase:	depending on the samples to be screened (for examples see below)
Sample application:	1 - 5 µL of sample and standard are applied as 8 mm bands, min 2 mm apart, 8 mm from lower edge of plate.
Development:	10x10 cm or 20x10 cm Twin Trough Chamber, saturated for 20 min (filter paper), 5 mL (respectively 10 mL) developing solvent per trough. Developing distance: 70 mm from lower edge of plate. After development the plate is dried with a stream of cold air for 15 min.
Detection:	DPPH*. The plate is immersed in the reagent for 1 s, then dried for 1 min at room temperature in the fume hood. The dry plate is wrapped with aluminum foil (or stored in the dark) for 30 min. Examination is performed in white light.

Examples:

Screening of flavonoid containing drugs with DPPH

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig. 1 Screening for antioxidant activity of flavonoid containing drugs
Sample preparation was performed as described above (St. John's Wort was extracted at 60°C for 10 min). Standard preparation: rutin 0.02%; chlorogenic acid 0.01%; quercitrin 0.05%. Developing solvent: ethyl acetate, acetic acid, formic acid, water (100:11:11:27).

Track assignment: 1) chlorogenic acid, 2) quercitrin, 3) rutin, 4) Ginkgo leaf extract, 5) St. Johns Wort, 6) Great Mullein, 7) Ribwort plantain, 8) Rosemary, 9) Marjoram, 10) Basil, 11) Thyme, 12) Chamomile, 13) Peppermint, 14) Arnica, 15) Birch, 16) Hibiscus

Discussion: Many flavonoid containing drugs show strong antioxidant activity.

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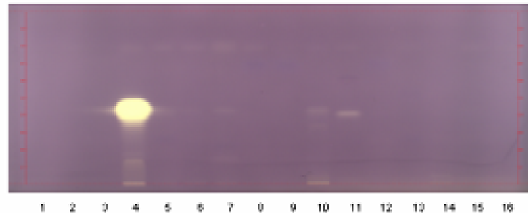
F38 cont.

APPLICATION NOTES

Screening of essential oil containing drugs with DPPH

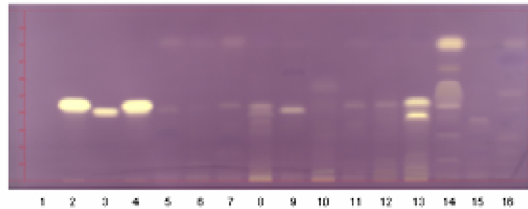


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig. 2a Screening of antioxidant activity of essential oil containing drugs

Sample preparation was performed as described above. Standard preparation in toluene: borneol 0.1%, caryophyllene 1 µL/mL, linalyl acetate 4 µL/mL. Developing solvent: toluene, ethyl acetate (95:5)

Track assignment: 1) borneol, 2) caryophyllene, 3) linalyl acetate, 4) Thyme oil, 5) Tea tree oil, 6) Pine oil, 7) Peppermint oil, 8) Anise oil, 9) Star anise oil, 10) Lemon oil, 11) Sage oil, 12) Fennel oil, 13) Lavender oil, 14) Roman chamomile oil, 15) Rosemary oil, 16) Niaouli oil



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Fig. 2b Application volumes were adjusted

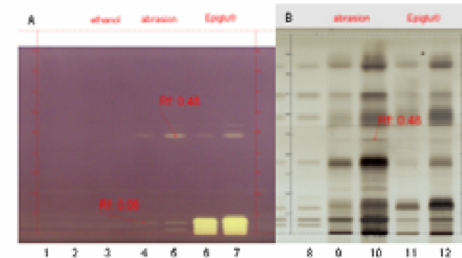
Standard preparation in toluene: carvacrol methylester 2 µL/mL, thymol 0.16%, carvacrol 1 µL/mL. Developing solvent: toluene, ethyl acetate (95:5). Application volumes: track 1 and 3: 1 µL, track 2 and 4: 0.2 µL, track 5-9: 10 µL, track 10-16: 20 µL.

Track assignment: 1) carvacrol methylester, 2) thymol, 3) carvacrol, 4) Thyme oil, 5) Sage oil, 6) Neroli oil, 7) Lemon oil, 8) Peppermint oil, 9) Rosemary oil, 10) Chamomile oil, 11) Sweet orange oil, 12) Manuka oil (Leptospermum scoparium), 13) Tea tree oil, 14) Pine oil, 15) Niaouli oil, 16) Anise oil

Discussion: If during screening no or too strong antioxidant activity is detectable (Fig. 2a), the application volume must be adjusted (Fig. 2b). Compounds, present in small concentration, may show antioxidant activity.

APPLICATION NOTES

DPPH-Screening of compounds sampled from human skin



1 2 3 4 5 6 7 8 9 10 11 12

Fig. 3 DPPH-Screening of compounds sampled from human skin[1]

Plate A: First development with toluene to 80 mm; after drying second development with hexane, TMBE, acetic acid (80:20:1) to 40 mm; chamber saturation for 10 min, humidity control at 33 % rH for both developing steps[2].

Track assignment: 1) and 8) standard mix of skin fatty acids: (from the bottom) 1,2 dipalmitoyl-sn-glycerol, cholesterol, palmitic acid, triolein, stearylpalmitate, cholesteryl-palmitate, squalen; 2-3) skin lipids extracted with ethanol, 4-5) and 9-10) skin lipids after abrasion, 6-7) and 11-12) skin lipids removed with Epiglu®

Plate B: the plate is derivatized with a copper (II) sulfate reagent[2] instead of DPPH[1]

Discussion: A non defined compound of human skin (Rf = 0.48) shows antioxidant activity. This compound was not extracted from the skin by ethanol (track 2-3). On track 6 and 7 compounds with strong radical-scavenging activity at Rf = 0-0.07 are detectable. This effect is due to components of Epiglu®, a tissue adhesive for external use.

References:

[1] Rothenbühler K (2009) HPTLC zur qualitativen und quantitativen Analyse von Hautlipiden - Master work, University of Basel, Department of Pharmaceutical Sciences, Institute of Pharmaceutical Biology, p. 17.

[2] Handloser D, Widmer V, Reich E (2008) Separation of Phospholipids by HPTLC – An Investigation of Important Parameter. Journal of Liquid Chromatography & Related Technologies, 31, 1857-1870.

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Diploma thesis Manina Meier Uni Graz

- HPTLC MS of natural products
- → GA Berlin

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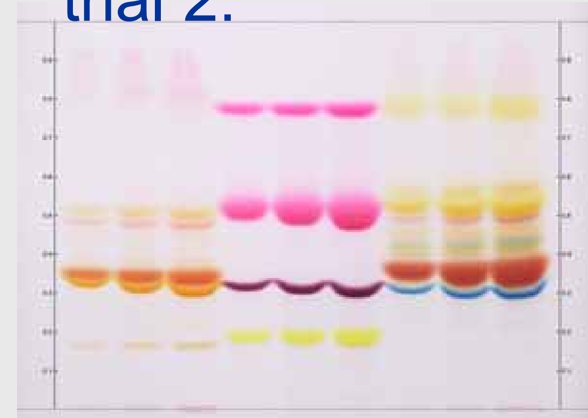
Pigments for cosmetics:

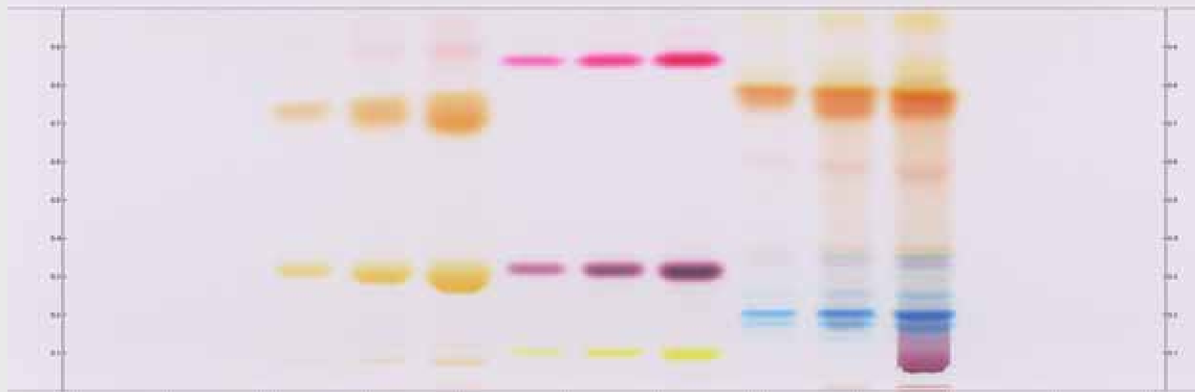
Starting point:

trial 1:

trial 2:



butanol, ethanol, 10% formic acid 50:10:15 (v/v/v)



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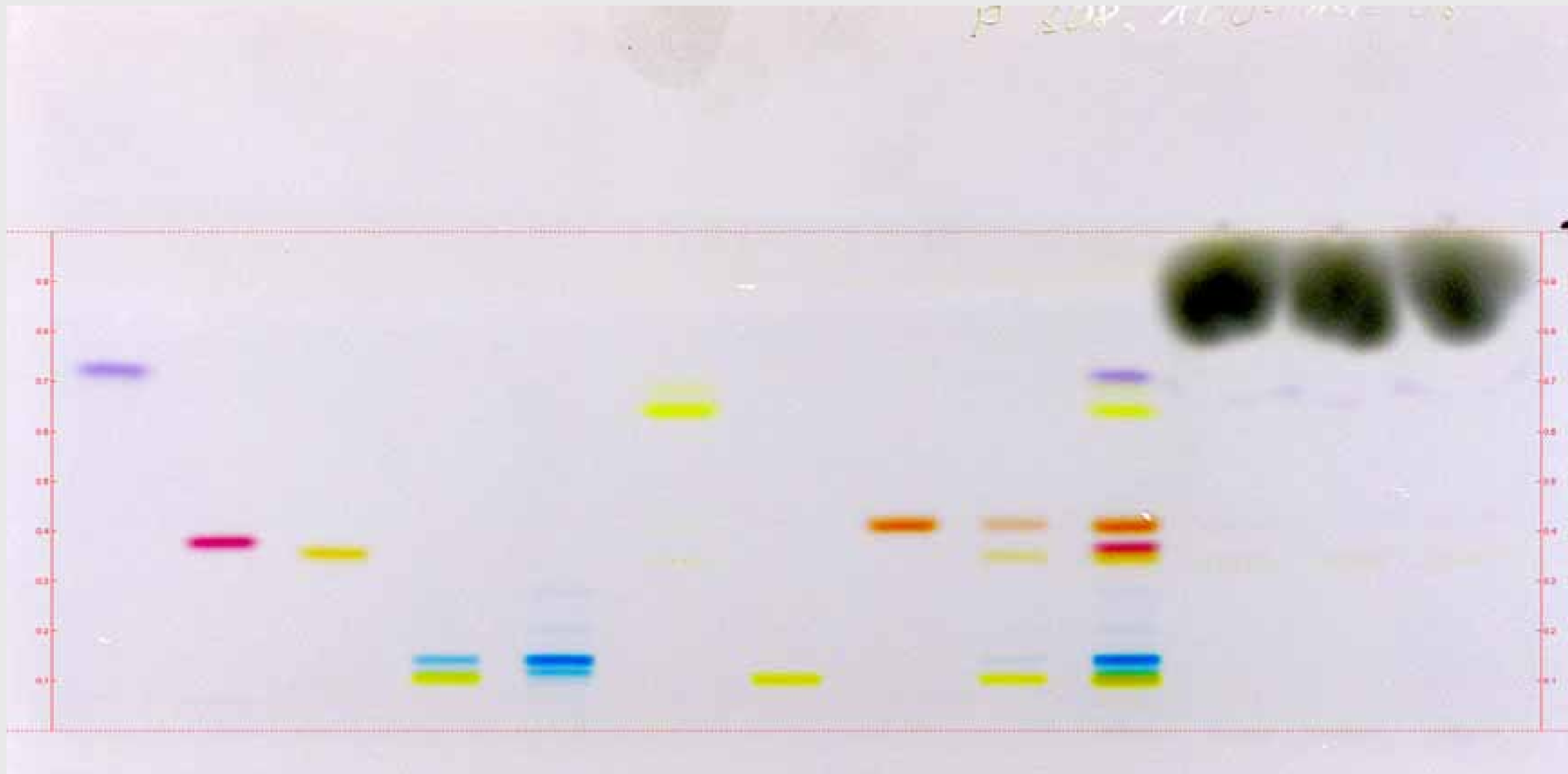
Colors for cosmetics:



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Colors for cosmetics: