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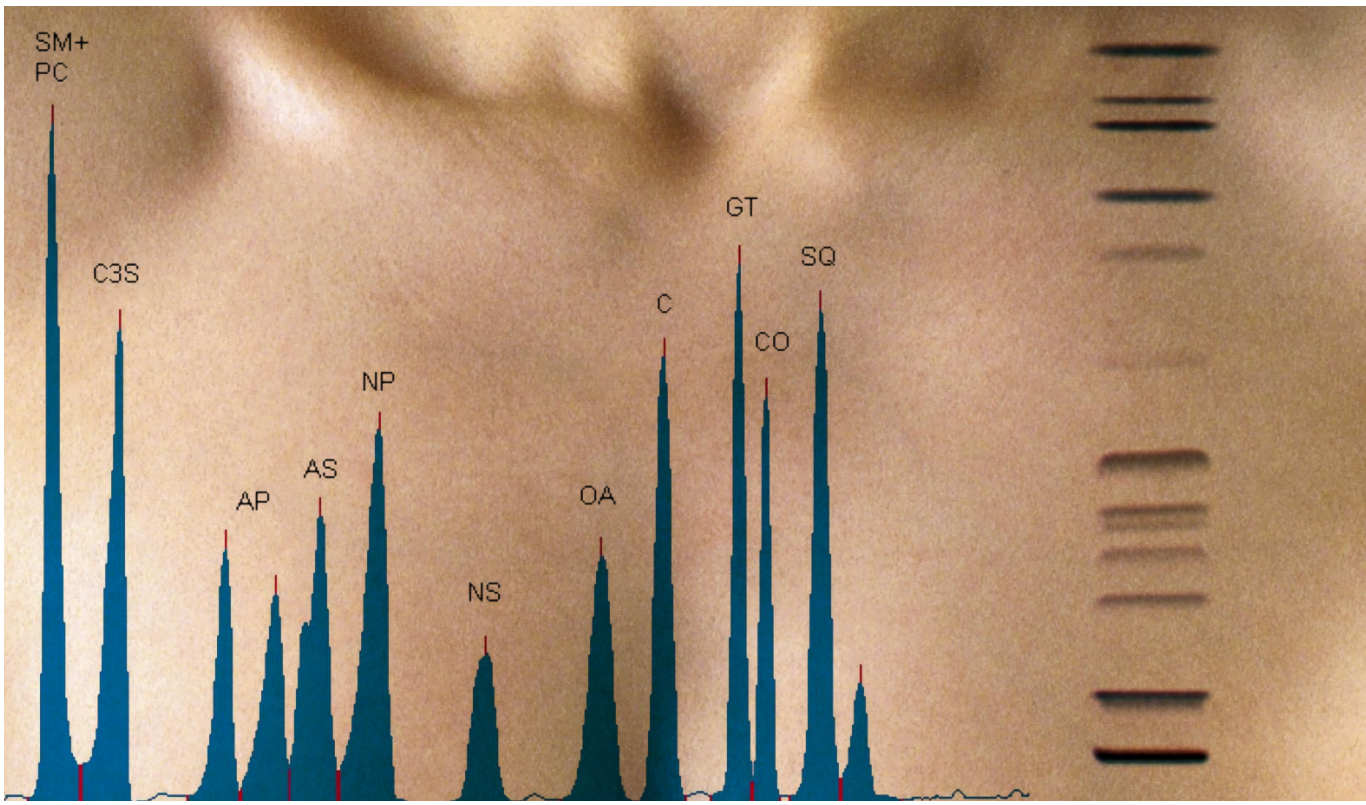


*AMD chromatogram of Stratum corneum lipids*

# The versatility of HPTLC – From Bio Analysis to the Detection of Pollutants in Water

CAMAG 105

CBS
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cbs@camag.com
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**IN THIS ISSUE**

**Procedures, applications**



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**Column: Know CAMAG**



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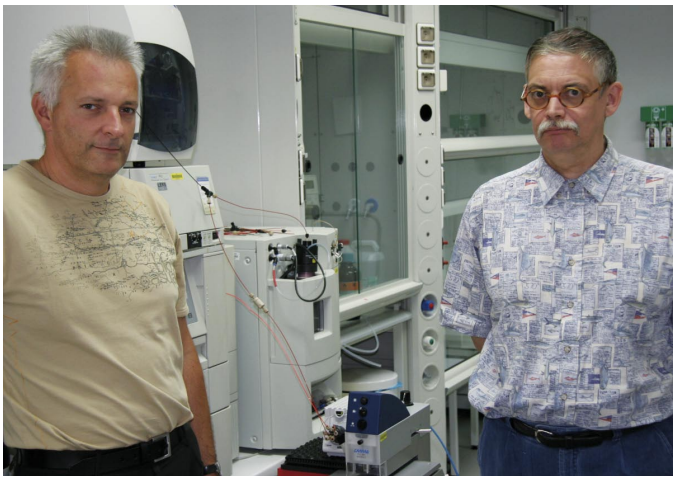
# TLC/HPTLC-ELSD-MS coupling



1

*Mr. François Bretin and Dr. Francis Maquin (right)*

The Lead Generation to Candidate Realization (LGCR) platform identifies small molecule leads and progresses them up to registration. LGCR Analytical Sciences (AnSci) is a new department in Sanofi-Aventis R&D, which is dedicated to support Business Divisions and Therapeutics Units. For LGCR-AnSci, Dr. Maquin* and F. Bretin in the research center in Vitry-sur-Seine use various analytical techniques (further locations are in Strasbourg, Chilly-Mazarin, Toulouse and Montpellier).

## Introduction

MS coupled to chromatography supports the chemist's decision for compound identification or follow-up in a synthesis mixture. Therefore, HPLC/UPLC-MS is mainly used, but TLC/HPTLC is also widely applied by the chemists as a rapid and reliable method to follow reaction processes.

**This is especially true for cases, when compounds remain on the column, due to high polarity or weak solubility, or when analyte detection is poor (no chromophore). In such cases, the HPTLC/MS platform with the new TLC-MS Interface is ideal for structural analysis. The TLC-MS Interface was complemented by a MS software controlled valve, adding some automation to the process, and by an Evaporative Light Scattering Detector (ELSD). The latter enables the analyst to distinguish between compounds that were not eluted from the plate from those that were not sufficiently ionized for MS detection.**

## Sample preparation

The samples were taken directly from the reactor and diluted with an appropriate solvent, usually an organic solvent of medium polarity.

## Standard preparation

Educts or known intermediate products were dissolved and diluted with an appropriate solvent.

## Chromatogram layers

TLC and HPTLC plates silica gel 60 $F_{254}$ 10 x 20 and 10 x 10 cm, respectively. If required, the plate size was reduced with the Smartcut.

## Sample application

Manually with disposable micropipettes of 5 to 20 µL volumes.

*Note: The Nanomat 4 is recommended to ensure precise positioning without damage of the layer. Especially for polar extracts, small volumes should be applied (<2 µL to obtain sharp start zones).*

## Chromatography

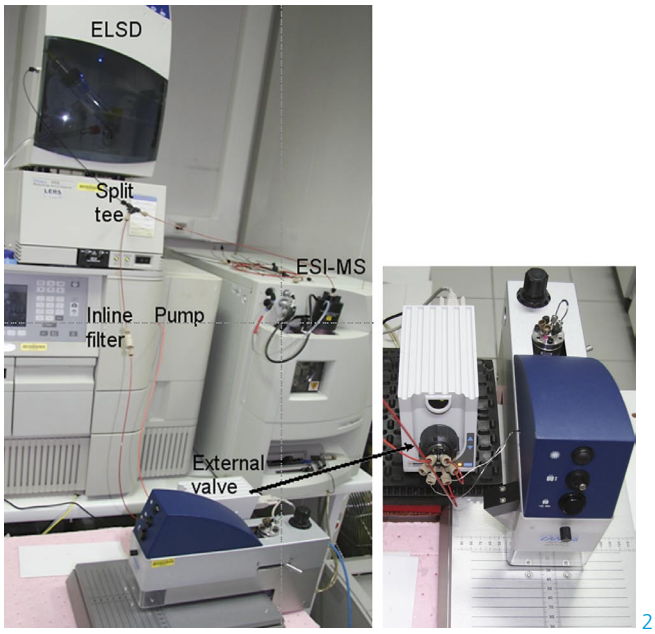
In a twin-through chamber, e.g., with mixtures of methanol and dichloromethane/ethyl acetate, or ethyl acetate and heptane/cyclohexane; the ratios depend on the compound mixtures.

## Derivatization

Compounds with neither UV/Vis-activity nor native fluorescence can be derivatized by non-destructive derivatization reagents, e.g., the primuline or berberine reagents for lipophilic compounds, and directly eluted with the TLC-MS interface into the MS. However, for destructive derivatizations, e.g., based on strong acidic carbonization reactions, on both plate sides, the outer track was cut, derivatized, and the respective bands were marked by extrapolation.

## Recording of MS spectra

The flow rate of the eluent (methanol – water 95:5, 0.4 mL/min) was split by a tee, and 0.2 mL/min were pumped to the MS (Micromass ZQ, Waters) and 0.2 mL/min to the ELSD (Sedere Sedex 85 LT). The TLC-MS Interface was equipped with either a round or oval elution head and connected via an external automated valve (MXP7900-000, Rheodyne) to the pump (Alliance 2695, Waters) and ESI-MS. In the transfer tube to the detectors, an inline filter was integrated (frit porosity 0.2 µm, A 356/504, Upchurch IDEX). The recording of mass spectra was performed in the positive/negative electrospray mode; for evaluation the MS software (Mass Lynx V4.0/Open Lynx, Waters) was used.



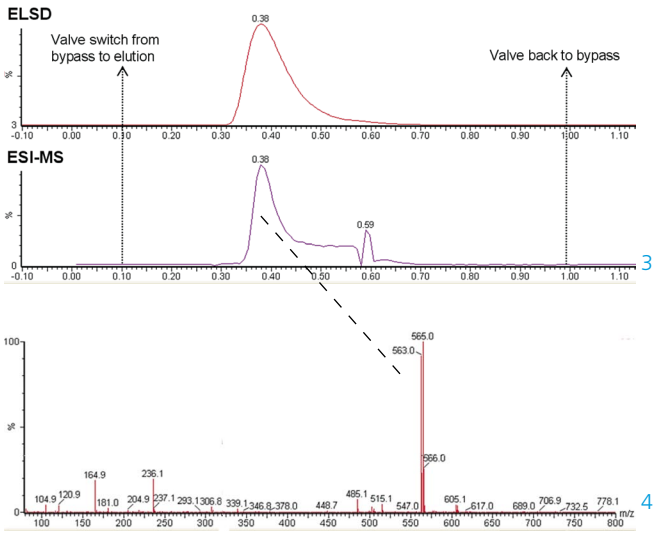

2

*TLC/HPTLC-ELSD-MS system configuration*

## Results and discussion

In our experiments, a lot of organic structures were not detected by DAD, so the capability of detection of the TLC/HPTLC-DAD-MS configuration was improved by substituting the DAD with the ELSD. As universal detector, the ELSD confirms more reliably the elution of compounds that are not UV/Vis-active.

Once the elution head was lowered onto the zone of interest and an ID number was entered in the software, valve switching was effected remotely, followed by an automatic acquisition of both the MS and ELSD signal. This minor automation resulted in the zones being eluted into the detector, and then the eluent being subsequently switched back to bypass (waste). The following protocol made cross contamination, which of course is dependent on the substance structure and zone concentration, less likely. For the given connecting tube length and internal diameter, the external valve was set 10 s in bypass, then 1.5 min in elution mode and finally 15 s in bypass. For a high throughput, the elution time was set below one minute.




*Elution profiles obtained by ELSD and ESI-MS (top) and mass spectrum of the eluted zone (bottom)*

An intensive use of the TLC/HPTLC-ELSD-MS instrumentation requires frit cleaning twice a week. The requirement of a cleaning cycle is indicated by an increased pump pressure likely from clogging of the inline filter frit (> 10 MPa).

To conclude, our experience underlines the interest in HPTLC/TLC-MS in an advanced analytical environment. The automatization, reliability of the data, and speed were the convincing arguments, which fully comply with our routine analytical needs.

Further information is available on request from the authors.

Contacts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- Mr. Pierre Bernard-Savary, Chromacim SAS, Pommiers la Placette, France
- Mr. Henri Gangloff, Sedere SA, Alfortville, France
- Mrs. Véronique de Nailly, BCP Instruments, Irigny, France



## CAMAG TLC Scanner 4

Classical densitometry uses monochromatic light in the form of a slit of selectable length and width to scan the tracks of a chromatogram, measuring the diffusely reflected light. The TLC Scanner 4 – the successor of TLC Scanner 3 – is the most advanced workstation for densitometric evaluation of Thin-Layer Chromatograms currently available.

Analysts can now benefit from a wider spectral range for measurements from 190 to 900 nm. The optimized positioning stage and its open access allow for robust use.

For the screening of water samples in this application, densitometric determination is performed by the multi-wavelength scan covering the wavelength range between 190 and 300 nm. In combination with AMD the whole polarity range of UV-active compounds is detected. This procedure is proven to be helpful in routine search for potential water contaminants.

# Determination of the glycoalkaloids α-solanine and α-chaconine in potatoes at different steps of potato processing


6

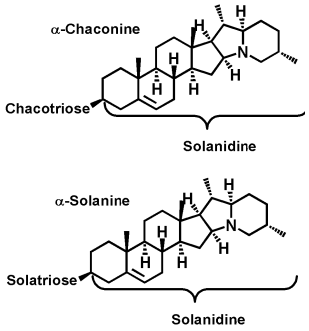

7

*Dr. Jens Mäder, Prof. Dr. Lothar W. Kroh*

Prof. Dr. Kroh*, TU Berlin, and his group, concentrate on carbohydrate chemistry and food analysis, especially in Maillard reaction chemistry, caramelization and melanoidin formation. A further research field of both authors is the characterization of bioactive compounds of processed and unprocessed food. Dr. Mäder is now head of product development at Milchwerke Mittelelbe, Stendal. This study [1] was performed by Dr. Mäder, Ms Hanschen and Ms Zietz (both not pictured) during their graduation.

## Introduction

The potato as a plant of the *Solanaceae* family contains bioactive steroidal alkaloids, which are deterrent to herbivores and pathogens and reported to be membrantoxic, embryotoxic and teratogenic for mammalians. They influence the flavor of fresh and processed potatoes from a bitter taste toward a burning sensation at higher concentrations [2]. At the moment no limit values are fixed in Europe, and potatoes, as the most important food source of glycoalkaloids, are regarded as safe for humans if they do not contain more than 200 mg total alkaloids per kg of fresh weight. Both main alkaloids α-solanine and α-chaconine exist at high levels in the tuber peel in a ratio of 1:2 to 1:7.




*Chemical structure of the potato alkaloids α-solanine and α-chaconine*

The potato is one of the world's most important food crops. Quality-management systems demand food safety and traceability as they often follow the *from farm to fork policy* of regulatory authorities. Therefore, easy to use, inexpensive, and reliable analytical methods are needed to evaluate the content of toxicologically relevant compounds like glycoalkaloids after harvest and during potato storage and processing.

**HPTLC is especially suited for effective and rapid determination of alkaloids [4] because up to 16 samples can be simultaneously separated without any matrix influence on the quality of the results. Consequently, all processing stages and by-products from one batch can be monitored and traced simultaneously within one analysis on one plate.**

## Sample preparation

Samples were collected at different stages of the production chain in a commercial potato flake process. After fourfold extraction of the lyophilized, ground potato powder with methanol - acetic acid 99:5, the extracts were concentrated and directly applied.

## Standard solutions

5 mg each of α-solanine and α-chaconine were dissolved in 25 mL methanol – acetic acid 99:1, mixed 1:1 and diluted 1:20.

## Chromatogram layer

HPTLC plate silica gel 60 (Merck), 20 x 10 cm

## Sample application

Bandwise with Automatic TLC Sampler 4, band length 5 mm, track distance 9.2 mm, distance from the lower edge 8 mm, application speed 70 nL/s, application volume 2–22 µL

## Chromatography

In the Horizontal Developing Chamber after 15 min chamber saturation with dichloromethane – methanol – ammonia (2.5 %) 70:30:4.4, migration distance of 75 mm from the lower edge of plate

## Post-chromatographic derivatization

With the Chromatogram Immersion Device III the plate was dipped after 25 min drying time at 90 °C into the Carr-Price reagent (70 g $SbCl_3$ is dissolved in 250 mL of a mixture of acetic acid – dichloromethane 1:3), followed by heating on the TLC Plate Heater at 110 °C for 3 min.

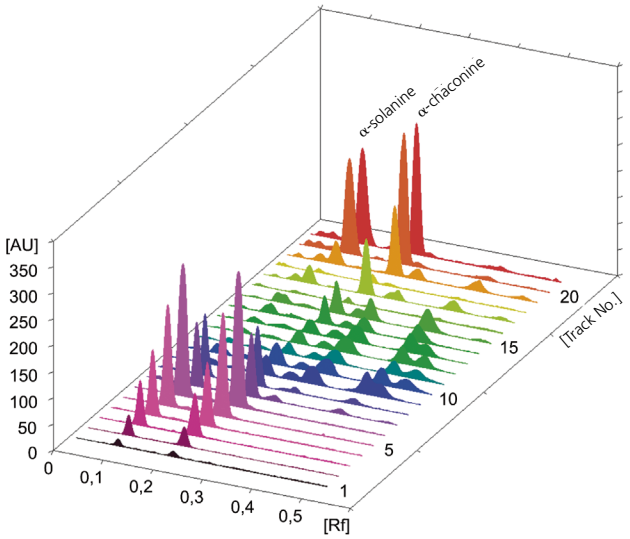
## Densitometry

Absorption measurement at 560 nm with TLC Scanner 3 and winCATS software within 15 min after derivatization, before the glycoalkaloid zones change the color from red to violet.

## Results and discussion

The densitogram clearly shows the good separation of α-solanine and α-chaconine in the different sample extracts. The extremely sensitive and selective coloring of both glycoalkaloids by dipping into the Carr-Price reagent allowed a limit of detection and quantification of 5–15 (depending on the matrix) and 30 ng/band, respectively, with a recovery rate between 94 and 105 %. Linear calibration was very satisfactory in the range of 30 to 700 ng/band ($r$ = 0.9998, sdv = 2.5 %), polynomial even up to 1500 ng/band ($r$ = 0.9999, sdv = 1.5 %).

In conclusion, this method ideally complies with the analytical demands mentioned in the introduction.

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*Densitogram of standard substances (track 1–6) and sample extracts of different steps of potato processing (track 7–21)*

Further information is available from the author on request.

*Prof. Dr. Lothar W. Kroh, Institut für Lebensmitteltechnologie und Lebensmittelchemie, TU Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, lothar.kroh@tu-berlin.de

### Literature

[1] J. Mäder *et al*. J Planar Chromatogr 22 (2009) 43.

[2] S.L. Sinden , K.L. Deahl and B.B. Aulenbach, J Food Sci 41 (1976) 520.

[3] A.J.A. Essers *et al.* Environ Toxicol Phar 5 (1998) 155.

[4] J. Mäder, H. Rawel and L.W. Kroh, J Agric Food Chem 57 (2009) 6292.

# 1H-Benzotriazole and tolyltriazole in the aquatic environment

9 *Dr. Wolfram Seitz, Stefan C. Weiss, Alexander Müller and Dr. Wolfgang Schulz (from left to right)*

10 *Dr. Walter H. Weber*

Lead by Dr. Weber* the laboratory for operation control and research of Zweckverband Landeswasserversorgung (LW) in Langenau, Germany, is active in the analysis of drinking water as well as in the monitoring of ground and waste water from the water protection area Donauried-Hürbe and of surface water from the surrounding area. HPTLC, preferably with automated multiple development (AMD), and online hyphenation of HPTLC-MS are part of the methods applied in routine analysis [1–3].

## Introduction

Within the scope of a monitoring program of raw water resources used by LW, comprehensive chemical, physicochemical and microbiological analyses are performed regularly. In addition non-target screening analyses for the detection of not yet considered contaminants or unknown substances were performed.

**In one of the HPTLC/AMD screening tests of extracts from secondary effluents and of surface and ground water samples the resulting chromatograms showed a zone which could not be identified as any of the substances known from target analysis at LW.**

## Sample preparation

Analyte enrichment from a 100 mL water sample was done by solid phase extraction (SPE) using 0.2 g of Isolute ENV+ sorbent at pH 3 or 7. The SPE sorbent was conditioned consecutively with 6 mL each of n-hexane, acetone, methanol and water (pH = 3 or 7). After drying 6 mL of methanol were used for elution and after evaporation to dryness the residue was taken up in 200 µL of methanol.

## Layer

HPTLC plates Lichrospher $F_{254}$, 20 x 10 cm (Merck) were pre-cleaned with 2-propanol and dried for 30 min at 120 °C on the TLC Plate Heater.

## Sample application

With Automatic TLC Sampler (ATS4) as area of 6 x 3 mm

## Chromatography

In the AMD 2 system with a 25-step gradient starting in isocratic mode with 5 steps acetonitrile – dichloromethane 1:1 for focusing, followed by 15 steps from acetonitrile – dichloromethane 1:1 to dichloromethane and then in 5 steps from dichloromethane – *n*-hexane 4:1 to *n*-hexane. The final migration distance was 80 mm.

## Densitometric evaluation

Multi-wavelength scan at 190, 200, 220, 240, 260, 280 and 300 nm with the TLC Scanner and winCATS software

## Documentation

Under UV 254 nm with the TLC Visualizer

## Results and discussion

During routine screening of extracts from secondary effluents as well as of surface and ground water samples a high peak was observed in the chromatograms. To further investigate this peak the unknown zone was eluted from the HPTLC plate by means of the TLC-MS Interface and analyzed by Nano LC followed by QTOF-MS ($ESI^+$). The analysis revealed that the zone consisted of two substances, peak A at RT = 3.2 min and peak B at RT = 3.4 min.

*continuation on page 9*

# Know CAMAG

One of CAMAG's goals is to provide worldwide in a variety of venues, a continuous flow of information on the state of up to date knowledge of contemporary planar chromatography (see editorial CBS 104). Through one-day workshops at universities, technical training schools, official governmental institutes, and at private companies through in-house training of laboratory personnel, this goal is a reality.

In Germany, CAMAG Berlin has held such seminars for over ten years, now extended into Austria and Benelux. The USA has a similar program and India has an even more aggressive program. In all other countries these are organized by CAMAG Switzerland. If you are interested, contact info@camag.com or telephone +41 61 4673434.

## About one such seminars, recently held by CAMAG Berlin, Professor Manfred Gey of the University of Applied Sciences Zittau/Görlitz reports:

11 *Prof. Gey, Ms Werther, Ms Dr. Gey, Dr. Zieloff (from left)*


12 *Practical session*

### 1. The beginning: How we got started!

Lectures and practical sessions on analytical chemistry for our students concentrated on Separation Sciences, i.e. instrumental bio-analysis, protein and carbohydrate analysis, hyphenating techniques, etc. Meanwhile, contemporary TLC/HPTLC has enhanced the scope of our "HPLC oriented" laboratories considerably.

A related institute offered us their CAMAG equipment, which fortunately for us had been mistakenly considered to be non-functional. CAMAG provided generous and efficient support. First they checked the TLC Scanner free of charge, and found it fully functional. Then they instructed our personnel in the operation of the equipment and the use of the software. For this help we are thankful to Dr. Natsias, Ms Werther, Dr. Zieloff and Mr. Nachtwey, all from CAMAG Berlin.

### 2. Workshop on contemporary TLC/HPTLC 17./18. May 2010 in Zittau

Very soon our cooperation with CAMAG led to the plan to organize a workshop on TLC/HPTLC for students and co-workers from our Faculty for Mathematics & Natural Sciences. There were 30 participants, considered a very good turnout, since all regular lectures and practical sessions ran on in parallel. Dr. Zieloff and Ms Werther demonstrated their outstanding knowledge in their lectures and experimental sessions. The participants were given the opportunity to optimize separations themselves. All results were critically evaluated and discussed. Meanwhile the workshop has now been incorporated in our teaching program as a fixed element.

All in all, the event was considered a great success. **Once again a big thank you to CAMAG!**

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# CBS

Liebe Freunde

Dieser CBS erscheint leider mit Verspätung. In der Zeit, in der die Bearbeitung der September-Ausgabe in das Endstadium rückt, war ich als Mitorganisator stark eingebunden in die Organisation des 39. Deutschen Lebensmittelchemikertages in Stuttgart-Hohenheim. Dieser erwies sich für die Verbreitung der HPTLC in der Lebensmittelanalytik als erfreulich ergiebig, vor allem im Workshop der jungen Lebensmittelchemiker.

Erfreulich ist, dass viele international tätige Unternehmen einen stark ansteigenden Bedarf an Lebensmittelanalytik haben, für die die HPTLC vorzügliche Voraussetzungen bietet. Grossen Nachholbedarf auf diesem Gebiet haben Länder wie China, Indien oder Thailand, in denen Lebensmittelsicherheit verstärkt gesetzlich gefordert wird.

Wegen der wachsenden Bedeutung der Lebensmittelanalytik stammen drei Anwendungsbeiträge dieses CBS aus diesem Bereich im weiteren Sinne. Übrigens ist der Studiengang Lebensmittelchemie einzigartig in Deutschland - international gibt es nichts Vergleichbares.

Die Vorbereitungen zum HPTLC Symposium in Basel, 6.–8. Juli 2011, schreiten zügig voran. Informationen (Call for Papers) finden Sie auf der letzten gelben Seite. Sehen wir uns in Basel? Ich würde mich freuen!

Herzlichst

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

This CBS issue has been delayed a bit, but in my defense, I would like to mention that during the hot final phase of the September issue, I was co-organizing the 39th German Food Congress. It was held in Stuttgart-Hohenheim this year and attended by over 600 participants. I think the congress was quite fruitful for the progress of HPTLC in food analysis, especially due to a workshop for the Young Food Chemists.

It was noted that many international companies reported an increase in the need for more food analyses, for which HPTLC offers excellent preconditions. There is great pent-up demand in countries like China, India, Thailand, and other countries where food safety is more and more forced by law.

Due to the increased demand for food analysis, three articles in this CBS relate to this field. By the way the study of food chemistry is a German unicum – there is no comparable study in other countries.

Please keep in mind that preparations for the HPTLC Symposium in Basel, 6–8. July 2011 proceed. Information (Call for Papers) can be found on the last yellow page. Do we meet in Basel? I would enjoy it!

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG SEPTEMBER 2010 105

# THE CBS CLASSIFICATION SYSTEM

1. **Reviews and books**
   a) Books on TLC
   b) Books containing one or several chapters on TLC
   c) Books containing frequent TLC information spread over several chapters of other information
2. **Fundamentals, theory and general**
   a) General b) Thermodynamics and theoretical relationship
   c) Relationship between structure and chrom. behaviour
   d) Measurement of physico-chemical and related values
   e) Optimization of solvent systems
   f) Validation of methods
3. **General techniques** (unless they are restricted to the application within one or two classification sections)
   a) New apparatus/techniques for sample preparation
   b) Separation material
   c) New apparatus for sample application/dosage
   d) New apparatus/techniques for chromatogram development
   e) New apparatus/techniques for pre- or post-chromatographic derivatization
   f) New apparatus/techniques for quantitative evaluation
   g) New apparatus/techniques for other TLC steps (distinguished from section 4)
4. **Special techniques**
   a) Automation of sample preparation/application
   b) Automation of complex chromatogram developing techniques
   c) Automation, computer application in quantitative chromatogram evaluation
   d) Combination of TLC with other chromatographic techniques
   e) Combination of TLC with other (non-chromatographic) techniques...MS, IR...etc.
5. **Hydrocarbons and halogen derivatives**
   a) Aliphatic hydrocarbons
   b) Cyclic hydrocarbons
   c) Halogen derivatives
   d) Complex hydrocarbon mixtures
6. **Alcohols**
7. **Phenols**
8. **Substances containing heterocyclic oxygen**
   a) Flavonoids
   b) Other compounds with heterocyclic oxygen
9. **Oxo compounds, ethers and epoxides**
10. **Carbohydrates**
    a) Mono- and oligosaccharides, structural studies
    b) Polysaccharides, mucopolysaccharides, lipopolysaccharides
11. **Organic acids and lipids**
    a) Organic acids and simple esters
    b) Prostaglandins
    c) Lipids and their constituents
    d) Lipoproteins and their constituents
    e) Glycosphingolipids (gangliosides, sulfatides, neutral glycosphingolipids)
12. **Organic peroxides**
13. **Steroids**
    a) Pregnane and androstane derivatives
    b) Estrogens
    c) Sterols
    d) Bile acids and alcohols
    e) Ecdysones and other insect steroid hormones
14. **Steroid glycosides, saponins and other terpenoid glycosides**
15. **Terpenes and other volatile plant ingredients**
    a) Terpenes
    b) Essential oils
16. **Nitro and nitroso compounds**
17. **Amines, amides and related nitrogen compounds**
    a) Amines and polyamines
    b) Catecholamines and their metabolites
    c) Amino derivatives and amides (excluding peptides)
18. **Amino acids and peptides, chemical structure of proteins**
    a) Amino acids and their derivatives
    b) Peptides and peptidic proteinous hormones
19. **Proteins**
20. **Enzymes**
21. **Purines, pyrimidines, nucleic acids and their constituents**
    a) Purines, pyrimidines, nucleosides, nucleotides
    b) Nucleic acids, RNA, DNA
22. **Alkaloids**
23. **Other substances containing heterocyclic nitrogen**
    a) Porphyrins and other pyrroles
    b) Bile pigments
    c) Indole derivatives
    d) Pyridine derivatives
    e) other N-heterocyclic compounds
24. **Organic sulfur compounds**
25. **Organic phosphorus compounds** (other than phospholipids)
26. **Organometallic and related compounds**
    a) Organometallic compounds
    b) Boranes, silanes and related non-metallic compounds
    c) Coordination compounds
27. **Vitamins and various growth regulators** (non-peptidic)
28. **Antibiotics, Mycotoxins**
    a) Antibiotics
    b) Aflatoxins and other mycotoxins
29. **Pesticides and other agrochemicals**
    a) Chlorinated insecticides
    b) Phosphorus insecticides
    c) Carbamates
    d) Herbicides
    e) Fungicides
    f) Other types of pesticides and various agrochemicals
30. **Synthetic and natural dyes**
    a) Synthetic dyes
    b) Chloroplasts and other natural pigments
31. **Plastics and their intermediates**
32. **Pharmaceutical and biomedical applications**
    a) Synthetic drugs
    b) Pharmacokinetic studies
    c) Drug monitoring
    d) Toxicological applications
    e) Plant extracts, herbal and traditional medicines
    f) Clinico-chemical applications and profiling body fluids
33. **Inorganic substances**
    a) Cations
    b) Anions
34. **Radioactive and other isotopic compounds**
35. **Other technical products and complex mixtures**
    a) Surfactants
    b) Antioxidants and preservatives
    c) Various specific technical products
    d) Complex mixtures and non-identified compounds
36. **Thin-layer electrophoresis**
37. **Environmental analysis**
    a) General papers
    b) Air pollution
    c) Water pollution
    d) Soil pollution
38. **Chiral separations**

XX. (abstract number underlined) refers to HPTLC related publication or application using HPTLC materials

## 1. Reviews and books

105 001 P.K. JAISWAL (Central Agmark Laboratory, Govt. of India, North Ambazari Road, Nagpur 440 010, India): High-performance thin-layer chromatography in food analysis. CBS Publishers & Distributors Pvt Ltd, New Dehli, 2010. Short review on planar chromatography in food analysis. Certain well-known applications are mentioned but important publications and recent scientific data is lacking.

food analysis, HPTLC 1a

105 002 Monika JANICKA*, A. WASIK, J. NAMIESNIK (*Gdansk University of Technology, Chemical Faculty, Department of Analytical Chemistry, 80-223 Gdansk, Poland, mjanicka@hotmail.com): Analytical procedures for determination of cocaine and its metabolites in biological samples. TrAC 29, 209-224 (2010). The dangerous effects of cocaine on living organisms and current problems associated with its wide use and availability have led to the development of various analytical procedures. This review discusses analytical methods proposed during the past ten years for the verification of cocaine and its metabolites in biological samples. Challenges regarding sample preparation and extraction techniques are described. Different approaches using thin-layer chromatography are discussed, and especially the possibility of simultaneous determination of parent, metabolite and interfering drugs in urine samples, as well as the identification of markers for combined consumption of cocaine and alcohol.

toxicology, comparison of methods, review 1, 22

105 003 A. ZEB*, M. MURKOVIC (*Institute for Biochemistry, Graz University of Technology, Graz, Austria; Alamzeb01@yahoo.com): Thin-Layer Chromatographic analysis of carotenoids in plant and animal samples. J. Planar Chromatogr. 23, 94-103 (2010) This review describes available data on analysis of carotenoids by TLC. Petroleum ether, acetone, and hexane are the major mobile phases used for TLC. This technique was found to have the potential to be the first choice for analysis of carotenoids in biological samples. The uses of other, orthogonal chromatographic methods, for example HPLC, MS, scanning densitometry, and image analysis with TLC can enable precise analysis of carotenoids. The review consists of the following parts: 1. Introduction; 1.1 Function of carotenoids; 1.2 Occurrence of carotenoids; 2. Analysis of carotenoids; 2.1 Sampling; 2.2 Sample preparation; 2.3 Extraction of carotenoids; 2.4 Saponification; 2.5 Chromatographic analysis; 3. Thin-layer chromatographic analysis of carotenoids; 3.1 TLC of carotenoids from microbial and animal sources; 3.2 TLC of carotenoids from plant sources; 3.3 Normal-phase TLC analysis of carotenoids; 3.4 Reversed-phase TLC analysis of carotenoids; 3.5 TLC analysis of carotenoids with scanning densitometry; 4. Advantages of TLC in carotenoid analysis; 5. Conclusion and future studies. 134 References.

food analysis, review 1, 30b

## 2. Fundamentals, theory and general

105 004 Rodica Domnica BRICIU*, Agata KOT-WASIK, A. WASIK, J. NAMIESNIK, C. SARBU (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos Str., No 11, 400028 Cluj Napoca, Romania): The lipophilicity of artificial and natural sweeteners estimated by reversed-phase thin-layer chromatography and computed by various methods. J. Chromatogr. A 1217 (23), 3702-3706 (2010). Evaluation of the chromatographic behavior of some artificial and natural sweeteners by HPTLC on RP18, RP18W, RP8, cyano and amino phases with mixtures of acetonitrile - water in different volume proportions. The lipophilicity is given through chromatographic descriptors such as RM0, mean of RM (mRM), and scores of RM values corresponding to the first principal component (PC1/RM). In addition, scores and loadings resulting from covariance matrix of retention data provide new information about similarity and differences of investigated compounds and between the stationary phase and the mobile phases. The experimental lipophilicity indices estimated from retention data were correlated with com-

puted values, via computer software and internet module. Results were in accordance at a highly significant statistical level.

quality control food analysis, HPTLC 2c

105 005 K. FERENCZI-FODOR, B. RENGER*, Z. VÉGH (*Vetter Pharma-Fertigung GmbH & Co. KG, Schuetzenstrasse 87, 88212 Ravensburg, Germany; bernd.renger@vetter-pharma.com): The frustrated reviewer - recurrant failures in manuscripts describing validation of quantitative TLC/HPTLC procedures for analysis of pharmaceuticals. J. Planar Chromatogr. 23, 173-179 (2010). Many manuscripts and already published articles on analytical procedures to be used in pharmaceutical quality control are characterized by several typical methodological failures and misconceptions. The autors present a collection of typical failures, misconceptions, and misleading data from articles published over the last two years in seven well-known chromatographic publications and provide at the same time a list of references describing optimum approaches to validation of specific TLC/HPTLC procedures. In particular, method specificity, linearity, accuracy, and precision very often are not determined properly and in accordance with best practise.

quality control, HPTLC, quantitative analysis, validation of methods 2f

105 006 T. HANAI (Health Research Foundation, Institut Pasteur 5F, Sakyo-ku, Kyoto 606-8225, Japan; thanai@attglobal.net): Quantitative in silico analysis of retention in normal phase liquid chromatography. J. Liq. Chromatogr. Relat. Technol. 33, 297-304 (2010). Retention in normal phase liquid chromatographic was quantitatively analyzed in silico using as samples sleeping medicines in acidic, basic, and neutral organic solvent mixtures. Hydrogen bonding is the major contribution in the retention that was supported by a high correlation coefficient between log k and hydrogen bonding energy values calculated using a molecuar mechanics program. TLC on silica gel with chloroform - acetone 9:1, benzene - acetic acid 9:1 and dioxane - benzene - aqueous ammonium hydroxide 20:75:4.

2c

## 3. General techniques

105 007 V. BEREZKIN*, Svetlana KHREBTOVA, Natalia KULAKOVA (*Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences, Leninsky pr. 29, Moscow, 119991, Russia): Four-dimensional TLC on plates with open and closed adsorbent layers. Chromatographia 71 (9-10), 907-911 (2010). Description of four-stage TLC, a new version of multidimensional (multi-stage) planar chromatography. In the first stage a multicomponent mixture is fractionated into three groups of chromatographic zones (fractions) by TLC where the adsorbent is deposited on an aluminum foil. In the second stage, the TLC plate is dried and cut by scissors into three narrow strips containing the separated fractions. In the next stages the isolated fractions on each of these strips („daughter“ plates) are separated into individual components with an appropriate mobile phase and by using a solvent flow perpendicular to the flow of the mobile phase. The practical application of the method is shown using as an example the separation of a mixture of dyes. The method can be expanded for separation of mixtures containing four or more fractions. This four-dimensional version of multidimensional TLC was carried out on plates with both open (conventional) and closed adsorbent layers.

3d

105 008 J.E. CLARK*, Susan OLESIK (*The Ohio State University, Department of Chemistry, 120 West 18th Ave, Columbus, OH 43210, USA): Electrospun glassy carbon ultra-thin layer chromatography devices. J. Chromatogr. A 1217 (23), 4655-4662 (2010). Development and application of electrospun glassy carbon nanofibers for ultra-thin layer chromatography (UTLC). The carbon nanofiber stationary phase was created through electrospinning and pyrolysis of SU-8 2100 photoresist, which resulted in glassy carbon nanofibers with diameters of 200-350 nm that form a

mat structure with a thickness of 15 µm. The chromatographic properties of UTLC devices produced from pyrolyzed SU-8 heated to temperatures of 600, 800, and 1000 °C were investigated. By use of Raman spectroscopy and scanning electron microscopy the physical and molecular structure of the nanofibers at each temperature was determined. The carbon UTLC devices were suitable for the analysis various dye mixtures and also allowed separation of three FITC-labeled essential amino acids (lysine, threonine, phenylalanine). The electrospun glassy carbon UTLC plates showed good retention properties, plate number values above 10000, and physical and chemical robustness for a range of mobile phases.

doping, HPTLC, qualitative identification, quantitative analysis 3b

105 009 P. PLOCHARZ*, Anna KLIMEK-TUREK, T.H. DZIDO (*Department of Physical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland): Pressurized planar electrochromatography, high-performance thin-layer chromatography and high-performance liquid chromatography - Comparison of performance. J. Chromatogr. A 1217 (23), 4868-4872 (2010). Comparison of the kinetic performance, measured by plate height, of high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC) and pressurized planar electrochromatography (PPEC). HPTLC on RP18W with mobile phases composed of acetonitrile - buffer. The HPLC column was packed with the same adsorbent, which was scrapped from an RP18W HPTLC plate. An additional HPLC column was packed with C18-type silica based (LiChrosorb RP18) adsorbent of 5µ m particle diameter. The plate height of both HPLC and PPEC systems depends on the flow velocity and the migration distance of the mobile phase. As test solution prednisolone succinate was used. The best performance was obtained by the PPEC system. The separation efficiency of the systems was investigated and confirmed by use of a test component mixture composed of six hormones.

quality control, HPTLC, comparison of methods, pressurized planar electrochromatography 3

## 4. Special techniques

105 010 P. HOFFMANN, M. HULSEWIG, S. DUVAR, H. ZIEHR, M. MORMANN, J. PETER, A. FRIEDRICH, H. KARCH, J. MUTHING* (*Institute of Hygiene and Interdisciplinary Center for Clinical Research, University of Munster, Munster, Germany, jm@uni-muenster.de): On the structural diversity of Shiga toxin glycosphingolipid receptors in lymphoid and myeloid cells determined by nanoelectrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom. 24, 2295-2304 (2010). HPTLC of glycosphingolipids (GSLs) on silica gel with chloroform - methanol - water 120:70:17. The plate was overlaid with Shiga toxin (Stx), and the microbes were detected with primary anti-Stx and appropriate alkaline phosphatase labeled secondary antibodies. Bound antibodies were visualized by color development using 0.05 % 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt in glycine buffer. The GSLs were extracted from the plate and analyzed by electrospray ionization mass spectrometry (ESI-MS). Using the combination of a TLC overlay assay and nanoESI-QTOF-MS, the structural characterization of the functional Stx1 receptors of Raji and THP-1 cells is reported.

pharmaceutical research, HPTLC 4e

105 011 A. MUELLER, S. WEISS, W. SCHULZ*, W. SEITZ, R. ALBERT, W. RUCK, W. WEBER (*Zweckverband Landeswasserversorgung, Betriebs- und Forschungslaboratorium, Am Spitzigen Berg 1, 89129 Langenau, Germany, schulz.w@lw-online.de): Combination of different liquid chromatography/mass spectrometry technologies for the identification of transformation products of rhodamine B in groundwater. Rapid Commun. Mass Spectrom. 24, 659-666 (2010). HPTLC of rhodamine B and five de-ethylated transformation products (N,N,N‘-tryethylrhodamine (1), N,N‘-dyethylrhodamine (2), N,N-dyethylrhodamine (3), N-ethylrhodamine (4), and rhodamine (5)) in groundwater on silica gel by automated multiple development with a 23-step gradient based on methanol (with the addition of formic acid) and dichloromethane. The drying

time after each step was 2 min. For detection by bioluminescence the plate was dipped into a suspension of Vibrio fischeri for 2 s at a speed of 3 cm/s. The $hR_f$ were 72, 66, 60, 53, 48, and 36 for compounds (1) - (5). Combination of different separation and detection techniques enabled a fast and effective screening of the groundwater sample.

environmental, HPTLC, qualitative identification, postchromatographic derivatization, AMD 4e

105 012 A. MUSKEN, J. SOUADY, K. DREISEWERD, W. ZHANG, U. DISTLER, J. PETER, H. MILLER, H. KARCH, J. MUTHING* (*Institute of Hygiene and Interdisciplinary Center for Clinical Research, University of Munster, Munster, Germany, jm@uni-muenster.de): Application of thin-layer chromatography/infrared matrix-assisted laser desorption/ionization orthogonal time-of-flight mass spectrometry to structural analysis of bacteria-binding glycosphingolipids selected by affinity detection. Rapid Commun. Mass Spectrom. 24, 1032-1038 (2010). HPTLC of glycosphingolipids (GSLs) on silica gel with chloroform - methanol - water 120:70:17. The plate was overlaid with GSL-specific bacteria, and the microbes were detected with primary antibodies and appropriate alkaline phosphatase labeled secondary antibodies, and by in situ MS analysis of bacteria-specific GSL receptors. The thin-layer chromatography infrared matrix-assisted laser desorption/ionization orthogonal time-of-flight mass spectrometry (TLC/IR-MALDI-o-TOF-MS) method represents one of the most powerful approaches for the detection of GSL receptors of microorganisms.

pharmaceutical research, HPTLC 4e

105 013 K. NIMPTSCH, R. SUESS, T. RIEMERA, A. NIMPTSCH, M. SCHNABELRAUCH, J. SCHILLER (*University of Leipzig, Medical Faculty, Institute of Medical Physics and Biophysics, Härtelstr. 16-18, 04107 Leipzig, Germany): Differently complex oligosaccharides can be easily identified by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry directly from a standard thin-layer chromatography plate. J. Chromatogr. A 1217 (23), 3711-3715 (2010). Oligosaccharides (derived from dextran, alginate, hyaluronan and chondroitin sulfate) were characterized by matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) directly on a normal phase TLC plate. The applied oligosaccharides were either commercially available or obtained from the polysaccharides by HCl-induced hydrolysis. TLC was followed by MALDI-TOF MS subsequent to matrix deposition. The high quality mass spectra obtained allow for unequivocal assignments. The high content of formic acid in the solvent does not cause major problems but is responsible for the partial formylation of the analyte and a minor N-acetyl loss from hyaluronan and chondroitin sulfate.

qualitative identification, HPTLC, oligosaccharides 4e

105 049 W. SCHWACK et al., see section 30

## 6. Alcohols

105 014 Anna NIESTROJ (Silesian University, Institute of Chemistry, 9 Szkolna Street, 40-006 Katowice, Poland; annaniestroj@wp.pl): Comparison of methods for calculation of the partition coefficients of selected aliphatic compounds. J. Planar Chromatogr. 23, 198-200 (2010). Proposition of new methods for calculation of the partition coefficients of aliphatic compounds from experimental $R_f$ values and the numerical values of selected topological indexes. The experimental partition coefficient (log Pexp) of cetyl alcohol was determined for the n-octanol-water system. Numerical values obtained were compared with theoretical values from a database (AlogPs, AC_logP, AB/LogP, ALOGP, milogP, and XLOGP2). HPTLC of cetyl alcohol, stearyl alcohol, palmitic acid, stearic acid, alpha-hydroxypalmitic acid, and 12-hydroxystearic acid on RP18 with methanol and with methanol - water 19:1 in a horizontal chamber at room temperature. Detection after visualization in iodine vapor.

comparison of methods, qualitative identification 6, 11a

## 7. Phenols

105 015 T. HOFMANN*, T. RÉTFALVI, L. ALBERT, P. NIEMZ (*University of West Hungary, Department for Chemistry, Ady Endre u. 5, 9400 Sopron, Hungary; hofmannt@emk.nyme.hu): High-performance thin-layer chromatographic assessment of thermally modified wood. J. Planar Chromatogr. 23, 227-229 (2010). HPTLC of phenolic compounds (syringic acid, vanillic acid, pyrocatechin, thymol, sinapic acid, 2,6-dimethoxyphenol, resorcin, guaiacol) on silica gel with diisopropyl ether - formic acid 9:1 in an unsaturated twin trough chamber. OPLC of sugars (sucrose, galactose, glucose, mannose, fructose, arabinose, xylose, ribose, rhamnose, stachyose, raffinose, maltose) on silica gel with acetonitrile - water 17:3. Detection of phenolic compounds by spraying with Folin-Ciacalteau reagent and of sugars by spraying with aniline - diphenylamine reagent.

agricultural, HPTLC, qualitative identification 7

105 016 Sayyada KHATOON*, H. SINGH, K. SINGH, A. K. GOEL (*Pharmacognosy and Ethnopharmacology Division, Council for Scientific and Industrial Research, National Botanical Research Institute, Rana Pratp Marg, Lucknow-226001, India; sayyadak@yahoo.com): TLC evaluation and quantification of phenolic compounds in different parts of Dendrophthoe falcata (Linn. f.) Etting. J. Planar Chromatogr. 23, 104-107 (2010). TLC of (+)-catechin, ellagic acid, quercetin, and ferulic acid on silica gel with toluene - ethyl acetate - fomic acid 6:4:1 in a twin-trough chamber previously saturated for 30 min at 25 °C. Other mobile phase compositions were ethyl acetate - methanol - water 10:1:1, n-butanol - acetic acid - water 4:1:5 (upper layer), n-butanol - ethanol - water 20:5:11, n-butanol - acetic acid - water 6:2:1, toluene - ethyl acetate - formic acid 5:5:1 and 7:3:1, and toluene - ethyl acetate - methanol - formic acid 140:60:1:1. Quantitative determination by absorbance measurement at 320 nm. Detection by dipping in anisaldehyde reagent, followed by drying and heating at 110 °C for 5 min. Characteristic bands of (+)-catechin, ellagic acid, quercetin, and ferulic acid were observed at $hR_f$ 35, 41, 63 and 66, respectively. Precision (CV, $n$ = 7) was 0.39, 0.44, 0.55 and 0.16 % and repeatability (CV, $n$ = 7) 0.65, 0.95, 0.37 and 0.18 %, respectively. LOD was 54, 340, 48 and 78 ng/band, and LOQ 4.1, 6.5, 1.6 and 1.5 µg/band, respectively. Linear regression was 0.9993 (100-500 ng/band), 0.9883 (1000-5000 ng/band), 0.9989 (100-500 ng/band) and 0.9987 (100-500 ng/band), respectively.

*Note of the editor: The reported LOQ are unusally high compared to the LOD. The reported CV (standard deviation divided by mean) also arises questions.*

herbal, traditional medicine, densitometry 7

105 017 M.M. PANDEY, S. RASTOGI, A. K. S. RAWAT* (*Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow-226001, India; rawataks@rediffmail.com): Optimization of an HPTLC method for separation and identification of phenolic compounds. J. Planar Chromatogr. 23, 108-111 (2010). HPTLC of gallic acid, ferulic acid, syringic acid, catechin, protocatechuic acid and vanillin on silica gel with toluene - ethyl acetate - formic acid 8:2:1 in a twin-trough chamber previously saturated for 15 min. Quantitative determination by absorbance measurement at 280 nm. Accuracy was between 96.3 and 90.7 %, repeatability between 0.65 and 0.93 %, inter-day precision between 0.80 and 0.90 %, intra-day precision between 0.72 and 0.95 %, and precision between 0.87 and 0.92 %. LOD and LOQ were about 100 and 400 ng/band, respectively. Starting at LOQ, the correlation coefficients were between 0.988 and 0.997.

herbal, traditional medicine, HPTLC 7

## 8. Substances containing heterocyclic oxygen

105 018 V. GLAVNIK, B, SIMONOVSKA, Irena VOVK* (*National Institute of Chemistry, Laboratory

for Food Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia; irena.vovk@ki.si): Comparison of TLC and HPLC methods used for analysis of (-)-epicatechin and its dimer procyanidin B2 in chocolate. J. Planar Chromatogr. 23, 230-232 (2010). HPTLC of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-epicatechin gallate, procyanidin B2, procyanidin A2, and methylxanthines (theobromine and caffein) on cellulose with n-propanol - water - acetic acid 20:80:1 in a horizontal chamber. Detection by dipping for 1 s into 4-dimethylaminocinnamaldehyde detection reagent. Quantitative determination by absorbance measurement at 655 nm. By densitometry LOD for (-)-epicatechin and procyanidin B2 was 0.2 and 2 ng/zone, respectively; LOQ was 0.4 ng and 4 ng/zone, respectively. These limits were lower by a factor 50 for (-)-epicatechin and by a factor of 10 for procyanidin B2 than those obtained by HPLC. The TLC method gave a more accurate result for the (-)-epicatechin content of baking chocolate than the HPLC method which was also more time-consuming.

food analysis, comparison of methods, HPTLC, densitometry, quantitative analysis 8a

105 019 J. SUN (Sun Jia) , Y. YUE* (Yue Yongde), F. TANG (Tang Feng), X. GUO (Guo Xuefeng) (*International Centre for Bamboo and Rattan, No. 8 Futong Dongdajie), Wangjing, Chaoyang District, Beijing 100102, Cina; yueyd@icbr.ac.cn): Simultaneous HPTLC analysis of flavonoids in the leaves of three different species of bamboo. J. Planar Chromatogr. 23, 40-45 (2010). HPTLC of flavonoids (vitexin, isovitexin, orientin, isoorientin, rutin) on polyamide at 23 +/- 2°C and 40 % relative humidity with the three-component mobile phase A-B-C 33:67:8, where A is dodecyl sulfate - n-butanol - n-heptane 147:158:28, B is water, and C is formic acid. The solvent mentioned gave the best resolution of vitexin ($hR_f$ 61), isovitexin ($hR_f$ 21), orientin ($hR_f$ 28), isoorientin ($hR_f$ 34), and rutin ($hR_f$ 52). Detection by spraying with 1 % aluminium trichloride in ethanol followed by waiting for approximately 1h. Quantitative determination by fluorescence measurement at 366 nm. Precision was found to be 0.98, 0.91, 1.02, 1.04 and 0.87 % for isovitexin, rutin, orientin, isoorientin, and vitexin, respectively. Average recoveries (at three different concentrations) were between 98.9 and 100.6 % from P. pubescens, P. glauca and P. yixingensis.

HPTLC, densitometry, quantitative analysis, qualitative identification 8a

## 9. Oxo compounds, ethers and epoxides

105 020 P. DEEPIKA, K. N. SABHARWAL, N. SIVARAMAN, T. G. SRINIVASAN*, P. R. VASUDEVA RAO (*Fuel Chemistry Division, Chemistry Group, Indira Gandhi Centre for Atomic Research, Kalpakkam, Tamil Nadu 603 102, India; tgs@igcar.gov.in): Development of a chromatographic procedure for the purification of 1,2-diketone. J. Liq. Chromatogr. Relat. Technol. 33, 97-108 (2010). TLC of octane-4,5-dione on silica gel with hexane, dichloromethane, and hexane - dichloromethane 1:1. Detection by placing the plate in a iodine chamber.

qualitative identification 9

## 10. Carbohydrates

105 021 J. GEISSER*, Evamaria KRATZ (*Chemical and Veterinary Investigation Laboratory (CVUA), Weissenburger Str. 3, 76187 Karlsruhe, Germany, Juergen.Geisser@cvuaka.bwl.de): Determination of aloe vera gel in cosmetics. CBS 104, 13-15 (2010). HPTLC of aloeverose, glucose and galactose in aloe vera products on silica gel (impregnated with NaH2PO4) with acetone - isopropanol - 0.1 M formic acid 2:2:1 (double development for products with high glucose content). Detection by immersion in 4-aminobenzoic acid reagent (1 g 4-aminobenzoic acid in 36 mL acetic acid, with 40 mL water, 2 mL 85 % phosphoric acid and 120 mL acetone), followed by heating at 100 °C for 10 min. Densitometric evaluation by fluorescence measurement at 366 nm. The limit of quantification was approx. 3 % in aloe vera products. *RSD* for polynomial calibration is 2.1 % for mannose in the range of 8-80 ng/band and 4.0 % using matrix calibration.

herbal, HPTLC 10b

105 022 P. MANDAL, A. MISRA* (*Division of Molecular Medicine, Bose Institute, A.J.C. Bose Birth Centenary Campus, Kolkata, India, akmisra69@gmail.com) : Concise synthesis of the pentasaccharide O-antigen corresponding to the Shiga toxin producing Escherichia coli O171. Bioorg. Chem. 38, 56-61 (2010). TLC of 4-methoxyphenyl glycoside on silica gel with acetonitrile - methanol - water 4:2:1. Detection by spraying with warm 2 % ceric sulphate in 2N sulfuric acid. The $hR_f$ of 4-methoxyphenyl glycoside was 30.

pharmaceutical research, qualitative identification 10b

105 023 K. SONG, J. SHIM, J. PARK, S. KIM, Y. KIM, W. BOOS, K. PARK* (*Department of Biology, University of Incheon, Incheon, Republic of Korea, parkkh@incheon.ac.kr): Transglycosylation properties of maltodextrin glucosidase (MaIZ) from Escherichia coli and its application for synthesis of a nigerose-containing oligosaccharide. Biochem. Biophys. Res. Commun. 397, 87-92 (2010). Preparative TLC of the hydrolysis reaction of maltodextrin glycosidase on various substrates (maltotriose, alpha-, beta-, gamma-cyclodextrins and cycloamylose) on silica gel with ethyl acetate - methanol - acetic acid - water 12:3:3:1. The structure of the products was determined by MALDI-TOF/MS, 13C NMR, and enzymatic analysis.

pharmaceutical research, preparative TLC 10b

105 024 C. YANG (Yang Cheng), J. GUAN (Guan Jia), J.-S. ZHANG (Zhang Jiang-sheng), S.-P. LI*(Li Shao-ping) (*Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China; lishaoping@hotmail.com): Use of HPTLC to differentiate among the crude polysaccharides in six traditional chinese medicines. J. Planar Chromatogr. 23, 46-49 (2010). HPTLC of polysaccharides (with galactose, glucose, mannose, arabinose, ribose, xylose, rhamnose, galacturonic acid, and glucuronic acid as standards) before and after hydrolysis on silica gel with chloroform - n-butanol - methanol - water - acetic acid 9:25:10:3:3 with chamber saturation for 30 min at room temperature. Detection by spraying with aniline - diphenylamine - phosphoric acid reagent and heating at 130 °C for 10 min. Quantitative determination by absorbance measurement at 380 nm. Ninhydrin reagent was used for detection of other compounds.

pharmaceutical research, herbal, traditional medicine, HPTLC, densitometry 10b

## 11. Organic acids and lipids

105 025 P. KUSHWALI*, Sheeja EDWIN, K. VARSHNEY, E. JARALD, S. AHMAD, A. DAUA (*TIFACORE in Green Pharma, B. R. Nahata College of Pharma, Mhow-Neemuch Rd., Mandsaur (M.P.), India): Estimation of curcumin and 3-acetyl-11-keto-a-boswellic acid in a marketed herbal product rheumax using HPTLC. International Seminar on Herbal Drug Research, PN-028 (2009). HPTLC of curcumin and 3-acetyl-11-keto-a-boswellic acid in the herbal product Rheumax (contains Curcuma longa, Boswellia serrata, Tinospora cordifolia and Vitex negundo) on silica gel with chloroform - methanol 37:3 for curcumin and n-hexane - ethyl acetate 1:1 for the acid. Quantitative determination by absorbance measurement at 430 nm for curcumin and 254 nm for the acid. The method was linear in the range of 100-500 ng/band for curcumin and 1500-4000 ng/band for the acid.

traditional medicine, quantitative analysis, HPTLC 11a

105 014 Anna NIESTROJ, see section 6

105 026 K. RIZWANBASHA*, M. SHANMUKHA, K. RAMRAO, N. MUNJUNATHA, V. SENTHIL, M. SAMANTA (*JSS College of Pharmacy, Dept. of Pharmaceutics, Oaty, India): Design and development of triphala fast dispersable tablets and its characterization. International Seminar on Herbal Drug Research, PN-064 (2009). HPTLC of gallic acid as marker compound in triphala

fast dispersable tablets on silica gel with ethyl acetate - toluene - methanol - glacial acetic acid 75:20:3:2. Results from HPLC analysis were comparable. The method was suitable for routine quality control of dispersable tablets formulation.

herbal, densitometry, HPTLC 11a

105 027 Karin ROTHENBUEHLER, E. REICH*, M. HAMBURGER (*CAMAG Laboratory, Sonnenmattstr. 11, 4132 Muttenz, Switzerland): Validated HPTLC method for skin lipids. CBS 104 5-6 (2010). HPTLC of skin lipids (squalene, triolein, palmitic acid, 1,2-dipalmitoyl-sn-glycerol, stearyl palmitate, cholesteryl palmitate, and cholesterol) on silica gel (prewashed with methanol) first with toluene to a developing distance of 80 mm, then with n-hexane - t-butyl methyl ether - acetic acid 80:20:1 to 45 mm in a twin-trough chamber saturated for 20 min. Detection by dipping in copper(II)sulfate reagent followed by heating for 30 min at 140 °C. Densitometric absorbance measurement at 350 nm. Stability of standards and samples during 3 h in solution and on the plate was good. The differences of the $hR_f$ values of 7 compounds on 3 plates was good (< 3). The precision of measurement was lowest on HPTLC LiChrospher silica gel plates (*RSD* of < 5 %, n=9). The linear range for all but two compounds was between 100 and 350 ng/band and correlation coefficients were > 0.9975.

HPTLC, pharmaceutical research 11c

## 13. Steroids

105 028 K. BOBER (Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, 4 Jagiellonski Street, PL-41-200, Sosnowiec, Poland; bober@sum.edu.pl): Densitometry application for evaluation of the visualizing agents for dehydroepiandrosterone. J. Liq. Chromatogr. Relat. Technol. 31, 2673-2685 (2008). TLC of dehydroepiandrosterone on silica gel with chloroform - acetone 17:3 with chamber saturation for 30 min. Detection by dipping into 0.05 % aqueous solutions of visualizing agents (methylene violet, gentian violet, janus blue, methylene blue, malachite green, rhodamine B) and methanolic chloramine T/methanolic sulfuric acid solution. Quantitative determination by densitometry at 200 nm (before derivatization and for methylene violet), 657 nm (gentian violet), 487 nm (janus blue), 488 nm (malachite green), 580 nm (rhodamine B), and 361 nm (chloramine T). LOD was 0.29 µg/5 mL (without visualizing agent, with chloramine T and with rhodamine B), 4.19 µg/5 mL with methylene violet, 1.10 µg/5 mL with gentian violet, 2.14 µg/5 mL with janus blue, and 4.19 µg/5 mL with malachite green.

densitometry, quantitative analysis 13a

## 15. Terpenes and other volatile plant ingredients

105 029 B. QIN, J. EAGLES, F. MELLON, P. MYLONA, L. PEÑA, A. OSBOURN* (*Sainsbury Laboratory, Norwich NR4 7UH, UK, anne.osbourn@bbsrc.ac.uk): High throughput screening of mutants of oat that are defective in triterpene sythesis. Phytochemistry. 71, 1245-1252 (2010). TLC of squalene (1), 2,3-oxidosqualene (2) and beta-amyrin (3) in the roots of Avena strigosa on silica gel with hexane - chloroform 23:2, hexane - chloroform 1:1, and hexane - acetone 4:1 for (1), (2) and (3), respectively. Detection by exposure to iodine vapor. Quantitative determination by GC-MS.

pharmaceutical research, HPTLC, quantitative analysis, densitometry 15a

105 030 A. VARMA, Neeta SHRIVASTAVA* (*B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Sarkhej-Gandhinagar Highway, Thaltej, Ahmedabad-380054, Gujarat, India; neetashrivastava_perd@yahoo.co.in): Micro scale procedure for analysis of andrographolide in Andrographis paniculata leaves. J. Planar Chromatogr. 23, 50-55 (2010). HPTLC of andrographolide on silica gel with chloroform - methanol - ethyl acetate 12:3:2 in a twin-trough chamber previously saturated for 15 min. Quantitative determination by absorbance measurement at 223 nm. The relative standard deviation of intra-day and inter-day analysis was in the ran-

ge of 0.56-1.33 %. Lineartiy was given between 200-700 ng/band; the correlation coefficient was 0.9998 and the *RSD* 0.97 %. The limits of detection and quantification were 60 and 150 ng/band. Average recovery was 98.8 +- 0.41 %.

pharmaceutical research, herbal, traditional medicine, HPTLC, densitometry, quantitative analysis 15a

## 17. Amines, amides and related nitrogen compounds

105 063 R. DEEPA et al., see section 32

105 087 B. MEHTA et al., see section 32

105 113 Jyoti SHRIVASTAVA et al., see section 32

## 18. Amino acids and peptides, chemical structure of proteins

105 031 P. VENKATAKRISHNAN, E. NAKAYASU, I. ALMEIDA, R. MILLER* (*Department of Biological Sciences, University of Texas at El Paso, El Paso, Texas, The United States of America, tmiller2@utep.edu): Arginase activity in mitochondria - an interfering factor in nitric oxide synthase activity assays. Biochem. Biophys. Res. Commun. 394, 448-452 (2010). HPTLC of [14C]-L-arginine and [14C]-L-citrulline of a nitric oxide synthase conversion assay in mitochondria rat liver, on silica gel with butanol - acetic acid - water 3:1:1. Detection by dipping in a solution of 2 % ninhydrin in acetone, followed by heating on a plate heater for 1 min. After visualization, the plates were exposed to X-ray film for 4.5 days.

pharmaceutical research, HPTLC, quantitative analysis, autoradiography 18a

## 19. Proteins

105 032 K. NISHIYAMA*, M. MAEDA, M. ABE, T. KANAMORI, K. SHIMAMOTO, S. KUSUMOTO, T. UEDA, H. TOKUDA (*Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan, nishiyam@iwate-u.ac.jp): A novel complete reconstitution system for membrane integration of the simplest membrane protein. Biochem. Biophys. Res. Commun. 394, 733-736 (2010). TLC of liposomes containing membrane protein integrase (MPIase), on silica gel with ethanol - chloroform - water 7:3:4. Detection by spraying with a solution of anisaldehyde - concentrated sulphuric acid - acetic acid 3:4:1. The $hR_f$ value of MPIase was 35.

pharmaceutical research, qualitative identification 19

## 21. Purines, pyrimidines, nucleic acids and their constituents

105 033 E. MINCSOVICS (OPLC-NIT Ltd, Andor Street 60, 1119 Budapest, Hungary, and Corvinus University, Faculty of Horticultural Sciences, Department of Genetics and Plant Breeding, Budapest, Hungary; emil.mincsovics@t-online.hu): Increased detection sensitivity in fully off-line OPLC by bilateral band compression. J. Planar Chromatogr. 23, 190-192 (2010). After sample application as bands and fully off-line OPLC separation followed by drying, the separated bands were compressed bilaterally, in parallel, perpendicular to the direction of development, by use of a strong eluent and capillary driven chromatography. To introduce the eluent for band compression onto the layer a simple manual tool equipped with parallel foam strips was constructed. OPLC of a black tea leaf extract and caffeine, theophylline, and theobromine on silica gel (prewashed with acetonitrile - water 17:3) with toluene - acetic acid 3:2. Quantitative determination by densitometry at 280 nm. After band compression 20 ng/zone theophyllin and theobromine could be detected by densitometry in the xanthine standard mixture at a loading of 20 µg/10 mm. These compounds were not visible in the original, uncompressed chromatogram.

herbal, quantitative analysis, densitometry 21a

105 034 K. VINODKUMAR*, T. VETRICHELVAN (*Dept of Pharmaceutical Analysis, Adhi Parasakthi College of Pharmacy, Tamil Nadu, India): Method development for risedronate sodium hemi pentahydrate by UV spectroscopic method and high-performance thin-layer chromatography. IPA Convention, 2010, RA-PO 26. HPTLC of risedronate sodium on silica gel with water - methanol - 25 % ammonia 20:3:3. Densitometric evaluation at 262 nm. The method was linear in the range of 3-6 ng/band, recovery was 98.6 %. Results were comparable with HPLC results. The advantage of the HPTLC method is the simultaneous analysis of several samples.

pharmaceutical research, quality control, densitometry, comparison of methods, quantitative analysis, HPTLC 21a

## 22. Alkaloids

105 002 Monika JANICKA et al., see section 1

105 035 J. KENNEDY, J. WISEMAN* (*Prosolia Inc., Indianapolis, IN 46202, USA, wiseman@prosolia.com): Direct analysis of Salvia divinorium leaves for salvinorin A by thin layer chromatography and desorption electrospray ionization multi-stage tandem mass spectrometry. Rapid Commun. Mass Spectrom. 24, 1305-1311 (2010). HPTLC of salvinorin A (1), salvinorin C (2), divinatorin B (3), and salvinorin B (4) in the leaves of Salvia divinorium on silica gel with methyl tert-butyl ether - hexane 3:1 as mobile phase. Detection by desorption electrospray ionization multi-stage tandem mass spectrometry (TLC/DESI-MS). The $hR_f$ were 49, 64, 85, and 95 for compounds (1) - (4), respectively. The use of a simple HPTLC protocol in combination with DESI-MS allowed for improved detection of different species of salvinorin in the leaf extracts.

toxicology, herbal, HPTLC, qualitative identification 22

## 23. Other substances containing heterocyclic nitrogen

105 036 M. DOLOWY*, A. NIESTRÓJ (*Department of Analytical Chemistry, Faculty of Pharmacy, Silesian University of Medicine, 4 Jagiellonska St., PL-42-200 Sosnowiec, Poland; mdolowy@wp.pl): Densitometric determination of ursodeoxycholic acid in pharmaceutical formulations in form of tablets and capsules. J. Liq. Chromatogr. Relat. Technol. 33, 109-117 (2010). TLC of ursodeoxycholic acid on silica gel with n-hexane - ethyl acetate - acetic acid 22:22:5 at room temperaure in a horizontal chamber. The $hR_f$ value was 48. Detection by spraying with 10 % sulfuric acid and heating at 120 °C for 20 min. Quantitative determination by densitometric scanning at 360 nm. LOD and LOQ was 0.014 and 0.041 mg/spot, respectively. The linearity range was 0.030-0.120 mg/spot. The correlation coefficient r was 0.0063. The accuracy of the quantitative analysis of ursodeoxycholic acid in extracts from examined pharmaceutical formulations was 95.3 % and 97.2 %, and the precision was 5.29 and 6.15 % in tablets and capsules, respectively.

quality control, densitometry, quantitative analysis 23b

105 037 Anna PETRUCZYNIK (Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland; annapetruczynik@poczta.onet.pl): Effect of chromatographic conditions on separation and system efficiency in HPTLC of selected quinoline standards on cyanopropyl stationary pases. J. Planar Chromatogr. 23, 56-64 (2010). HPTLC of thirteen quinolines (quinoline, isoquinoline, 2,4-dihydroxyquinoline, 8-hydroxyquinoline, 5,6-benzoquinoline, 2,2‘-diquinoline, 8-methylquinoline, 5-aminoquinoline, 1-ethylquinoline, 2-chlorquinoline, 6-nitroquinoline, 2,6-dimethylquinoline, 5-hydroxyquinoline) on cyano phase with different mobile phases prepared from mixtures of methanol, acetonitrile, 2-propanol, tetrahydrofuran, and dioxane with addition of buffer at different pH, ion-pairing reagents, or silanol blockers. Detection under UV light at 254 nm and by densitometric absorbance measurement at 254 nm. Improved peak symmetry was observed for mobile phases containing ion-pairing reagent. The most symmetrical peaks were obtained by use of mobile phase containing diethylamine as silanol blocker.

qualitative identification, HPTLC, densitometry 23e

105 038 T.M. SHAH*, S.S. SAVLE, M.T. CHHABRIA, I.S. RATHOD, C.J. SHISHOO, P. S. BRAHMKSHATRIYA (*Dept. of Pharmaceutical Chem. & Dept. of Q. A., L. M. College of Pharmacy, Navranga, Ahmedabad 380009, India): Pharmacokinetic study of a novel antihyperlipidemic agent LM-13765-A prodrug. Ind. J. Pharma. Science 71 61, 644-650 (2009). HPTLC of the novel antihyperlipidemic agent LM-13765 in rabbit plasma on silica gel (pre-washed with methanol) with benzene - methanol 4:1 in a saturated chamber at 25 °C. Densitometric evaluation at 314 nm. The method was linear in the range of 12.5-100 ng/band. Also HPTLC of LM-13765-C, a metabolite of the parent compound LM-13765, on silica gel with toluene - methanol 23:2. Densitometric evaluation at 274 nm. Details are provided on the complete extraction procedure for the extraction of the parent compound and its metabolite from plasma.

pharmaceutical research, clinical routine analysis, HPTLC, densitometry, quantitative analysis 23b

## 24. Organic sulfur compounds

105 039 K. PLUTA*, B. MORAK-MLODAWSKA, M. JELEN, R. KORLACKI (*Department of Organic Chemistry, The Medical University of Silesia, Jagiellonska 4, 41-200 Sosnowiec, Poland; pluta@slam.katowice.pl): TLC separation of isomeric diazinodithiins and diazinyl sulfides as the Smiles rearrangement products. J. Liq. Chromatogr. Relat. Technol. 31, 3020-3031 (2008). TLC of twelf thioazines on silica gel with chloroform - ethanol 10:1 and chloroform, and on aluminum oxide with dichloromethane and benzene - chloroform 1:1 in a chamber saturated for 30 min. Detection under UV 254 and 366 nm. The retention parameters were measured and then calculated as separation factors deltaRf, RS, and alpha. The $hR_f$ values were correlated with the dipole moments of thioazines and the symmetry of diazinodithiins.

qualitative identification 24

## 27. Vitamins and various growth regulators

105 040 A. HOSU, Claudia CIMPOIU*, M. SANDRU, L. SESERMAN (*Faculty of Chemistry and Chemical Engineering, „Babes-Bolyai“ University, 11 Arany Janos, 400028 Cluj-Napoca, Rumania; ccimpoiu@chem.ubbcluj.ro): Determination of the antioxidant activity of juices by thin-layer chromatography. J. Planar Chromatogr. 23, 14-17 (2010). TLC of vitamin C of different concentrations (0.45, 0.50, 0.55, 0.60, and 0.65 mg/mL) and juice (e. g. orange with grapefruit, orange with apple and carrot, „multi-fruit“) mixed with a methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) on silica gel. Images of plates were evaluated using a specific computer software. The equivalence of the results obtained using the procedure described was demonstrated by applying the traditional UV-visible spectrophotometry.

*Note from editor: The title is misleading. This is not TLC as there is no chromatography at all. Silica gel is used as carrier and not as stationary phase for a separation.*

food analysis, qualitative identification 27

## 28. Antibiotics, Mycotoxins

105 041 M. BROSZAT, C. WELLE, M. WOJNOWSKI, H. ERNST, B. SPANGENBERG* (*University of Offenburg, Institute of Process Engineering, Badstrasse 24, 77652 Offenburg, Germany; Spangenberg@FH-Offenburg.de): A versatile method for quantification of aflatoxins and ochratoxin A in dried figs. J. Planar Chromatogr. 23, 193-197 (2010). HPTLC of ochratoxin A, aflatoxin B1, G1, B2, and G2 on silica gel with t-butyl methyl ether - water - methanol - cyclohexane 48:1:2:1 in an unsaturated horizontal developing chamber and on RP18 with methanol - 4 % aqueous zinc sulfate solution - ethyl methyl ketone 5:5:1. After development the silica gel plate was dipped for 2 s in silicone oil - hexane 1:2 which enhanced aflatoxin fluorescence by a factor of 2 and ochratoxin A fluorescence by a factor of 3-10. RP18 plates were developed to a distance of 75 mm in an unsaturated vertical chamber. Averaged densitograms were obtained in the emission wavelength range from 445 to 485 nm. Sample pretreatment was by modified QuEChERS (Quick, Easy; Cheap, Effectice, Rugged, Safe) extraction with tetahydrofuran or acetone. Linearity was

in the range of 3 to 100 pg/zone for aflatoxins B2 and G2, 10 to 350 pg/zone for aflatoxins B1 and G1, and 0.25 to 2.5 ng/zone for ochratoxin A. LOQ for the aflatoxins were between 13 and 35 pg/zone (equivalent to to 1.5 and 2.5 ppb); for ochratoxin A it was 970 pg/zone (56 ppb).

toxicology, HPTLC, densitometry, quantitative analysis 28b

105 042 Michele HOELTZ*, J. WELKE, I. NOLL (*Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91570-901 Porto Alegre - RS, Brasil, michelehoeltz@yahoo.com.br): Photometric procedure for quantitative analysis of aflatoxin B1 in peanuts by thin-layer chromatography using charge coupled device detector. Quim. Nova 33, 43-47 (2010). HPTLC of aflatoxin B1 in peanuts on silica gel with chloroform - acetone 99:1. Quantitative determination by absorbance measurement at 366 nm, using a CCD camera followed by image processing using the software ImageJ. Linearity was between 0.8 and 4.8 ng/zone. The intra-day and inter-day precisions had a *RSD* lower than 5.2 %. LOD was 0.4 ng/zone while LOQ was 1.2 µg/kg. The average recovery was 94.9 %. The proposed method is a simple, efficient and low cost tool for quantitative analysis of aflatoxin B1 in peanut samples.

food analysis, HPTLC, quantitative analysis, densitometry 28b

105 043 Juliane WELKE, M. HOELTZ, H.A. DOTTORI, I.B. NOLL (*Instituto de Ciencia e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Av. Bento Goncalves, 9500, 91570-901, Porto Alegre, RS, Brasil; juliwelke@yahoo.com.br): Rapid, simple, and economical method for quantification of ochratoxin A in red wine. J. Planar Chromatogr. 23, 116-118 (2010). HPTLC of ochratoxin A on silica gel with toluene - ethyl acetate - formic acid 6:3:1 in a saturated chamber. Detection by spraying with ethanolic sodium bicarbonate solution (6 g sodium hydrogen carbonate, 100 mL water, 20 mL ethanol); after drying, evaluation and videodensitometry under 366 nm. The linear regression coefficient of the calibration for OTA in the concentration range 0.8 to 12 ng/zone was 0.9992. The mean recovery was 92 +- 8.9 %. Recovery from wine samples at levels of 0.5, 2, and 5 µg/L was 84, 90, and 102 %, respectively, and the respective relative standard deviations were 5.7, 8, and 7 %. LOD was 0.32 ng/spot and LOQ 0.1 µg/L.food analysis, HPTLC

quantitative analysis 28b

105 044 Juliane WELKE*, Michele HOELTZ, H. DOTTORI, I. NOLL (*Instituto de Ciência e Tecnologia de Alimentos, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91570-901 Porto Alegre - RS, Brasil, juliwelke@yahoo.com.br) : Determination of ochratoxin A in wine by thin-layer chromatography using charge coupled device. J. Braz. Chem. Soc. 21, 441-446 (2010). HPTLC of ochratoxin A in wine on silica gel with toluene - ethyl acetate - chloroform - formic acid 6:3:1. Quantitative determination by absorbance measurement at 366 nm, using a CCD camera followed by images processing using the software ImageJ. Linearity was between 0.8 and 32 µg/L. The intra-day and inter-day precisions had a *RSD* lower than 9.9 % and 11.5 %, respectively. LOD was 16 ng/zone while LOQ was 100 ng/zone. The proposed method is a simple, efficient and low cost tool for quantitative analysis of ochratoxin A in wine samples.

food analysis, HPTLC, quantitative analysis, densitometry 28b

## 29. Pesticides and other agrochemicals

105 045 A. FITTLER*, B. KOCSIS, Z. MATUS, L. BOTZ (*Pharmaceutical Institute and Central Pharmacy, Faculty of General Medicine, University of Pécs, Honvéd u. 3., Pécs 7624, Hungary; andras.fittler@aok.pte.hu): A sensitive method for thin-layer chromatographic detection of amphotericin B. J. Planar Chromatogr. 23, 18-22 (2010). TLC of amphotericin B on silica gel with chloroform - methanol - borate buffer (pH 8.3) 4:5:1 in a chamber pre-saturated for 20 min. Detection under UV 366 nm. The $hR_f$ of the main component was 46, and of the minor component 31.

Quantitative determination by absorbance measurement at 385 nm. Direct bioautography with Candida albicans proved to be the most sensitive method, with a detection limit of 0.8 ng per spot. For densitometric evaluation of plates at 385 nm ten times more substance is required.

pharmaceutical research, quality control, densitometry, quantitative analysis, comparison of methods 29e

105 046 G. OROS, T. CSERHÁTI* (*Research Institute of Materials, and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary; tevi@chemres.hu): Relationship between the calculated physicochemical parameters and reversed phase thin-layer chromatographic retention behavior of carboxamide fungicides and related compounds. J. Liq. Chromatogr. Relat. Technol. 33, 880-893 (2010). TLC of 6 carboxamide fungicides and 11 phenylbenzamide derivatives on silica gel and aluminium oxide impregnated by overnight predevelopment in n-hexane - paraffin oil 19:1 with mixtures of methanol - water, acetonitrile - water, tetrahydrofurane - water, and acetone - water with the concentration of organic modifier varying in steps of 5 %. Detection under UV light. The RMO and b values related to the molecular lipophilicity and to the specific hydrophobic surface area (b) of the solutes were calculated separately for each RP-TLC system and for each analyte. The correlations between the physicochemical parameters measured were calculated by linear regression analysis.

environmental, qualitative identification 29e

105 047 T. TUZIMSKI (Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Lublin, 6 Staszica Street, 20-081 Lublin, Poland; tomasz.tuzimski@umlub.pl): New procedure for analysis of complex mixtures by use of multidimensional planar chromatography in combination with diode-array scanning densitometry and high-performance liquid chromatography coupled with diode-array detection. J. Planar Chromatogr. 23, 184-189 (2010). Multidimensional planar chromatography on monolayer or multiphase plates and modern fiber optical TLC densitometer scanners with DAD is especially useful for correct identification of components of difficult, complicated mixtures, e.g. pesticides in plant extracts (after preliminary clean-up and concentration by, e. g., solid-phase extraction). TLC of clofentezine [3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine] in Herba Thymi on silica gel in a horizontal chamber with tetrahydrofuran - n-heptane 3:7 in the first direction, then with ethyl acetate - n-heptane 1:4 in the second direction. Detection in the range of 200 to 600 nm with a TLC-DAD scanner. Also TLC of thyme herb extracts on silica gel and on RP18 plates with tetrahydrofuran - n-heptane 3:7 in the first direction and with methanol - water 7:3 in the second direction. LOD and LOQ were 0.23 and 0.70 µg/band, respectively, in TLC-DAD and 0.35 and 1.06 µg/mL, respectively, in HPLC-DAD. Average recoveries from the spiked plant material samples were 80.1 % and 100.5 % at 2.5 µg/g and 95.1 % at 5 µ/g measured at 202 nm.

agricultural, herbal, densitometry, quantitative analysis 29

105 048 R. ZAKRZEWSKI*, W. CIESIELSKI (*Department of Instrumental Analysis, University of Lódz, Pomorska 163, 90-236 Lódz, Poland; robzak@chemul.uni.lodz.pl): Thin layer chromatography with post-chromatographic iodine-azide reaction for thiuram analysis in food samples. J. Liq. Chromatogr. Relat. Technol. 31, 2657-2672 (2008). TLC and HPTLC of thiuram on silica gel with methanol or dichloromethane in a saturated horizontal chamber for 15 min. Detection by spraying with improved and non-improved iodine-azide, iodine, and copper(II) reagents and by evaluation under UV 254 nm. For derivatization the developed plates were sprayed with freshly prepared mixtures of sodium azide and starch solution adjusted to pH 6.0 (5 mL of 10 % aqueous sodium azide solution and 12.5 mL of 2 % aqueous starch solution were mixed and adjusted to pH 6.0 with 0.1 mol/L hydrochloric acid solution; the solution was diluted to 25 mL with water) and exposed to iodine vapor for 5 s. Quantitative analysis by scanning with an office scanner (PC scanner) and after detection with improved iodine-azide reagent densitometric measurement at

483 nm. The $hR_f$ value of thiuram was 29. LOD were 3 and 0.5 pmol per spot using a iodine-azide detection system in TLC and HPTLC, respectively. Linearity was in the range of 2-8 pmol per spot; the correlation coefficient r was 0.9981. Results obtained by use of a PC scanner were comparable to measurements using a TLC densitometer.

food analysis, postchromatographic derivatization, HPTLC, quantitative analysis, densitometry 29e

## 30. Synthetic and natural dyes

105 049 W. SCHWACK*, Elodie PELLISSIER (*University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany, wschwack@uni-hohenheim.de): Determination of unauthorised fat-soluble azo dyes in spices by HPTLC. CBS 103, 13-15 (2009). HPTLC of azo dyes (Sudan I, II, III, IV, B, Sudan orange G, Sudan red 7B, Para red) in spice samples on caffeine impregnated silica gel with isohexane - methyl ethyl ketone 5:1 with chamber saturation for 10 min. Densitometric absorption measurement at 390, 415, 500, 525 and 550 nm. The limits of detection were approx. 10 mg/kg. Confirmation of suspected compounds in samples by comparison of UV spectra. TLC-MS analysis in positive ESI mode further confirms positive findings.

food analysis, HPTLC, quantitative analysis, densitometry, TLC-MS 30a, 4e

105 050 R. SKIBINSKI*, L. KOMSTA (*Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland, robert.skibinski@am.lublin.pl): Validation of NP-HPTLC and RP-HPTLC methods with videodensitometric detection for analysis of ziprasidone in pharmaceutical formulations. J. Planar Chromatogr. 23, 23-27 (2010). HPTLC of ziprasidone (5-[2-[4-(1,2-benzothiazol-3-yl)piperazin-1-yl]ethyl]-6-chloro-1,3-dihydroindol-2-one) on silica gel with hexane - dioxane - propylamine 5:45:2 up to 9 cm (under saturated conditions) and on RP8 with tetrahydrofuran - phosphate buffer (pH 9.0) 1:1 up to 4.5 cm (under unsaturated conditions), both in horizontal chambers. Quantitative determination by videodensitometry at 254 nm. Calibration was linear in the range 0.2-1.2 and 0.1-1.1 µg/spot ziprasidone for NP-HPTLC and RP-HPTLC, respectively. The intra-day precisions for 0.4-1.2 µg/spot on NP-HPTLC was 2.0 to 5.2 % and on RP-HPTLC 4.0 to 6.1 %; the respective inter-day precision for NP-HPTLC was 2.0 to 6.7 % and for RP-HPTLC 4.1 to 7.1 %. LOD/LOQ on NP-HPTLC was 0.03/0.09 µg/spot; using RP-HPTLC, LOD/LOQ was 0.02/0.06 µg/spot. The specificity of the methods was confirmed by comparison of $hR_f$ values (74 +/- 2 in NP-HPTLC and 36 +/- 1 in RP-HPTLC, n=12).
quality control, HPTLC

densitometry 30a

105 051 B. YUANGSOI*, O. JINTASATAPORN, P. TABTHIPWON, C. KAMEL (*Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand; bundyu@kku.ac.th): Comparative pharmacokinetics after feeding fancy carp (Cyprinus carpio) with diets containing carotenoids from natural sources (tea (Camellia sinensis), mulberry (Morus alba), and cassava (Manihot esculenta) leaf). J. Planar Chromatogr. 23, 219-224 (2010). TLC of carotenoids (lutein, beta-carotene), astaxanthin and tannin on silica gel with petroleum ether - diethyl ether - acetone 15:3:2 in a twin-trough chamber saturated for 30 min at room temperature. Quantitative determination by densitometric absorbance measurement at 450 nm. The $hR_f$ values of lutein in tea, mulberry, and cassava leaf were 19, 22, and 19 and corresponded to lutein standard. The least polar zone had an average $hR_f$ value of 98, 98, and 96 for tea, mulberry, and cassava leaf, respectively, and was identical with beta-carotene standard.

food analysis, densitometry, quantitative analysis 30b

105 052 A. ZEB*, M. MURKOVIC (*Institute for Biochemistry, Graz University of Technology, Graz, Austria; Alamzeb01@yahoo.com): High-performance thin-layer chromatographic method for

monitoring the thermal degradation of ß-carotene in sunflower oil. J. Planar Chromatogr. 23, 35-39 (2010). HPTLC of beta-carotene on silica gel (prewashed with methanol) with petroleum ether - hexane - acetone 2:3:1 in a saturated twin-trough chamber. Quantitative determination by absorbance measurement at 450 nm. Linearity was between 100 and 600 ng/band. LOD and LOQ were 0.11 and 0.37 ng/band, respectively. Average intra-day precision and inter-day-precision were 0.54 % and 0.50 %, respectively.

food analysis, HPTLC, quantitative analysis, densitometry 30b

105 003 A. ZEB et al., see section 1

## 32. Pharmaceutical and biomedical applications

105 053 R. ARAVIND*, J. SAJAN, K. BINDU, A. BINDU (*Dept. of Pharmaceutical Science, Cheruvandoor Campus, Kottayam, Kerala, India): Quantitative determination of quercetin present in the leaves of Cinnamomum malabatrum (Burman) B using HPTLC method. Abstract No. 259, 61st IPC (2009). HPTLC of quercetin in alcoholic extracts of the leaves of Cinnamomum malabatrum on silica gel with toluene - acetone - formic acid 36:12:5. Quantitative absorbance measurement at 254 nm. The alcoholic extract was found to contain 10.02 mg/g of quercetin. The total flavonoid content was estimated using a colorimetric method with aluminum chloride. Results were in good correlation with the HPTLC method.

pharmaceutical research, quality control, herbal, HPTLC, comparison of methods, quantitative analysis 32e

105 083 Amelia M. AVACHAT*, S. B. BHISE (*Department of Pharmaceutics, Sinhgad College of Pharmacy, Off Sinhgad Road, Vadgaon (Bk.), Pune-411 041, India; prof_avachat@yahoo.com): Stability-indicating validated HPTLC method for simultaneous analysis of rifabutin and isoniazid in pharmaceutical formulations. J. Planar Chromatogr. 23, 123-128 (2010). HPTLC of rifabutin and isoniazid on silica gel with dichloromethane - acetone - methanol 20:7:2 in a twin-trough chamber previously saturated for 25 min. Quantitative determination by absorbance measurement at 504 nm for rifabutin ($hR_f$ 84) and at 262 nm for isoniazid ($hR_f$ 48). The linearity range was 10-70 and 5-35 µg/mL, the correlation coefficient was 0.9991 and 0.9989, the precision (*RSD*, $n = 6$) 0.90 % and 0.71 %, and precision on different days (*RSD*, $n = 3$) was 0.89 % and 1.01 %. LOD was 180 µg and 90 µg/zone, LOQ was 540 and 270 µg/zone, and the system suitability (*RSD*, $n = 6$) was 1.41 % and 1.86 % for rifabutin and isoniazid, respectively.

quality control, HPTLC, quantitative analysis, densitometry 32a

105 054 D. BAHETI*, P. SHINDE, M. AGRAWAL, R. BANGAR (*Sitabai Thite College of Pharmacy, Pune, Maharashtra, India): Quantitative estimation of withanolide A in marketed polyherbal spansules. Abstract No. C-372, 61st IPC (2009). HPTLC of withanolide A in extracts and spansules dosage form on silica gel with toluene - ethyl acetate - formic acid 8:6:1. Densitometric evaluation at 254 nm. Withanolide A was well separated with an $hR_f$ value of 14. The linearity range was 40-200 ng/band.

pharmaceutical research, quality control, herbal, densitometry, HPTLC, quantitative analysis 32e

105 055 G. BANSAL*, Reecha MADAAN, S. KUMAR (*S. D. College of Pharmacy, Barnala, Punjab, India): Pharmacognostic investigation on Actaea spicata L. Abstract No. C-274, 61st IPC (2009). TLC of petroleum ether and chloroform extracts of Actaea spicata roots on silica gel with n-hexane - chloroform 9:1 (for petroleum ether extracts) and toluene - ethyl acetate - glacial acetic acid 80:50:15:1 (for chloroform extracts). Detection by spraying with 50 % methanolic sulfuric

acid followed by heating at 105 °C. Petroleum ether extracts showed 2 bands, whereas chloroform extracts showed 3 bands.

quality control, herbal, qualitative identification 32e

105 056 Bharati BARMECHA*, D. GHANAWAT, P. SHINDE, Smita SHELKE (*Sitabai Thite College of Pharmacy, Pune, Maharashtra, India): Simultaneous determination of gallic acid and piperine by high-performance thin-layer chromatography. Abstract No. C-338, 61 IPC (2009). HPTLC of gallic acid and piperine in combined formulation on silica gel with toluene - ethyl acetate - formic acid 16:8:1. Quantitative absorbance measurement at 320 nm. The method was linear in the range of 200-800 ng/band for gallic acid and 50-350 ng/band for piperine.

pharmaceutical research, quality control, herbal, densitometry, HPTLC, quantitative analysis 32e

105 057 P. BHARATI, A. VINODINI. A. S. REDDY, P. S. DEVI* (Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, India; sitadevi@iict.res.in): Development and validation of a planar chromatographic method with reflectance scanning densitometry for quantitative analysis of anastrozole in the bulk material and in tablet formulations. J. Planar Chromatogr. 23, 79-83 (2010). HPTLC of anastrozole (2-[3-(1-cyano-1-methyl-ethyl)-5-(1H-1,2,4-triazole-1-yl-methyl)-phenyl]-2-methyl-propanenitrile) on silica gel (prewashed with methanol) with toluene - acetone - ammonia 60:40:3 in a twin-trough chamber previously saturated for 20 min. Quantitative determination by absorbance measurement at 200 nm. Recovery of anastrozole from 1mg tablet formulations ranged from 98.9 to 101.5 %. Intra-day and inter-day precision were 1.34 % and 1.59 %, respectively. LOD and LOQ were 71 and 214 ng/band. The correlation coefficient for the anastrozole calibration was 0.9983 over the range of 500-1500 ng/band (peak area).

quality control, densitometry, quantitative analysis, HPTLC 32a

105 058 K. BOBER (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, PL-41-200, Sosnowiec, Poland, bober@sum): Densitometric analysis of selected fluorquinolines. J. Liq. Chromatogr. Relat. Technol. 33, 778-785 (2010). TLC of ofloxacin and pefloxacin on silica gel with acetonitrile - formic acid - water 40:3:7 in chamber saturated for 30 min. Quantitative determination by densitometry at 295 nm for ofloxacin and at 280 nm for pefloxacin. The regression equations were achieved using computer program STATISTICA 7.1.

quality control, densitometry, quantitative analysis 32a

105 059 M. BOMBAYWALA*, D. MOHALE, A. CHANDEWAR (*P. WADHWANI College of Pharmacy, Yavatmal, Mah, India): Bioassay guided fractionation of Lagenaria siceraria for antihyperlipidemic activity. International Seminar on Herbal Drug Research, PN-009 (2009). Bioassay guided fractionation of Lagenaria siceraria was carried out on a silica gel column with solvents in ascending order of polarity. Each fraction obtained was subjected to preparative TLC on silica gel with n-butanol - methanol - water 3:1:1. Four bands with different Rf values were collected and active compounds were extracted and screened for antihyperlipidemic activity.

pharmaceutical research, traditional medicine, clinical chemistry research, herbal, preparative TLC 32e

105 060 A. BORKAR*, S. MULGUND, A. GAJBHAR, K. JAIN (*Sinhgad College of Pharmacy, Pune, Maharashtra, India): HPLC-PAD and HPTLC methods for quantitative and chromatographic fin-

gerprint analysis of Embella ribes (Vidanga) Churna formulation. Abstract No. F-10, 61st IPC (2009). HPTLC of Embella ribes Churna formulation on silica gel with chloroform - ethyl acetate - formic acid 5:4:1 in a twin trough chamber. Densitometric measurement of embelin at 291 nm. The method was linear in the range of 600-1800 ng/band with recovery value of 99.1-101.2 %. The formulation was also analyzed by HPLC and results were found to be comparable. p h a r - maceutical research, quality control

herbal, densitometry, HPTLC, comparison of methods, quantitative analysis 32e

105 061 P. CHANDRA*, A. RATHORE, L. SATHIYANARAYANAN, K. MAHADIK (*Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India): Development of validated HPLC and HPTLC methods for simultaneous determination of levocetirizine dihydrochloride and montelukast sodium in bulk drug and pharmaceutical dosage form. Abstract No. F-18, 61st IPC (2009). HPTLC of levocetirizine dihydrochloride and montelukast sodium in bulk and tablet dosage formulation on silica gel with toluene - ethyl acetate - methanol - 25 % ammonia 5:14:5:2. Both drugs were well resolved with $hR_f$ values of 31 for levocetirizine and 44 for montelukast. Quantitative evaluation at 231 nm. The method was linear in the range of 500-2500 ng/band for levocetirizine and 1000-5000 ng/band for montelukast. Both drugs were also analysed by HPLC on RP18 column and results were comparable with HPTLC.

pharmaceutical research, quality control, HPTLC, densitometry, comparison of methods 32a

105 062 K. DATTA, A. SINGH*, A. MUKHERJEE, B. BHAT, B. RAMESH, A. BURMAN (*Molecular Oncology Lab, Dabur Research Foundation, Sahibabad, India, singhat@dabur.com): Eclipta alba extract with potential for hair growth promoting activity. J. Ethnopharmacol. 124, 450-456 (2010). HPTLC fingerprinting of Eclipta alba on silica gel with chloroform - ethanol - water 35:10:2. Densitometric evaluation at 254 nm. Major compounds identified were coumestants and wedelolactone, with hair growth promoting activity.

traditional medicine, herbal, HPTLC, qualitative identification 32e

105 063 R. DEEPA*, K. MADHURI, R. SHANMUGAM, G. SADAGOBAN (*J.S.S. College of Pharmacy, Ooty, T.N., India): Estimation of harmaline in Peganum harmala by HPTLC. International Seminar on Herbal Drug Research, PN-011 (2009). HPTLC of harmaline in alcoholic extracts of Peganum harmala on silica gel with chloroform - acetone - diethyl amine 5:4:1 in a saturated chamber at 25 °C. The $hR_f$ of harmaline was 50. Quantitative determination by absorbance measurement at 351 nm.

pharmaceutical research, herbal, densitometry, quantitative analysis 32e, 17a

105 064 A. DHOBI*, N. VEKARIYA, G. PATEL, R. DHOLAKIYA, C. SHASHTRY (*Shree Dhanvantary Pharmacy College, Kim, Gujarat, India): Development and validation of analytical method for simultaneous determination of telmisartan and amlodipine besylate in bulk and tablets by HPTLC. Abstract No. F-159 61st IPC (2009). HPTLC of telmisartan and amlodipine besylate on silica gel with tetrahydrofuran - dichloroethane - methanol - 25 % ammonia 30:10:5:2. Both compounds were well resolved with $hR_f$ values of 22 and 45 for telmisartan and amlodipine besylate respectively. Densitometric evaluation at 326 nm. The method was found to be linear in the range of 1200-7200 ng/band for telmisartan and 400-1400 ng/band for amlodipine besylate.

quality control, densitometry, HPTLC, quantitative analysis 32a

105 065 Tatjana DJAKOVIC-SEKULIC*, V. DESPOTOVIC, G. USCUMLIC (*Department of Chemistry, Biochemistry, and Environmental Protection, University of Novi Sad, Faculty of Sciences, Trg

Dositeja Obradovica 3, 21000 Novi Sad, Republic of Serbia; tatjana.djakovic-sekulic@dh.uns.ac.rs): Quantitative structure-retention relationships study of the retention data of 5,5-disubstituted hydantoins. J. Planar Chromatogr. 23, 201-207 (2010). TLC and HPTLC of 17 5,5-disubstituted hydantoins on silica gel with ethyl acetate - toluene (with 30-60 % ethyl acetate) and acetonitrile - toluene (with 30-50 % acetonitrile) and on RP18 with methanol - water (with 56-80 % methanol) and acetonitrile - water (with 30-60 % acetonitrile) at room temperature without chamber saturation. Detection under UV 254 nm. The effect of the structures of the derivatives on their retention in both normal and reversed-phase modes was investigated by use of QSRR and molecular descriptors. Cross-validation indicated the best models are reliable QSRR models.

qualitative identification, postchromatographic derivatization 32a

105 066 N. DUBEY*, N. DUBEY, R. MEHTA, A. SALUJA (*Sophisticated Instrumentation Center for Applied Research and Testing, Vallabh Vidya Nagar, Gujarat, India and Devi Ahilya Vishwavidyalaya, School of Pharmacy, Indore, Madhya Pradesh, India; nidhidubeympharm@yahoo.com): Selective determination of aconitine in polyherbal oils containing Aconitum chasmanthum using high-performance thin-layer chromatography. J. AOAC Int. 93, 1617-1621 (2010). HPTLC of aconitine on silica gel with ethyl acetate - ethanol 3:1 at 22 °C in a saturated twin-trough chamber. The $hR_f$ value of aconitine was 33. Quantitative determination by absorption measurement at 238 nm. LOD and LOQ was 20 and 70 ng/band, respectively. The linearity with respect to peak area was in the range of 300 to 1800 ng/band with an r of 0.9991. The repeatability (*RSD*) was 0.85 %; and the inter-day and intra-day precision (*RSD*) was 1.01-1.38 and 1.04-1.34 %, respectively.

quality control, herbal, traditional medicine, HPTLC, densitometry, quantitative analysis 32e

105 067 S. DWIVEDI*, J. BORKAR, A. SAOJI, P. YEOLE (*Institute of Pharmaceutical Education and Research, Wardha, Maharashtra, India): Phytochemical screening and evaluation by TLC and UV spectrophotometer of polyherbal tablets. Abstract No. C-495, 61st IPC (2009). Screening of different phytoconstituents in a polyherbal tablet formulation. TLC of n-hexane, chloroform and methanol extracts of the tablets on silica gel with n-hexane - ethyl acetate 7:3; chloroform-methanol 9:1, and chloroform - glacial acetic acid - methanol - water 8:40:15:10. Evaluation under UV 254 nm as well as under UV 366 nm after spraying with different reagents: 20 % sulfuric acid, aniline-hydrogen phthalate reagent, anisaldehyde-sulfuric acid reagent, and vanillin-sulfuric acid reagent for the detection of piperine and andrographide, the active constituents present in formulations like Tefroliv Forte tablets. Other constituents (tannins etc.) were analyzed by UV spectrophotometry.

pharmaceutical research, quality control, herbal, densitometry, qualitative identification 32e

105 068 M. FAIYAZUDDIN*, J. ALI, S. AHMAD, N. AHMAD, J. AKHTAR, S. BABOOTA (*Formulation Research Laboratory, Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India, and Department of Pharmaceutics, Faculty of Pharmacy, Integral University, Lucknow-226026, Uttar Pradesh, India; md.faiyazuddin2008@g.mail.com): Chromatographic analysis of trans- and cis-citral in lemongrass oil and in a topical phytonanocosmeceutical formulation, and validation of the method. J. Planar Chromatogr. 23, 233-236 (2010). HPTLC of trans-citral and cis-citral in lemongrass oil on silica gel with toluene - ethyl acetate 17:3 in a twin-trough chamber saturated for 15 min (at 25 °C and 55 % RH). Detection by spraying with vanillin-sulfuric acid reagent. Quantitative determination by absorbance measurement at 595 nm. Intra-day and inter-day precision were evaluated by replicate ($n = 6$) analysis of samples (trans-citral at 450, 900, and 1800 ng/band, and cis-citral at 470, 940, and 1880 ng/band). The linear range was 225-3600 ng/band for trans-citral, and 470-3760 ng/band for cis-citral. The correlation coefficient r was 0.9933 for trans-citral and 0.9937 for cis-citral. Intra-day precision ($n = 6$) was < 3.56 and 5.66 % for trans- and cis-citral, respectively. Inter-day precision was assessed to be < 3.47 and 5.52 % for trans- and cis-citral by repeating the intra-day assay on three different days.

Repeatability of sample application and peak-area measurement was 0.98 %, determined by performing six replicate analyses of the same band (1800 ng/band trans-citral and 1880 ng/band cis-citral). The *RSD* of recovery of trans and cis-citral was in the ranges 1.36-3.25 and 1.64-3.47, respectively.

herbal, quality control, cosmetics, HPTLC, quantitative analysis, densitometry 32e

105 069 M. GANDHIMATHI*, M. SARAVANA KUMAR, R. BAGHLA (*College of Pharmacy SRIPMS, Coimbatore, TN, India): RP-HPTLC method for the in vitro estimation of edaravone in human plasma. IPA Convention, 2010, RA-PO 03. HPTLC of edaravone in samples of human plasma (purified by liquid-liquid extraction) on RP-18 with n-butanol - methanol - diethyl ether 1:8:1. The compound was well resolved with an $hR_f$ value of 81. Densitometric evaluation at 240 nm. The limit of detection and quantification was 25 ng and 150 ng respectively. The linearity was 600-2400 ng/band. Recovery (%) based on analysis of spiked sample was more than 65 %.
pharmaceutical research, clinical chemistry research, HPTLC, densitometry,

quantitative analysis 32c

105 070 A. GANTAIT, S. PANDIT, N. K. NEMA, P. K. MUKJERJEE* (*Jadavpur University, School of Natural Product Studies, Kolkata-700 032, India; naturalproductm@gmail.com): Quantification of glycyrrhizin in Glycyrrhiza glabra extract by validated HPTLC densitometry. J. AOAC Int. 93, 492-495 (2010). HPTLC of glycyrrhizin on silica gel with chloroform - methanol - water 130:72:15 in a twin-trough chamber saturated for 30 min. Detection under UV 254 nm and after spraying with anisaldehyde-sulfuric acid reagent. The $hR_f$ value of glycyrrhizin was 22. Quantitative determination by densitometry at 254 nm. The linearity was between 0.96-4.80 µg/spot, the correlation coefficient was r = 0.99904 and the standard deviation was 2.52 %. Average recovery was 99.6 %. LOQ and LOD was 246 and 81 ng/spot.

herbal, traditional medicine, HPTLC, densitometry, quantitative analysis 32e

105 071 Tatana GONDOVÁ*, D. HALAMOVÁ, K. SPACAYOVÁ (*Department of Analytical Chemistry, Faculty of Science, P. J. Safárik University, Moyzesova 11, Kosice SK-040 ß1, Slovak Republic; tatana.gondova@upjs.sk): Simultaneous analysis of new antidepressants by densitometric thin-layer chromatography. J. Liq. Chromatogr. Relat. Technol. 31, 2429-2441 (2008). TLC of citalopram, sertraline, fluoxetine, and fluvoxamine on silica gel with acetone - benzene - 25 % ammonia 10:9:1 in a twin-trough chamber saturated for 15 min. Detection under UV 254 nm. The $hR_f$ value was 28 for fluoxetine, 44 for citalopram, 56 for fluvoxamine, and 68 for sertraline (with a standard deviation less than 0.02 % in all cases). Quantitative determination by absorbance measurement at 240 nm. The calibration curve was linear in the range of 500-5000 ng/spot for all analyzed compounds; correlation coefficients were found to be more than 0.998 for all drugs (except the 0.991 for fluvoxamine). LOD was 40 ng/spot for citalopram and 50 ng/spot for fluoxetine, fluvoxamine, and sertraline, respectively. LOQ was found to be 130 and 160 ng/spot for citalopram and fluoxetine, respectively. Intra-assay precision (%*RSD*) was within the range of 0.52-0.87 % and 1.34-1.84 % for citalopram and fluoxetine, respectively, inter-day precision for the analyses conducted on three consecutive days was below 1.8 % and 2.5 % for citalopram and fluoxetine, respectively. The recoveries (*RSD*) of citalopram ad fluoxetine were found to be in the range of 99.3-100.3% (0.8 %) and 99.4-100.4 % (1.9%), respectively.

quality control, densitometry, quantitative analysis 32a

105 072 Anna GUMIENICZEK*, A, BERECKA (*Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland; anna.gumieniczek@umlub.pl): Quantitative analysis of glicazide and glipizide in tablets by a new validated and stability-indicating RPTLC method. J. Planar Chromatogr. 23, 129-133 (2010). TLC of glicazide and glipizide on RP18 silica gel with 60 % acetonitrile in

pH 2.3 phosphate buffer in an unsaturated horizontal chambers at room temperature. Detection and quantitative determination by absorbance measurement at 215 nm. Linearity was in the range of 0.8-1.8 µg/zone for both drugs and the correlation coefficients r were 0.998 for gliazide ($hR_f$ 38) and 0.993 for glipizide ($hR_f$ 51). LOD and LOQ were 50 and 200 ng/zone, respectively, for glicazide and 60 and 300 ng/zone for glipizide.

quality control, densitometry, quantitative analysis 32a

105 073 K.R. GUPTA*, M.R. TAJNE, S.G. WADODKAR (*Bhoyar College of Pharmacy, Near Dragon Palace Temple, New Kamptee-441 002, Dist: Nagpur (M.S.), India; krishnargupta@rediffmail.com): A validated high-performance thin-layer chromatographic method for the quantification of sertraline in tablets. J. Planar Chromatogr. 23, 134-136 (2010). TLC of sertraline on silica gel (prewashed with methanol) with chloroform - ethyl acetate - triethylamine 25:15:1 in a twin-trough chamber previously saturated for 10 min at room temperature. Quantitative determination by absorbance measurement at 279 nm. The $hR_f$ for sertraline was 40. Intra-day precision and inter-day precision was 99.84 % *RSD* and 99.92 % *RSD*, respectively. A good linear relationship between response (peak area) and amount was obtained over the range 2.7-7.9 µg/band.

quality control, HPTLC, densitometry, quantitative analysis 32a

105 074 D. HALAMOVA*, M. BADANICOVA, V. ZELENAK, T. GONDOVA, U. VAINIO (*Department of Inorganic Chemistry, Faculty of Science, P.J. Safarik University, Moyzesova 11, Slovac Republic, dasa.halamova @upjs.sk): Naproxen drug delivery using periodic mesoporous silica SBA-15. Appl. Surf. Sci. 256, 6489-6494 (2010). TLC of naproxen released from a SBA-15 mesoporous silica drug delivery system on silica gel with benzene - tetrachloromethane - acetic acid 7:1:1. Quantitative determination by absorbance measurement at 260 nm at different time intervals. The $hR_f$ value of naproxen was 50.

pharmaceutical research, densitometry, quantitative analysis 32a

105 075 Purnima HAMRAPURKAR*, S. PAWAR, M. PHALE (*Department of Pharmaceutical Analysis, Prin. K. M. Kundnani College of Pharmacy, Jote Joy Building, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai-400 005, India; phamrapurkar@gmail.com): Quantitative HPTLC analysis of phyllanthin in Phyllanthus amarus. J. Planar Chromatogr. 23, 112-115 (2010). HPTLC of phyllanthin on silica gel (prewashed with methanol) with hexane - toluene - ethyl acetate 2:2:1 in a twin-trough chamber saturated at 25-30 °C and 40-50 % relative humidity. Quantitative determination by absorbance measurement at 206 nm. LOD was 70 ng/mL, LOQ 200 ng/mL.The linear calibration range was 200-1200 ng/mL. Repeatability (*RSD*, $n = 3$) was 0.18-0.59 % with a correlation coefficient of 0.999. Intra-day and inter-day precision studies showed the CV was less than 2.0 %, indicating the method was precise: %*RSD* at 200, 600, and 1200 ng/mL was between 0.43 and 1.51 % for intra-day precision, and between 0.59 and 1.73 % for inter-day precision. The intra-day recovery for 200, 600 and 1200 ng/mL was between 102.3 % and 99.9 %, and the inter-day recovery between 102.5 % and 99.9 %, respectively.

traditional medicine, herbal, HPTLC, densitometry, quantitative analysis 32e

105 076 R. JAYAPRAKASAM*, M. SASIKALA, M.. GANDHIMATHI, M. SUKUMAR, T. RAVI (*College of Pharmacy, Sri Ramkrishna Inst. of Para Med. Science, Coimbatore, T.N., India): HPLC & HPTLC fingerprinting and in-vitro antioxidant studies of various extracts, isolated compounds and formulations of Eugenia jambolana Lam. International Seminar on Herbal Drug Research, PN-013 (2009). HPTLC of ethyl acetate extracts of seeds of Eugenia jambolana on silica gel with chloroform - acetone - formic acid 150:33:17. The antioxidant activity of different extract was assessed by DPPH method. The proposed method was applied to plant extract as well as to polyherbal formulation. The ethyl acetate extract was found to have good antioxidant activity. For

HPLC fingerprint profiling a RP18 column was used with 80 % methanol as mobile phase.

herbal, comparison of methods, qualitative identification 32e

105 077 A. JOHNSON, A. KUMAR, S. RASHEED, S. CHANDRIKA, A. CHANDRASEKHAR, S. BABY*, A. SUBRAMONIAM (*Phytochemistry and Phytopharmacology Division, Tropical Botanical Garden and Research Institute, Pacha-Palode, Kerala, India, sabulal@gmail.com): Antipyretic, analgesic, anti-inflammatory and antioxidant activities of two major chromenes from Melicope lunu-ankenda. J. Ethnopharmacol. 130, 267-271 (2010). HPTLC of evodione and leptonol from the leaves and influorescences of Melicope lunu-ankenda on silica gel with chloroform - methanol 1:1. Detection by spraying with anisaldehyde - sulfuric acid reagent, followed by heating at 105 °C for 5 min. Quantitative determination by absorbance measurement at 580 nm.

traditional medicine, herbal, HPTLC, quantitative analysis, densitometry 32e

105 078 V. KADAM*, Varsha JADHAV, Sapana KAMBLE, A. PAHADE (*Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, Maharashtra, India): Development and validation of HPTLC method for determination of 3-hydroxy androstane (16,17-C) (6-methyl 2'-1-hydroxy-isopropene-1-yl)-4,5,6 H-pyran in herbal formulation. Abstract No. C-231, 61st IPC (2009). An HPTLC method is reported for determination of 3-OH-androstane-(16,17-C) (6-methyl-2-1-hydroxy-isopropene-1-yl)-4,5,6 H-pyran, a phyto constituent of Eugenia jambolana. The compound was isolated by ethanolic extraction, identified by melting point, IR, and NMR, and used as marker. HPTLC on silica gel with toluene - ethyl acetate 17:3. Densitometric evaluation at 366 nm. The method was linear in the range of 1000-5000 ng/band. It can be used for routine quality control of Eugenia jambolana seeds and herbal formulation.

pharmaceutical research, quality control, herbal, HPTLC, quantitative analysis 32e

105 079 A. KHATIB*, A. C. HOEK, S. JINAP, M. Z. I. SARKER, I. JASWIR, R. VERPOORTE (*Center of Excellence for Food Safety Research, Faculty of Food Science and Technology, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malysia; alfikhatib@hotmail.com): Application of two dimensional thin layer chromatography pattern comparison for fingerprinting the active compounds in the leaves of Vitex trifolia Linn possessing anti-tracheospasmolytic activity. J. Liq. Chromatogr. Relat. Technol. 33, 214-224 (2010). TLC of ethanolic extracts of the leaves of Vitex trifolia on silica gel with chloroform - methanol 9:1 in the first direction and ethyl acetate - chloroform - methanol 7:7:11 in the second direction. Detection under UV light at 254 and 366 nm and by spraying with anisaldehyde-sulfuric acid reagent.

traditional medicine, herbal 32e

105 080 V. KUMAR*, S. VARGHESE, H. JOHN (*Dept. of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore, T.N., India): Development of validated HPTLC & HPLC methods for estimation of citicoline sodium in tablet dosage form. IPA Convention, 2010, RA-PO 35. HPTLC of citicoline sodium in tablet formulation on silica gel with chloroform - methanol - water 3:7:3. The compound was well resolved with an $hR_f$ value of 53. Densitometric measurement at 280 nm. The method was linear in the range of 300-900 ng/band. HPLC analysis was performed on RP18 column using 1 % formic acid - methanol 19:1. Results obtained with either method were comparable.

pharmaceutical research, quality control, comparison of methods, quantitative analysis, densitometry 32a

105 081 P. LAHORKAR*, K. RAMITHA, V. BANSAL, D.B. ANANTHA NARAYANA (*Gerbal Research Laboratory, Hindustan Unilever Research Centre, 64 Main Raod, Whitefield, Bangalore

560066, India): A comparative evaluation of medicated oils prepared using ayurvedic and modified processes. Ind. J. Pharma. Science 71 61, 656-662 (2009). Medicated oils prepared both by Ayurvedic as well as modified process were evaluated for fingerprint profiling by HPLC and HPTLC. HPTLC of methanolic extracts of the oils on silica gel with chloroform methanol 9:1 and toluene - ethyl acetate 4:1 for general fingerprint profiling; with toluene - ethyl acetate - formic acid 5:4:1 for flavonoids and with toluene - ethyl acetate - diethyl amine 7:2:1 for alkaloids. Evaluation under 254 nm and 365 nm. Detection by treatment with NP-PEG reagent for flavonoids and Dragendorff reagent for alkaloids.

pharmaceutical research, traditional medicine, herbal, postchromatographic derivatization, qualitative identification, HPTLC 32e

105 082 B. LUKAS, C. SCHMIDERER, U. MITTEREGGER, J. NOVAK* (*Institute of Applied Botany and Pharmacognosy, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria, Johannes.Novak@vu-wien.ac.at): Arbutin in marjoram and oregano. Food Chem. 121, 185-190 (2010). TLC of arbutin in the leaves and flowers of Origanum majorana and Origanum vulgare on silica gel with ethyl acetate - methanol - water 77:13:10. Detection by spraying with 2.5 % dibromchinochlorimide solution in ethanol. The $hR_f$ of arbutin was 47.

food analysis, herbal, qualitative identification 32e

105 084 Ágnes M. MÓRICZ*, E. TYIHÁK, P.G. OTT (*Plant Protection Institute, Hungarian Academy of Sciences, Herman O. u. 15, 1022 Budapest, Hungary; moricz_am@nki.hu): Usefulness of transgenic luminescent bacteria in direct bioautographic investigation of chamomile extracts. J. Planar Chromatogr. 23, 180-183 (2010). TLC of chamomile extracts on silica gel with benzene - ethyl acetate 19:1 or chloroform - methanol - water 40:10:1 in an unsaturated chamber. Detection at 254 and 366 nm. For bioautographic evaluation bioluminescent Bacillus subtilis or Pseudomonas syringae pv. maculicola were used; visualization by dye a reagent was achieved by dipping the plate in an aqueous solution of MTT. Quantitative determination by densitometric scanning at 300 nm (before biological detection) or at 590 nm (after visualization of the bioautogram with MTT).

quality control, herbal, traditional medicine, qualitative identification, densitometry, quantitative analysis, bioautography 32e

105 085 A. MAKOWSKI*, E. ADAMEK, W. BARAN (*Medical University of Silesia, Faculty of Pharmacy, 4 Jagiellonska Street, 41-200 Sosnowiec, Poland; makowski.andrzej@gmail.com): Use of photocatalytic reactions to visualize drugs in TLC. J. Planar Chromatogr. 23, 84-86 (2010). TLC of 18 drugs (6 antibiotics [benzyl-penicillin procaine, benzyl-penicillin potassium, penicillic acid, tetracycline hydrochloride, oxytetracyline hydrochloride, chlortetracycline hydrochloride], 2 analgesics [aminophenazone, salicylamide], 2 anaesthetics [phenazone, procaine hydrochloride], and one each of anti-rheumatic [penicillamine], anti-inflammatory [metamizole sodium], antitussive [codeine phosphate], broncholytic [aminophylline], spasmolytic [papaverine hydrochloride], hypnotic [phenobarbital], sympathomimetic [ephedrine hydrochloride] and vitamin [ascorbic acid] drugs) on silica gel with butanol - anhydrous acetic acid - water 3:1:1. Detection by spraying with 10 mL each of a solution of (A) 0.25 g titanum dioxide in 0.1 mol/L potassium permanganate, (B) 0.25 g titanium dioxide in 1.0 mol/L potassium iodide, (C) 0.25 g titanium dioxide in 1.0 mol/L potassium bromide, which was the best (most sensitive) one, and (D) 0.25 g titanium dioxide in 1.0 mol/L potassium chloride. After spraying with reagents C and D, plates were illuminated for 10 min, sprayed with 0.1 mol/L silver nitrate solution and illuminated again for 3 min. In all experiments the TLC plates were illuminated by use of UV lamps with the radiation at 366 nm. LODs for most of the drugs studied were in the range 0.2-0.5 µg/spot.

qualitative identification 32a

105 086 Astha MEHTA*, A. THAKER (*Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, NMIMS University, Vile Parle-W Mumbai-400056, India; astha2212@gmail.com): Validated HPTLC method for assay of prednisolone in tablets and comparison with pharmacopoeial methods. J. Planar Chromatogr. 23, 208-211 (2010). HPTLC of prednisolone (and hydrocortisone as impurity) on silica gel (prewashed with methanol) with chloroform - methanol 19:1 in a twin-trough chamber previously saturated for 30 min. Quantitative determination by scanning densitometry at 250 nm. Linearity was in the range of 2-10 µg/band (r = 0.9967, calculated via peak area). LOD and LOQ was 200 and 600 ng/spot, respectively. Recovery was 100.0 % for prednisolone. Repeatability was 0.74 % and the inter-day ($n$ = 6) and intra-day ($n$ = 12) precision was 2.6 and 2. 7 %, respectively.

quality control, comparison of methods, densitometry, HPTLC, quantitative analysis 32a

105 087 B. MEHTA*, S. MORGE (*Dept. of Chem. University of Mumbai, Santacruz (E), Mumbai, 400098, India): Simultaneous determination of irbesartan and hydrochlorothiazide by HPTLC method. Indian Drugs 47(2), 71-74 (2010). HPTLC of irbesartan and hydrochlorothiazide on silica gel with acetone - chloroform - ethyl acetate - methanol 6:6:6:1. The plates were preconditioned for 10 min in a saturated chamber prior to development. The $hR_f$ value of irbesartan was 27 and of hydrochlorothiazide 37. The linearity was 1500-9000 ng/band and 125-750 ng/band for irbesartan and hydrochlorothiazide respectively. The average recovery for both drugs was 99.4-99.5 %.

pharmaceutical research, quality control, densitometry, quantitative analysis 32a,17c

105 088 Sigrid MENNICKENT*, J. CONTRERAS, C. REYES, M. VEGA, M. DE DIEGO (*Department of Pharmacy, Faculty of Pharmacy, University of Concepción, P. O. Box 237, Concepción, Chile; smennick@udec.cl): Validated instrumental planar chromatographic method for quantification of fluphenazine hydrochloride in injections. J. Planar Chromatogr. 23, 75-78 (2010). HPTLC of fluphenazine hydrochloride on silica gel (prewashed with methanol) with methanol - water 9:1 in a saturated twin-trough chamber. Quantitative determination by absorbance measurement at 306 nm. Linearity was in the range of 100 to 500 ng/µL with a correlation coefficient of 0.998. LOD and LOQ were 1.45 and 4.40 ng/zone, respectively. Intra-assay and inter-assay precision, expressed as relative standard deviation (*RSD*), were in the range 0.73-1.77 % ($n$ = 3) and 1.18-1.86 % ($n$ = 9), respectively. Recovery of fluphenazine hydrochloride was between 98.3 and 101.5 %, with *RSD* not higher than 1.87 %. The method was selective for fluphenazine hydrochloride and the preservatives in the injections.

quality control, HPTLC, densitometry, quantitative analysis 32a

105 089 E. MINCSOVICS*, N. TABANCA, Á. M. MÓRICZ, D. E. WEDGE, E. TYIHÁK (*OPLC-NIT Ltd, Andor Street 60, 1119 Budapest, Hungary, and Corvinus University, Faculty of Horticultural Sciences, Dept. Genetics and Plant Breeding, Budapest, Hungary; emil-mincsovics@t-online.hu): Preliminary investigation of Origanum onites essential oil by overpressured layer chromatography and BioArena. J. Planar Chromatogr. 23, 225-226 (2010). OPLC of oregano oil components (carvacrol, thymol, and linalool) on silica gel with dichloromethane. Detection under UV 254 nm, by spraying with vanillin-sulfuric acid reagent (0.1 g vanillin, 100 mL ethanol, and 2.2 mL 95-98 % sulfuric acid) and heating at 110 °C for 3 min, and in the BioArena system (the dried developed plates were dipped for 10 s into an aqueous cell suspension of the soil bacteria Bacillus subtilis and incubated for 2 h at 100 % rel. humidity and 30 °C). Visualizion of antimicrobial compounds was performed by immersing the plates for 5 s in an aqueous solution of MTT reagent (80 mg MTT and 100 mg Triton X-100 in 100 mL water). The layers were further incubated and and documented.

herbal, food analysis, traditional medicine, qualitative identification 32e

105 090 H. MISTRY*, S. SHUKLA, N. PRAJAPATI, B. JOGI (*Institute of Science and Technology for Adavanced Studies and Research, Gujarat, India): Standardization of Panchkol Churna by HPTLC method for the determination of piperine, plumbagine and zingiberine. Abstract No. C-258, 61st IPC (2009). HPTLC of piperine, plumbagine and zingiberine in Panchkol Churna, an ayurvedic preparation used for anorexia, distension and abdominal pain. HPTLC on silica gel with toluene - ethyl acetate 7:3. Densitometric evaluation at 340 nm for piperine, and at 420 nm for plumbagine and zingiberine. The $hR_f$ value of piperine, zingiberine and plumbagine was 31, 75, and 84.

pharmaceutical research, quality control, herbal, densitometry, HPTLC, quantitative analysis 32e

105 091 P. NIRALI*, K. MANVITHA, K. SALMA, A. SHABARAYA (*Srinivas College of Pharmacy, Mangalore, India): Determination of andrographolide in Andrographis paniculata extracts with and without human serum by HPTLC. Abstract No. C-161, 61st IPC (2009). An HPTLC method is reported for estimation of andrographolides bitter principles in Andrographis paniculata, popularly known as kalmegh. HPTLC of methanolic and water extracts on silica gel with chloroform - methanol 7:1 in a saturated twin trough chamber. Quantitative evaluation by absorbance measurement at 231 nm. The method was found to be linear in the range of 1-5 µg/band. Both extracts were found to contain andrographolides. Maximum yields of andrographolides were observed in extracts prepared by refluxing.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 32e

105 092 A. ÖZTUNC*, A. ÖNAL, S. E. TOKER (*Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, Turkey; aoztunc@istanbul.edu.tr): Detection of methamphetamine, methylenedioxymethamphetamine, and 3,4-methylenedioxy-N-ethylamphetamine in spiked plasma by HPLC and TLC. J. AOAC Int. 93, 556-561 (2010). TLC of methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) on silica gel with hexane - chloroform 1:9 and HPTLC on cyano phase with benzene - diethyl ether - petroleum ether (40-60 °) - acetonitrile - ethyl methyl ketone 4:7:7:1:1 with chamber saturation for 30 min. The $hR_f$ value of MA, MDMA, and MDAE on silica gel were 28, 23, and 36, repectively, and on cyano phase 35, 30, and 40, respectively. LOD on silica gel were 0.8, 0.6, and 1.2 µg/mL (in plasma) for MA, MDMA, and MDEA respectively.

doping, toxicology, HPTLC 32a

105 093 H.A. PANAHI*, A. RAHIMI, E. MONIRI, A. IZADI, M. M. PARVIN (*Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran, Iran; panahi20002000@yahoo.com): HPTLC separation and quantitative analysis of aspirin, salicylic acid, and sulfosalicylic acid. J. Planar Chromatogr. 23, 137-140 (2010). HPTLC of aspirin, salicylic acid, and sulfosalicylic acid on silica gel (prewashed with methanol-chloroform 1:1 and impregnated with 2 % boric acid in ethanol) with chloroform - methanol - ammonia - water 120:75:2:6 in a chamber previously saturated at 25 °C for 30 min. Detection and quantitative determination by densitometry at 254 nm. The $hR_f$ of aspirin, salicylic acid, and sulfosalicylic acid were 81, 61, and 24, respectively. The linear range was 100-1000 ng/band for all three compounds, and the correlation coefficients r were 0.97, 0.94, and 0.95, respectively. LOQ were 123, 95, and 61 ng/band, respectively, and the respective LOD were 37, 37, and 18 ng/band.

quality control, HPTLC, quantitative analysis, densitometry 32a

105 095 N. PATEL*, D. MODI, B. SHAH, P. RACHH (*Vidyabharati Trust College of Pharmacy, Surat, Gujarat, India): Estimation of ellagic acid in Eugenia jambolana Lam seed alcoholic extract by HPTLC method. Abstract No. C-497, 61st IPC (2009). HPTLC of ellagic acid in seeds of Eu-

genia jambolana Lam on silica gel with ethyl acetate - glacial acetic acid - formic acid - water 100:11:11:27. Densitometric evaluation at 254 nm. The method was linear in the range of 200-1200 ng/band. The alcoholic seed extract contained 11.03 % of ellagic acid and 21 % of total tannin (measured by chemical method).

pharmaceutical research, quality control, herbal, densitometry, HPTLC, quantitative analysis 32e

105 094 Prachi PATEL (M. P. Patel College of Pharmacy, Kheda, Gujarat, India): Quantification of vasicine and piperine in polyherbal formulation. Abstract No. C-47, 61st IPC (2009). HPTLC of piperine and vasicine in polyherbal cough formulations on silica gel with dioxane - toluene - ethyl acetate - methanol - 25 % ammonia 15:20:10:10:3. Quantitative determination by absorbance measurement at 304 nm. The method was validated for accuracy, precision, LOD, LOQ, linearity and specificity and was found to be linear in the range of 2-10 µg/band for both vasicine and piperine.

pharmaceutical research, quality control, herbal, HPTLC, densitometry 32e

105 096 S. PATHAN*, S. ALAM, G. JAIN, S. ZAIDI, S. AKHTER, D. VOHORA, R. KHAR, F. AHMAD (*Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, New Delhi, India,shadab.ahmad1@gmail.com): Quantitative analysis of safranal in saffron extract and nanoparticle formulation by a validated high-performance thin-layer chromatographic method. Phytochem. Anal. 21, 219-223 (2010). HPTLC of safranal in saffron extract and in a safranal-loaded nanoparticle formulation on silica gel with n-hexane - ethyl acetate 9:1. Quantitative determination by absorbance measurement at 310 nm. The $hR_f$ of safranal was 51. Linearity was between 0.5 and 5.0 µg/zone. The intra-day and inter-day precisions were 1.08-2.17 and 1.86-3.47 %, respectively. LOD was 50 ng/zone while LOQ was 150 ng/zone. The average recovery was 99.9 %. The proposed method provides significant advantages in terms of greater specificity and rapid analysis.

pharmaceutical research, herbal, HPTLC, densitometry, quantitative analysis 32a

105 097 R. PIETRAS*, D. KOWALCZUK (*Department of Medicinal Chemistry, Medical University of Lublin, 4 Jaczewskiego Str., 20-090 Lublin, Poland; rafal.pietras@umlub.pl): RP-TLC separation of antiarrhythmic drugs. Densitometric analysis of flecainide in tablets. J. Planar Chromatogr. 23, 65-69 (2010). TLC of some antiarrhythmic compounds (disopyramide phosphate, verapamil hydrochloride, flecainide acetate, mexiletine hydrochloride, and tocainide hydrochloride) on RP8 and RP18 silica gel with organic-aqueous mobile phases containing citrate or acetate buffers at different pH in a horizontal chamber. The best separation of individual and mixed drug standards was achieved with tetrahydrofuran - citrate buffer (pH 4.45) 3:7. Flecainide acetate was identified and quantified by UV densitometry at 225 and 310 nm. Linear relationships were obtained between peak height or peak area and amount in the range 6.0 to 12.0 µg/spot, the correlation coefficient r was 0.990. Precision (*RSD* 1.1-5.9 %) and accuracy (96.2-103.6 %) were satisfactory.

pharmaceutical research, quality control, densitometry, quantitative analysis 32a

105 098 S. PRASAD*, S. HEMALATHA, T. THITE, M. KRISHNAN (*Dept. of Pharmaceutics, Institute of Technology, Banaras Hindu Univ., Varanasi, U.P., India) : Identification and quantification of withaferin-A in different fractions of Withania coagulans dunal by TLC and HPTLC method. Abstract No. C-97, 61st IPC (2009). Chromatographic methods are reported for identification (TLC) and quantification (HPTLC) of withaferin-A in methanolic and chloroform extract of dried fruits of Withania coagulans. Chromatographic separation on silica gel with toluene - ethyl acetate - formic acid 5:5:1. The identification of withaferin-A in both chloroform and methanolic extracts was performed by comparison of $hR_f$ values and UV absorbance maxima (209

nm). Quantification was performed by absorbance measurement at 540 nm after spraying the developed plate with Liebermann-Burchard reagent. Methanolic extracts and chloroform extracts contained 3.67 mg/g and 2.10 mg/g of withaferin-A, respectively. No withaferin-A was found in hydroalcoholic extracts.

pharmaceutical research, quality control, densitometry, HPTLC, quantitative analysis 32e

105 099 Alina PYKA*, D. RUSEK, P. BOCHENSKA, D. GURAK (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, PL-41-200 Sosnowiec, Poland; alinapyka@wp.pl): Use of RP-TLC and theoretical computational methods to compare the lipophilicity of salicylic acid and its derivatives. J. Liq. Chromatogr. Relat. Technol. 33, 179-190 (2010). TLC of salicylic acid and its derivatives, namely acetylsalicylic acid, salicylanilide, salicylaldehyde, salicylamide, salicylhydroxamic acid, methyl salicylate, phenyl salicylate, 3,5-dinitrosalicylic acid, 2,5-dihydroxysalicylic acid, 3-aminosalicylic acid, 4-aminosalicylic acid, and 5-aminosalicylic acid, on RP8, RP18 and HPTLC on RP18 and cyano phase with methanol - water; the content of methanol in mobile phase was gradually varied by 5 % from 20-100 %. Development in a chamber saturated for 15 min. Quantitative determination by scanning densitometry in absorption mode at the respective absorption maximum. The $hR_f$ values were recalculated on the RM values. The chromatograms were repeated in triplicate and mean $hR_f$ values were calculated. The results indicate that the chromatographic parameter of lipophilicity determined on RP8 and cyano phase may be used as a measure of lipophilicity of the investigated salicylic acid and its derivatives.

HPTLC, quantitative analysis, densitometry 32a

105 100 Alina PYKA*, P. BOCHENSKA (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, PL-41-200 Sosnowiec, Poland; apyka@sum.edu.pl): Comparison of NP-TLC and RP-TLC with densitometry to quantitative analysis of ibuprofen in pharmaceutical preparations. J. Liq. Chromatogr. Relat. Technol. 33, 825-836 (2010). NP-TLC of ibuprofen standard and in tablet extracts on silica gel (prewashed with methanol) with n-hexane - ethyl acetate - acetic acid 150:50:7 and RP-TLC on RP18 with methanol - water 9:1 in a saturated twin-trough chamber. Quantitaitve determination by absorbance measurement at 200 and 224 nm for NP-TLC and RP-TLC analysis, respectively. The $hR_f$ values were 61 and 67. Linearity was between 2.50-12.50 and 2.50-12.50 µg/spot, the correlation coefficient (r) was 0.998 and 0.994. LOD was 0.60 and 1.00 µg/spot, and LOQ 12.50 and 12.50 µg/spot. Recovery was 98.2 % and 96.1 %, with a standard deviation of 1.21 and 1.45, and *RSD* 1.28 and 1.81 for NP-TLC and RP-TLC, respectively.

quality control, densitometry, quantitative analysis 32a

105 101 BHARGAVI, R. SHANMUGAN*, K. MADHURI (*Vels college of Pharmacy, Dept. of Pharmaceutics, Chennai, India): Estimation of withanolide-A in nicandra physaloides by HPTLC. International Seminar on Herbal Drug Research, PN-008 (2009). HPTLC of withaferin-A from Nicandra physaloides on silica gel with toluene - ethyl acetate - formic acid 10:3:1. The $hR_f$ value was 14. Quantitative determination by absorbance measurement at 213 nm.

pharmaceutical research, densitometry, quantitative analysis 32e

105 102 Tirumala RAJESH*, K.S. LAKSHMI, S. SHARMA, P. D. REDDY, S. LAKSHMI (*Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur-603203, Tamil Nadu, India, rajeshtirumala@hotmail.com): Use of a validated stability-indicating HPTLC method to study the degradation of rimonabant. J. Planar Chromatogr. 23, 148-155 (2010). HPTLC of rimonabant and degradation products on silica gel in a horizontal chamber with methanol - water 7:3. A compact band was obtained at $hR_f$ 71. Quantitative determination

by absorbance measurement at 250 nm. Linear regression analysis of calibration data revealed good linear relationship with r = 0.9985 in the linear working range of 100-800 ng/band.

quality control, HPTLC, densitometry, quantitative analysis 32a

105 103 T. RAJKUMAR*, B. SINHA (*Dept. of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Jharkhand, India): Chromatographic fingerprint analysis of budmunchiamines in Albizia by HPTLC technique. Abstract No. C-453, 61st IPC (2009). Fingerprint analysis of budmunchiamines, the main constituents in Albizia amara. Dried powdered leaves were extracted with petroleum ether (60-80 °C), chloroform, ethyl acetate and 90 % methanol by maceration for 48 h. TLC on silica gel with chloroform - methanol 19:1. Zones 2, 4 and 8 corresponded to the marcocylic alkaloids budmunchiamine A, B, and C, which was confirmed by FTIR, NMR and MS.

pharmaceutical research, quality control, herbal, densitometry 32e

105 104 J. RAO*, K. CHAUHAN, K. MAHADIK (*Poona College of Pharmacy, Bharati Vidyapeeth University, Pune, Maharashtra, India): Validated high-performance thin-layer chromatographic method for determination of diacerein in the presence of degradation products formed under ICH-recommended stress conditions. Abstract No. F-234 61st IPC (2009). A stability-indicating HPTLC method is reported for estimation of diacerein in the presence of its degradation products. HPTLC on silica gel with toluene - ethyl acetate - formic acid 50:30:1. The $hR_f$ value of diacerein was 39. Densitometric analysis at 254 nm. The method was found to be linear in the range of 100-600 ng/band. The method was validated regarding stability. The sample was subjected to different stress conditions (acid/base hydrolysis, oxidation, photolysis, thermal) and showed extensive degradation in alkaline medium and mild degradation under acidic and oxidative conditions. The method allowed separation of diacerein from different degradation products.

pharmaceutical research, quality control, densitometry, HPTLC, quantitative analysis 32a

105 105 Suparna ROY*, B. SINHA, MANIK (*Birla Institute of Technology, Ranchi, Jharkhand, India): Estimation of constituents from methanolic extract of Aloe vera by HPTLC technique. Abstract No. C-37, 61st IPC (2009). HPTLC of methanolic leaf extracts of Aloe vera (after purification with petroleum ether (60-80 °C)) on silica gel with toluene - ethyl acetate - glacial acetic acid - methanol 4:18:1:4. Under UV 254 nm six bands with $hR_f$ values of 12, 26, 34, 44, 62, and 84 were observed. These bands correspond to well known constituents of Aloe vera: aloeresin $hR_f$ 25, barbaloin $hR_f$ 33, aloe emodin $hR_f$ 43, emodin $hR_f$ 63. The bands with $hR_f$ values of 12 and 84 could not be identified. The reported finger print profiling can serve as potential technique for authentification and batch to batch consistency of herbal drugs.

pharmaceutical research, quality control, herbal, HPTLC, densitometry, qualitative identification, quantitative analysis 32e

105 106 P.K. SAINI, R.M. SINGH*, S.C. MATHUR, G.N. SINGH, C.L. JAIN, R.K. KHAR, A. HAFEEZ (*Indian Pharmacopoeia Commission, Ministry of H. and F. W., Government of India, Rajnagar, Ghaziabad (U. P.)-201 002, India; raman19662002@yahoo.co.in): A simple and sensitive HPTLC method for quantitative analysis of artemether and lumefantrine in tablets. J. Planar Chromatogr. 23, 119-122 (2010). HPTLC of artemether and lumefantrine on silica gel with n-hexane - ethyl acetate 4:1 at room temperature in a twin-trough chamber saturated for 15 min. Quantitative determination by densitometric scanning in reflectance mode at 357 nm. The method is linear ($r^2 > 0.995$), precise ($RSD < 2$ %), accurate (average recovery of 100.5 % for artemether and 99.5 % for lumefantrine), specific, and robust. LOD and LOQ for artemether were 50 and 150 ng/band, respectively, and those for lumefantrine were 300 and 900 ng/band, respectively.

quality control, HPTLC, densitometry, quantitative analysis 32a

105 107 L. SAWANT*, N. PANDITA (*School of Pharmacy and Technology Management, NMIMS University, Mumbai, Maharashtra, India): Development and validation of HPTLC densitometric quantification method for gallic acid from Phyllanthus emblica Linn. Abstract No. C-293, 61st IPC (2009). HPTLC of gallic acid in fruits of Phyllanthus emblica on silica gel with toluene - ethyl acetate - formic acid - methanol 15:15:4:1. The $hR_f$ value of gallic acid was 40. Quantitative absorbance measurement at 280 nm. The method was linear in the range of 40-240 ng/band. The method was reproducible and suitable for quality control.

pharmaceutical research, quality control, herbal, HPTLC, densitometry 32e

105 108 M. SAXENA, K. RAVIKANTH, A. KUMAR*, A. GUPTA, B. SINGH, A. SHARMA (*Phytochemistry and Analytical Laboratory, R and D Centre, Ayurvet Limited, Baddi, H.P., India, akumar@ayurvet.in): Purification of Azadirachta indica seed cake and its impact in nutritional and antinutritional factors. J. Agric. Food Chem. 58, 4939-4944 (2010). HPTLC of azadirachtin (1) and salannin (2) in the seeds of Azadirachta indica on silica gel with hexane - ethyl acetate1:3. Quantitative determination by absorbance measurement at 220 nm. The $hR_f$ values of (1) and (2) were 18 and 30, respectively. Linearity was between 50 and 200 ppm for both (1) and (2). Recovery was 99.9 % for (1) and 99.3 % for (2). The intermediate precision was 88.5 % and 87.5 % for (1) and (2), respectively (n=3). The HPTLC and HPLC methods gave comparable results.

food analysis, herbal, HPTLC, quantitative analysis, densitometry, comparison of methods 32e

105 109 M. SCHULZ*, S. MINARIK, C. WIRTH, M. OBERLE (*Merck KGaA, PC-RP-SIL, Frankfurter Str. 250, 64293 Darmstadt, Germany): Screening of unknown plant extracts by planar chromatogaphy. CBS 103, 10-12 (2009). HPTLC of plant extracts and standards chlorogenic acid, hyperoside, rutin, quercetin and kaempferol (0.1 % in methanol) on silica gel in a twin-trough chamber with ethyl acetate - formic acid - glacial acetic acid - water 100:11:11:27. Detection by spraying with various detection reagents: 1) natural products reagent, evaluation under UV 366 nm, 2) anisaldehyde reagent, evaluation under white light, 3) diphenyl-2-picrylhydrazyl reagent (DPPH), evaluation under white light, 4) rhodamine B reagent, evaluation under UV 366 nm, 5) Dragendorff reagent, evaluation under white light.

herbal, HPTLC, qualitative identification 32e

105 110 N. SHARMA, U. SHARMA, A. GUPTA, A. SINHA* (*Natural Plant Products Division, Institute of Himalayan Bioresource Technology (CSIR), Himachal Pradesh, India, aksinha08@rediffmail.com): Simultaneous determination of epicatechin, syringic acid, quercetin-3-O-galactoside and quercitrin in the leaves of Rhododendron species by using a validated HPTLC method. J. Food Comp. Anal. 23, 214-219 (2010) HPTLC of epicatechin (1), syringic acid (2), quercetin-3-O-galactoside (3), and quercitrin (4) in the leaves of Rhododendron species on RP18 with methanol - 5 % formic acid in water 1:1. Quantitative determination by absorbance measurement at 290 nm. The $hR_f$ values of (1), (2), (3), and (4) were 63, 47, 28 and 21, respectively. Linearity was between 200 and 1200 ng for (1), (3) and (4), and between 200 and 2400 ng for (2). The intra-day and inter-day precisions (expressed in terms of %*RSD*) for compounds (1) to (4) were in the range of 0.41-1.37 % and 0.67-2.04 %, respectively. LOD obtained for compounds (1) to (4) were 20, 40, 25 and 25 ng/zone, respectively while LOQ were 50, 115, 75 and 70 ng/zone, respectively. Recovery for all four compounds was in the range of 95.5-98.5 %.

herbal, HPTLC, densitometry, quantitative analysis 32e

105 111 A.N. SHIKOV*, Olga N. POZHARITSKAYA, Svetlana A. IVANOVA, V.G. MAKAROV, V.P. TIKHONOV, B. GALAMBOSI (*Saint Petersburg Institute of Pharmacy, 47/5, Piskarevskiy pr., 195067, St. Petersburg, Russia; alexs79@mail.ru): Improved and validated HPTLC method for

quantification of oenothein B and its use for analysis of Epilobium angustifolium L. J. Planar Chromatogr. 23, 70-74 (2010). Descripton of a selective and simple HPTLC method for quantification of oenothein B on the basis of the free gallic acid and total gallic acid content after acid hydrolysis. HPTLC of gallic acid on silica gel with benzene - methanol - acetic acid 90:16:8 in a glass chamber previously saturated with the mobile phase vapor for 20 min. Quantitative determination by absorbance measurement at 570 nm after derivatization with 1 % ethanolic iron(III) chloride solution. Average recovery of the active ingredient was in the range 95.4-104.6 %. Linearity was in the range of 440-2200 ng/band. The correlation coefficient r was 0.9991, LOD/LOQ were120/360 ng/band; repeatability (*RSD*) was 3.0 % and intermediate precision 1.0 %; intraday precision (*RSD*, $n = 6$, 440-2200 ng/band) was 3.8 to 5.2 % and interday precision 4.3 to 5.7 %. Both, precision and accuracy, were within acceptable limits for routine drug analysis (</= 15 %).

herbal, quality control, HPTLC, quantitative analysis, densitometry 32e

105 112 A. SHIKOV*, Olga POZHARITSKAYA, Svetlana IVANOVA, V. MAKAROV, Vera KOSMAN (*Saint Petersburg Institute of Pharmacy, 47/5, Piskarevsky prospect, 195067, St. Petersburg, Russia, spb.pharmacy@gmail.com): A comparison between HPLC and HPTLC for the separation and quantification of boswellic acids in Boswellia serrata extracts. CBS 104, 2-4 (2010). HPTLC of beta-boswellic acid (BA), acetyl-beta-boswellic acid (ABA), 11-keto-beta-boswellic acid (KBA), and acetyl-11-keto-beta-boswellic acid (AKBA) in Boswellia serrata extracts on silica gel with n-hexane - ethyl acetate - glacial acetic acid 16:5:1 in a twin-trough chamber saturated for 15 min. Quantitative determination by absorption measurement at 254 nm for KBA and AKBA, and at 560 nm for BA and ABA after derivatization by manual dipping in anisaldehyde reagent follwed by heating at 110 °C for 5 min. Results were compared with results from the HPLC analysis. By HPLC (injection volume 20 µL) the limits of detection for KBA and AKBA were 6-8 ng, and for BA and ABA 60-80 ng. By HPTLC (application volume 2 µL) the limits of detection for KBA and AKBA were 150 ng, and for BA and ABA 100 ng. Precision (%*RSD* of boswellic acids) by HPLC was between 6 and 18 % and by HPTLC below 2 % for AKBA and KBA, and around 10 % for BA and ABA after derivatization. Comparison of quantification of boswellic acid in extracts by either HPLC or HPTLC showed that both methods gave identical results. HPTLC is the method of choice for routine analysis because of lower solvent consumption and fast analysis time.

HPTLC 32e

105 113 Jyoti SHRIVASTAVA*, M. MAHADIK, S. DHANESHWAR (Poona College of Pharmacy, Dept. fo Pharmaceutics Chem., Bharati Vidyapeeth Univ., Mah., India): Validated HPTLC method development for simultaneous quantitation of thiocolchicoside and diclofenac in bulk drug and formulation. International Seminar on Herbal Drug Research, PN-017 (2009). HPTLC of thiocolchicoside and diclofenac sodium on silica gel with toluene - acetone - methanol - formic acid 500:200:200:1. Quantitative determination by absorbance measurement at 280 nm. The method was linear in the range of 160-800 ng/band (thiocolchicoside) and 1000-5000 ng/band (diclofenac sodium). The recovery was in the range of 99.2-100.9 for both compounds.

pharmaceutical, research, densitometry, quantitative analysis, HPTLC 32a,17c

105 114 S. SHUKLA*, A. SHUKLA, S. PANDYA (*Indukaka Ipcowala College of Pharmacy, New V V Nagar, Gujarat, India): A validated HPTLC method for the quantification of ursolic acid and luteolin in Lippia nodiflora Rich. Abstract No. C-454, 61st IPC (2009). HPTLC of ursolic acid and luteolin from aerial parts of Lippia nodiflora on silica gel with toluene - ethyl acetate - formic acid 70:30:3. The $hR_f$ value of luteolin was 34 and of ursolic acid 85. Densitometric analysis at 254 nm for luteolin and at 530 nm for ursolic acid after derivatization with natural products

reagent followed by PEG reagent. The recovery of both marker components was in the range of 98.6-100.5 %.

pharmaceutical research, quality control, herbal, densitometry, HPTLC, quantitative analysis 32e

105 115 Y. SUBUDHA*, K. VARSHNEY, S. EDWIN, S. AHMED (*B. R. Nahata College of Pharmacy, Mandsaur, M.P., India): Estimation of curcumin in a marketed herbal product Rheumax (Herbajules rumatis) using HPTLC. Abstract No. C-291, 61st IPC (2009). Quantitative determination of curcumin in the marketed herbal product Rheumax (Herbajules rumatis) containing extracts of Curcuma longa, Boswellia serrata, Tinospora cordifolia and Vitex negundo by TLC on silica gel with chloroform - methanol 37:3. The $hR_f$ value of curcumin was 59. Quantitative absorbance measurement at 430 nm. The linear regression between 100 and 500 ng/zone showed $r^2$ of 0.99984 and *RSD* of 1.58 % for curcumin.

pharmaceutical research, quality control, herbal, densitometry, quantitative analysis, HPTLC 32e

105 116 N. SURYAVAMSA*, S. MANIMARAN, G. ARUN, K. NITHYA, S. DHANBAL, T. PRAVEEN (*J.S.S. College of Pharmacy, Dept of Phytopharma & Phytomedicine, TIFA CORE HD, Mysore, T.N., India): Validation and determination of possible catechins present in Camellia sinensis collected from different place of India. International Seminar on Herbal Drug Research, PN-024 (2009). HPTLC of catechins and epicatechin in leaves of tea (Camellia sinensis on silica gel with toluene - ethyl acetate - formic acid 7:5:1. Quantitative determination by absorbance measurement at 254 nm. The leaves were found to contain 10-24 % of total polyphenols.

pharmaceutical research, herbal, densitometry, comparison of methods, HPTLC 32e,7

105 117 S.R. TAMBE*, R.H. SHINDE, L.R. GUPTA, V. PAREEK, S.B. BHALERAO (*Mahatma Gandhi Vidyamandir‘s Pharmacy College, Panchavati, Mumbai ,Agra Road, Nashik 422003, Maharashtra, India; santoshtambe@indiatimes.com): Development of LLE and SPE procedures and its applications for determination of olmesartan in human plasma using RP-HPLC and HPTLC. J. Liq. Chromatogr. Relat. Technol. 33, 423-430 (2010). HPTLC of olmesartan and zidovidine on silica gel with ethyl acetate - methanol - acetic acid 160:40:1 in a twin-trough chamber saturated for 10 min. Quantitative determination by densitometric scanning at 269 nm. The linearity range was 80-600 ng/zone. LOQ was 80 ng/zone, the correlation coefficient 0.9900 and 0.9820. Recovery was 90.1 and 79.6 %. The accuracy and precision of the method were determined by repeatability (intra-day) and intermediate precision (inter-day) for the set of quality control samples (low, mid, high) in replicate. The results revealed excellent intra- and inter-day accuracy and precision of the method, which was within the acceptable limit (accuracy (% RE) 11.89 and 6.76 (low), 2.53 and 3.83 (mid), and 0.65 and 7.14 (high); inter-day precision (CV): 3.29 and 3.00 (low), 1.02 and 1.51 (mid), and 1.11 and 0.69 (high); intra-day precision: 2.59 and 2.86 (low), 1.07 and 1.13 (mid), and 1.04 and 0.72 (high) - after LLE and SPE, respectively).

quality control, HPTLC, quantitative analysis, densitometry 32a

105 118 T. THOMAS*, R. JAYAPRAKASAM, M. GANDHIMATHI, T. RAVI (*College of Pharma., Sri Ramakrishna Institute of Para Medical Sciences, S. N. Raod, Coimbatore, T.N., India): HPTLC fingerprinting and evaluation of antioxidant activity of extracts of Cyperus rotundus Linn rhizomes. International Seminar on Herbal Drug Research, PN-051 (2009). HPTLC of four different extracts of rhizomes of Cyperus rotundus on silica gel with chloroform - benzene 1:1. Evaluation under 254 nm. Detection by treatment with ethanolic potassium hydroxide and evaluation of the fingerprint under daylight.

herbal, HPTLC 32e

105 119 M. TOMCZYK*, A. BAZYLKO, A. STASZEWSKA (*Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Bialystok, Bialystok, Poland, tomczyk@umwb.edu.pl): Determination of polyphenolics in extracts of Potentilla species by high-performance thin-layer chromatography photodensitometry method. Phytochem. Anal. 21, 174-179 (2010). HPTLC of tiliroside (1), methyl brevifolincarboxylate (2), and ellagic acid (3) in the aerial parts of Potentilla species on silica gel with toluene - ethyl formate - formic acid 6:4:1. Quantitative determination by absorbance measurement at 320 nm for (1), 287 nm for (2), and 280 nm for (3). The $hR_f$ values of (1), (2) and (3) were 9, 13, and 20, respectively. Linearity was between 50 and 500 ng/zone for (1), 50 and 520 ng/zone for (2), and 52 and 500 ng/zone for (3). The intra- and inter-day precisions (expressed in terms of CV %) were observed in the ranges 2.5-9.0 % and 2.4-11.1 %, respectively. LOD for (1) to (3) were 5, 10 and 34 ng/zone, respectively, while LOQ were 27, 17 and 44 ng/zone, respectively. The average recovery of (1), (2) and (3) was 101.1, 82.2 and 94.0 %, respectively.

herbal, HPTLC, densitometry, quantitative analysis 32e

105 120 Anna W. SOBANSKA*, E. BRZEZINSKA (*Department of Analytical Chemistry, Medical University of Lodz, ul. Muszynskiego 1, 90-151 Lodz, Poland; a.sob@poczta.onet.pl): Rapid HPTLC quantification of p-aminobenzoic acid in complex pharmaceutical preparations. J. Planar Chromatogr. 23, 141-147 (2010). HPTLC of p-aminobenzoic acid on silica gel (prewashed with the mobile phase) with diethyl ether - cyclohexane 5:1 in an unsaturated chamber. Quantitative determination by absorbance measurement at 270 nm. Detection by automatic spraying the following reagent solutions: 0.1 mol/L sodium nitrite solution, 0.1 mol/L hydrochloric acid, 0.03 mol/L ethanolic 8-hydroxyquinoline solution, and 10% sodium hydroxide solution. For each solution 2 spraying cycles were applied. After drying quantitative determination by densitometric scanning at 500 nm. Measured before derivatization at 270 nm calibration of p-aminobenzoic acid was perfomed between 50-1600 ng per spot. LOD was 170 ng/spot (calculated) and 50 ng/spot (estimated visually). LOQ (calculated) was 510 ng/spot. The correlation coefficient r was 0.9936 (peak area) and 0.9866 (peak height); the linear range was 200-1800 ng/spot. Measured at 500 nm after derivatization, LOD was 290 ng/spot (calculated) and 50 ng/spot (estimated visually). LOQ (calculated) was 870 ng/spot and 200 ng per spot (estimated visually). The correlation coefficient r was 0.9815 and 0.9538.

quality control, HPTLC, densitometry, quantitative analysis 32a

105 121 D. YENICELI, D. DOGRUKOL-AK* (*Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, 26470 Eskisehir, Turkey; dak@anadolu.edu.tr): A validated thin-layer chromatographic method for analysis of bupropion hydrochloride in a pharmaceutical dosage form. J. Planar Chromatogr. 23, 212-218 (2010). TLC of bupropion on silica gel with ethanol - chloroform - glacial acetic acid 30:10:1. The $hR_f$ value was 56. Quantitative determination by densitometry at 254 nm. Linearity was in the range 200-1000 ng/band (via peak area). The limits of detection and quantitation were 11 and 35 ng per band, respectively. The intra-day repeatability of the method was around 1-2 % *RSD*. Recovery was between 102.1 and 104.6 % and between 97.2 and 102.2 % for quality-control standards and for bupropion hydrochloride, respectively.

quality control, densitometry, quantitative analysis 32a

## 33. Inorganic substances

105 122 S.S. BOZKURT, I.K. CAVDAR, H.M. KURTBAY, M. MERDIVAN* (*Dokuz Eylul University, Faculty of Arts and Sciences, Chemistry Department, 35160 Buca Izmir; melek.merdivan@deu.edu.tr): Determination of trace metal ions using porphyrins as chelating agents by high-performance thin-layer chromatography - densitometry. J. Liq. Chromatogr. Relat. Technol. 33, 748-760 (2010). HPTLC of mercury(II), copper(II), nickel(II), cadmium(II), mercury(II), lead(II), cobalt(II), manganese(II), palladium(II), platinum(II), and zinc(II) as porphyrin complexes using newly synthesized tetra-(bromo-4-hydroxyphenyl) porphyrin (TBHPP), tetra-(4-phenoxyphenyl)

porphyrin (TPPP) and tetra-p-chloromethylphenyl porphyrin (CMPP) on silica gel with acetone - chloroform 1:4 for TBHPP and TPPP and dichloromethane - chloroform - hexane 1:1:3 for CMPP. The $hR_f$ values were determined for the metal-TBHPP, -TPPP, and -CMPP chelates. Detection was performed by absorbance measurement at 420 nm. The linear range was 3.6-60, 3.6-30, 1.2-30, 0.6-30, 0.6-30, 2.4-60 ng/µL, LOD was 0.90, 0.92, 0.36, 0.19, 0.16, and 0.41 ng/µL, and LOQ was 3.01, 3.06, 1.11, 0.54, 0.54, 0.54, and 1.36 ng/µL. Intermediate precision (*RSD*%, $n = 5$) (6 ng/µL) was 3.55, 4.25, 3.20, 3.85, 2.25, 0.45 % and the regression coefficient was 0.9908, 0.9904, 0.9944, 0.9961, 0.9972, and 0.9942. The $hR_f$ values were 68, 16, 83, 42, 67, and 76 for Hg-TBHPP, Zn-TBHPP, Cu-TBHPP, Co-TBHPP, Hg-TBHPP, and Cu-CMPP, respectively.

HPTLC, densitometry, quantitative analysis 33a

105 123 A. MOHAMMAD*, A. MOHEMAN (*Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University; Aligarh 202002, India;alimohammad08@gmail.com): Adsorption of zinc(II) and cadmium(II) on soil layers in TLC in the presence of surfactant-containing mobile phases. J. Planar Chromatogr. 23, 28-34 (2010). Plates with layers of specified soil were prepared by coating 20 cm × 3.5 cm glass plates by a soil slurry (soil-to-water ratio 1:2) to give layers 0.5 mm thick. The plates were then dried in air at room temperature (30 ± 2°C). TLC of Zn(II) and Cd(II) nitrate on soil plates with 1.0 M magnesium chloride solution in 9.9 mM CTAB (cetaltrimethylammonium bromide) as the optimum mobile phase (of 35 mobile phases). Detection by spraying with dithizone solution (0.1 % in carbon tetrachloride) to obtain dark pink and brick red spots of complexes. The smallest detectable amounts of Zn(II) and Cd(II) on soil layers were 0.69 and 1.0 µg, respectively, for distilled water and 9.0 mM CTAB solution, and 1.2 and 2.5 µg, respectively, for the optimum mobile phase.

qualitative identification 33a

105 124 A. MOHAMMAD*, S. HENA, A. MOHEMAN (*Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh-202 002, India; alimohammad08@gmail.com): Micellar TLC of inorganic ions: Simultaneous separation of lead(II), zinc(II), and cobalt(II) and spectrophotometric estimation of zinc(II). J. Planar Chromatogr. 23, 162-165 (2010). TLC of iron(III), copper(II), nickel(II), cobalt(II), cadmium(II), zinc(II), silver((I), lead(II), bismuth(III), mercury(II), titanium(IV), manganese(II), and chromium(VI) on silica gel with 0.02, 0.1, 0.2, and 1.0 M aqueous sodium dodecyl sulfate (SDS) (M1), 0.2 M SDS + 0.04 M tartaric acid (9:1, 1:1, and 1:9) (M2); 0.2 M SDS + 0.08 M tartaric acid (9:1, 1:1, and 1:9) (M3); 0.2 M SDS + 0.1M tartaric acid (9:1, 1:1, and 1:9) (M4); 0.2 M SDS + 0.08 M citric acid (1:1) (M5); 0.2 M SDS + 0.08 M formic acid(1:1) (M6); 0.2 M SDS + 0.08 M acetic acid (1:1) (M7); 0.2 M SDS + 0.08 M oxalic acid (1:1) (M8). Detection reagents used were: 0.5 % dithizone in carbon tetrachloride, 1.0 % aqueous potassium ferrocyanide, 1.0 % ethanolic dimethylglyoxim, 1:1 2.0 M aqueous sodium hydroxide in 30 % hydrogen peroxide, and a methanolic silver nitrate solution. Quantitative analysis of zinc(II) by spectrophotometry after extraction. The detection limits were 0.85, 0.05, and 1.5 µg respectively for lead(II), zinc(II), and cobalt(II). The in-situ detection of cations was more sensitive than detection in solution.

qualitative identification 33a

105 125 P.A.M. NAJAR*, R.N. CHOUHAN, M.T. NIMJE, K.V.R. RAO (*Jawaharlal Nehru Aluminium Research Development and Design Center, Wadi, Amaravati Road, Nagpur-440 023, India; najarp@gmail.com): Quantitative analysis of primary aluminium by densitometric thin-layer chromatography. J. Planar Chromatogr. 23, 156-161 (2010). TLC of aluminium, silicon and iron on microcrystalline cellulose, on silica gel and combinations with aluminium oxide. Mobile phases used were 1:1, 7:3, 9:1, and 8:2 mixtures of 10 % aqueous solutions of sodium chloride, potassium chloride, potassium bromide, and sodium formate with 10 % aqueous formic acid, and 1:8 mixtures of 1 % aqueous sodium molybdate with 3 %, 4 %, and 5 % hydrochloric acid.

Aluminium(III) was detected by spraying with 0.05 % aqueous aluminon (triammonium aurin tricarboxylate). Aqueous potassium ferrocyanide in 10 % aqueous sodium formate solution was used for the visualization of iron(II). Silicon was detected by spraying with 1 % sodium molybdate at pH 0.80-0.92. Quantitative determination by scanning densitometry at 402 nm for silicon, at 528 nm for aluminium and at 620 nm for iron. LOD was 7-10 ppm for aluminium(III), 2-5 ppm for iron(II), and 4-6 ppm for silicon(IV).

densitometry, quantitative analysis 33a

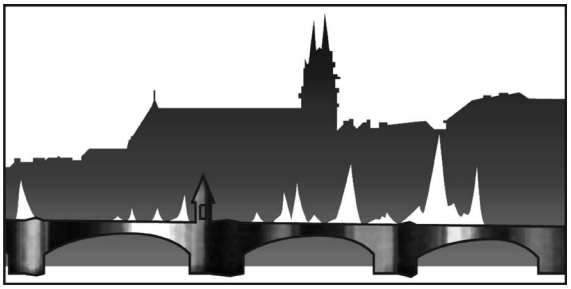
## 38. Chiral separation

105 126 Ravi BUSHAN*, Charu AGARWAL (*Department of Chemistry, Indian Istitute of Technology, Roorkee-247667, India; rbushfcy@iitr.ernet.in): Resolution of beta blocker enantiomers by TLC with vancomycin as impregnating agent or as chiral mobil phase additive. J. Planar Chromatogr. 23, 7-13 (2010). TLC of the enantiomers of racemic atenolol, metoprolol, propanolol, and labetalol on silica gel using vancomycin as chiral impregnating agent or as chiral phase additive. With vancomycin as impregnating agent, resolution of the enantiomers of atenolol, metoprolol, propanolol, and labetalol was achieved with acetonitrile - methanol - water - dichloromethane 7:1:1:1, acetonitrile - methanol - water 6:1:1, acetonitrile - methanol - water - dichloromethane - glacial acetic acid 14:2:2:2:1, and acetonitrile - methanol - water 15:1:1. With vancomycin as mobile phase additive, resolution of the enantiomers of metoprolol, propanolol, and labetalol was achieved using acetonitrile - methanol - 0.56 mM aqueous vancomycin (pH 5.5) 15:1:2, and acetonitrile - methanol - 0.56 mM aqueous vancomycin (pH 5.5) - dichloromethane 18:2:3:2, respectively. Chromatograms were developed in glass chambers previously equilibrated with the mobile phase at 16 +/- 2 °C for 10-15 min. Detection by exposure to iodine vapor. The detection limits were 1.3, 1.2, 1.5. and 1.4 µg for each enantiomer of atenolol, metoprolol, propanolol, and labetalol, respectively.

pharmaceutical research, quality control, qualitative identification 38

We would like to inform you about an outstanding event taking place, the

# International Symposium for High-Performance Thin-Layer Chromatography BASEL, Switzerland 06–08 July 2011



# Call for papers

This symposium will comprehensively inform scientists about the immense potential of HPTLC and its latest developments. Be up-to-date with the state-of-the-art. HPTLC is increasingly seen as a rational method in this age of ultra-rapid separations and an exchange of knowledge is foremost in sustaining this position. The scientific program will feature tutorials, invited keynote speakers, selected submitted lectures, panel discussions, and poster presentations. Contributions are invited from all areas of planar chromatography.

**Deadlines**

- Abstract submission (oral and poster): **1 March 2011**
- Final registration: **30 May 2011**

For abstract submission and electronic registration see **www.hptlc.com**. An abstract template (guideline) is available for download. After the abstract submission deadline the final program will be announced at this webpage, about the middle of March.

**Tentative program**
The program will run from Wednesday 6th 9:15 (registration starts at 8:00) until Friday 8th 15:30.

Wednesday 9:15-10:30 two parallel tutorials on *HPTLC analysis of herbal and medicinal plants* (Dr. Eike Reich) and *Hyphenations in HPTLC* (Dr. Gertrud Morlock) are planned prior to the formal opening of the symposium at 11:00. Dedicated panel discussions between sessions will provide a focus and forum for the exchange of information on the main themes of the symposium. Presentations will end by 13:00 on Friday 8th to allow for lunch and visit to the Novartis campus in the afternoon.

Among others, invited speakers include:

Fundamentals: Prof. Dr. Colin Poole, USA

Analysis of medicinal plants: Prof. Dr. Ikhlas Khan, USA, Prof. Dr. Zheng-Tao Wang, China

Analysis of phytopharmaceuticals and herbal products: Dr. Wanchai De-Eknamkul, Thailand, Dr. J. Madhusudana Rao, India

Analysis of cosmetics/active ingredients: Prof. Dr. Ingo Schellenberg, Germany

Food analysis: Prof. Dr. Mario Vega, Chile

Detection with bioassays: Dr. Irena Choma, Poland

HPTLC-MS: Dr. Gary Van Berkel, USA

Miniaturization: Prof. Dr. Michael Brett/Steven Jim, Canada

**Exhibition**
There will be a manufacturers' exhibition in the Congress Center in parallel with the seminar program.

**Social events**
Wednesday evening: Welcome cocktail at Ramada hotel
Thursday afternoon: Manufacturers' cocktail
Thursday evening: Official symposium dinner: Rhine cruise with a marvellous view of Basle
Friday afternoon: Visit to the new Novartis campus

**Location**
Wednesday/Thursday
Congress Center
Messeplatz 21
4005 Basel
www.congress.ch

Friday:
Novartis Pharma AG
Auditorium Fabrikstr. 6
4002 Basel
www.novartis.ch

Special accommodation rates are available for the Symposium:
RAMADA PLAZA
Messeplatz 12
4005 Basel
www.ramada.de/basel

**Fees**
The participation fee includes the full scientific program, lunches, coffee breaks, and all social events.

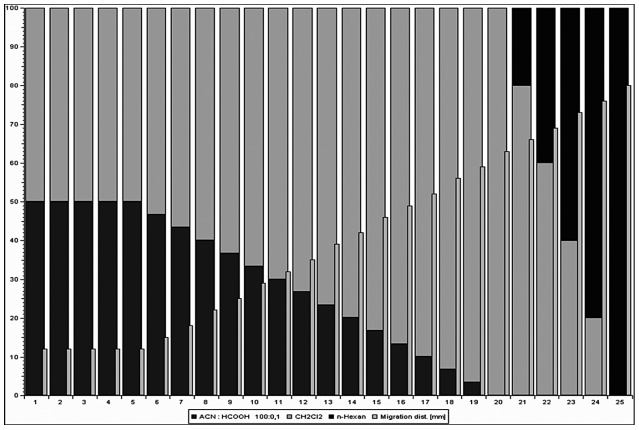
- Industrial 500 €
- Academic 400 €
- Students 200 €

Reduction of 100 € with ISPS or CCCM membership.

Many returning faces from previous symposia and new faces are cordially invited. We are looking forward to welcoming you at the conference.

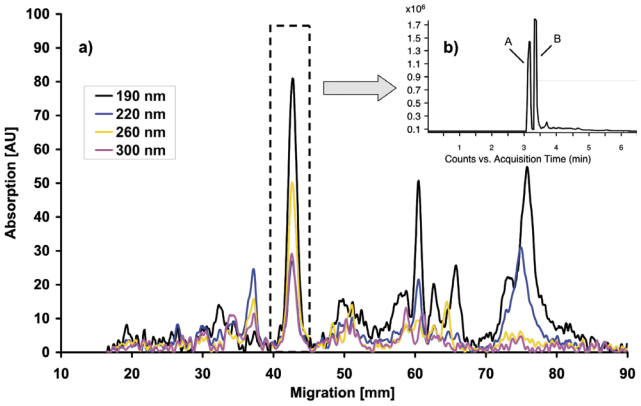
**Further information**
info@hptlc.com for general information
committee@hptlc.com for scientific matters
registration@hptlc.com for registration concerns

*continuation from page 7*



13

*25-step AMD 2 gradient for the screening of water samples*



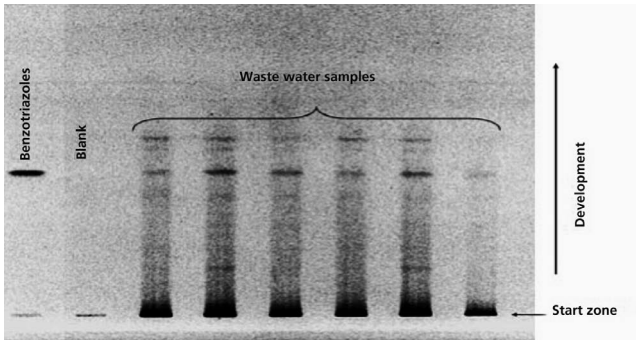

14

*HPTLC/AMD multi-wavelength scan of an extract from secondary effluents (a), Nano LC/QTOF-MS chromatogram of the extracted zone, peak A: 1H-benzotriazole; peak B: tolyltriazole (b)*

For the identification of the two unknown analytes a substance library (approx. 300 entries) created at the laboratory for operation control and research of the LW was used, which features molecular formula and exact mass of potential environmental contaminants described in literature. The unknown analytes were identified as 1H-benzotriazole and a mixture of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole.

Benzotriazoles are ubiquitous in the aquatic environment because they are used in a broad range of applications: in coatings and paints, as anticorrosive in copper and copper alloys, in coolants and lubricants for engines, as silver protection in detergents, as antifreeze and as aircraft de-icing fluid.

In 14 % of ground water samples ($n$ = 74) 1H-benzotriazole was detected at a maximum of 173 ng/L and in 18 % of samples tolyltriazoles were detected at a maximum of 75 ng/L. Concentrations in the river Danube and in some of its feeding rivers in the Ulm area were between 100 and 500 ng/L. In secondary effluents, the main source of benzotriazoles in the environment, concentrations of 1H-benzotriazole and tolyltriazoles can even exceed 10 µg/L. Comparison of water sampled from wastewater treatment plants, surface water and ground water showed that the ratio of the investigated substances shifted towards 1H-benzotriazole.

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*HPTLC/AMD chromatogram of SPE extracts from various secondary effluents and of the standard mixture 1H-benzotriazole/tolyltriazole*

Further information is available from the authors on request.

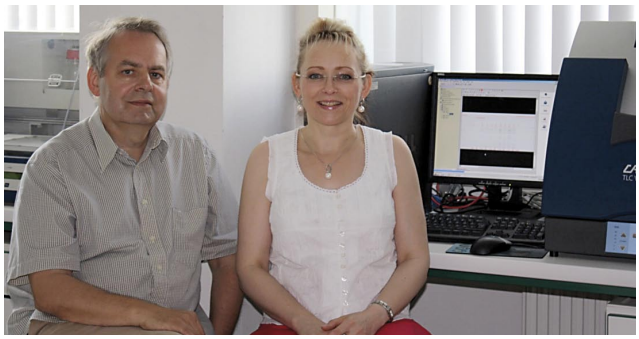
Contact: Dr. Walter H. Weber, Zweckverband Landeswasserversorgung, Betriebs- und Forschungslaboratorium, Am Spitzigen Berg 1, 89129 Langenau, Germany, weber.w@lw-online.de

[1] W.H. Weber *et al.* Vom Wasser 105 (1) (2007) 7

[2] W.H. Weber *et al.* Vom Wasser 107 (4) (2009) 16

[3] A. Müller *et al.* Rapid Commun Mass Spectrom 24 (2010) 659

# Optimization of an AMD 2 method for determination of *stratum corneum* lipids



*Prof. Dr. Ingo Schellenberg and Dr. Kathrin Kabrodt*

The workgroup of Prof. Schellenberg at the Institute of Bioanalytical Sciences (IBAS) is engaged in the isolation and production of plant extracts, which are used in foodstuffs, in nutriceuticals, as well as in cosmetic and pharmaceutical products. Further core competencies are the encapsulation of bioactive ingredients, lipids and flavors, flavors also investigated as to their importance for sensory quality. Parts of these investigations were done by Ms Julia Lüttich in the course of her Bachelor thesis.

## Introduction

Lipids are substances that are soluble in organic solvents due to their non polar properties. In *stratum corneum*, which represents the outer layer of skin, lipids play a key role in maintaining the barrier function of the skin, i.e. protection. Predominantly lipids of *stratum corneum* are ceramides, fatty acids and cholesterol. Ceramides consist of long-chain hydroxylated amine base called sphingoid, which are amide-linked to fatty acids. By various combinations of fatty acids and base types there are different ceramide groups. In human *stratum corneum* 9 ceramide groups have been identified up to now.

**An optimized AMD2 separation of various lipid standards that are found in native *stratum corneum* is presented below. The AMD system was already successfully used for these analyses (see CBS 77, 90 and 93). However, the described methods were rather time and solvent consuming.**

## Standard solutions

| Standard substances | Abbreviation | Supplier | Sample amount (mg) | Concentration of standard solution (mg/mL) |
|---|---|---|---|---|
| Ceramide NS | NS | Sederma* | 14,0 | 0,35 |
| Ceramide NP | NP | Evonik* | 4,8 | 0,12 |
| Ceramide AS | AS | Sigma-Aldrich | 4,8 | 0,12 |
| Ceramide AP | AP | Evonik* | 14,0 | 0,35 |
| Cholesteryl-3-sulfate | C3S | Sigma-Aldrich | 8,0 | 0,20 |
| Cholesterol | C | Sigma-Aldrich | 5,6 | 0,14 |
| Cholesteryloleate | CO | Sigma-Aldrich | 4,0 | 0,10 |
| Glyceryl trioleate | GT | Sigma-Aldrich | 6,0 | 0,15 |
| Phosphatidylcholine | PC | Sigma-Aldrich | 4,0 | 0,10 |
| Oleic acid | OA | Sigma-Aldrich | 3,6 | 0,09 |
| Squalene | S | Sigma-Aldrich | 8,0 | 0,20 |
| Sphingomyeline | SM | Sigma-Aldrich | 5,6 | 0,14 |

*Thanks to Sederma and Evonik for free supply of standards.

Each standard substance was weighted into a 2 mL volumetric flask each and filled up with methanol – chloroform 1:1 (stock solution). 500 µL of each stock solution were diluted to 10 mL with the same solvent (standard solution). 500 µL of each stock solution were put into a 10 mL volumetric flask and filled up with the same solvent (standard solution mix).

## Chromatogram layer

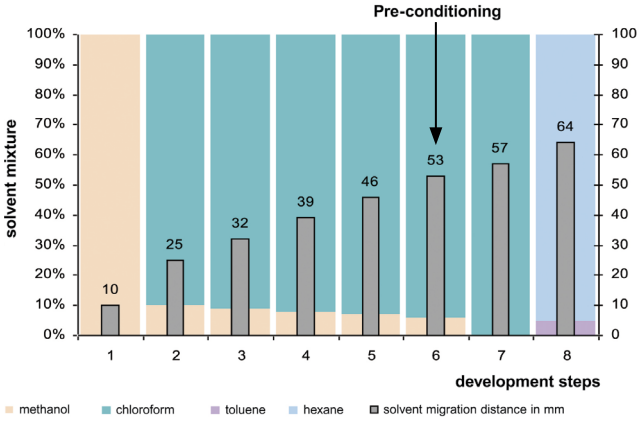
HPTLC plates 20 x 10 cm, silica gel 60 $F_{254}$, 0.1 mm for AMD (Merck); pre-washed twice with chloroform – methanol 2:1 (v/v) and dried at 120 °C in a drying oven for 30 min; storage in an exsiccator

## Sample application

Bandwise with Automatic TLC Sampler 4, 13 tracks, band length 8 mm, distance from the side 14 mm, distance from lower edge 8 mm, track distance 13.6 mm (automatically calculated), application volume 5 µL

## Chromatography

8-step gradient in AMD 2 system, pre-conditioning before step 6 with 4 M acetic acid, drying time 2 min, maximum migration distance 64 mm, duration 1.5 h, solvent consumption 60 mL

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## Post-chromatographic derivatization

With the Chromatogram Immersion Device III the plate was immersed in copper(II)sulfate reagent (aqueous solution of 10% copper(II)sulfate and 8% orthophosphoric acid), then dried and heated at 170 °C on the TLC Plate Heater for 8 min.

## Densitometry

Absorption measurement at 600 nm by TLC Scanner 3 with winCATS software

## Documentation

TLC Visualizer under white light (reflection mode)

## Results and discussion

The first step of the 8-step gradient (100 % methanol) was used to elute all polar substances and to focus them to a sharp line. Steps two to six separated cholesterol-3-sulfate and the different ceramides. Step seven (100 % chloroform) was used to separate cholesteryl oleate from glyceryl trioleate. In the last step with *n*-hexane – toluene 19:1, squalene was separated from cholesteryl oleate. To focus oleic acid, a pre-conditioning of the HPTLC plate with 4 molar acetic acid was necessary before step six was started.


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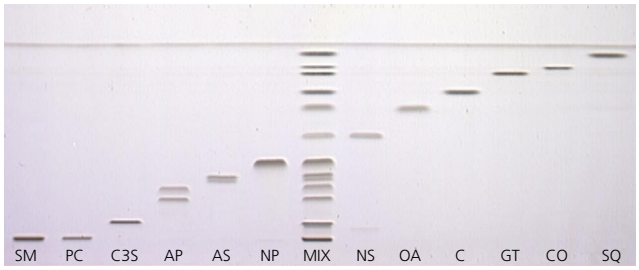
**CAMAG AMD 2 System**
(Automated Multiple Development)

AMD is used when the desired resolution is unattainable over the available separation distance by one step isocratic development. The combination of multiple and gradient development, and the individual pH adjustment of the layer (here just before step 6) leads to a focusing effect of the zones. Peak sharpness and resolution are improved.

In general, AMD is used for mixtures of components with a wide polarity range. But for this AMD application in the field of lipid analysis, especially the unpolar region of the gradient is of interest. From step to step, the polarity change of the solvent (only 2 %) is minor. This way, also compounds of slight polarity differences are separated. The migration distance increments of mostly 7 mm are high compared to 2 to 4 mm increments usually. For a baseline separation using 4 mm increments, a reduced substance amount per zone (e.g. by a factor of 4) would be necessary. Additionally, a final migration distance of 40 mm would significantly reduce the gradient time further on.

Phosphatidylcholine and sphingomyeline (at the start) could not be separated with this gradient. However, if one wants to separate both lipids, it is possible to supplement some polar steps at the beginning of the gradient.

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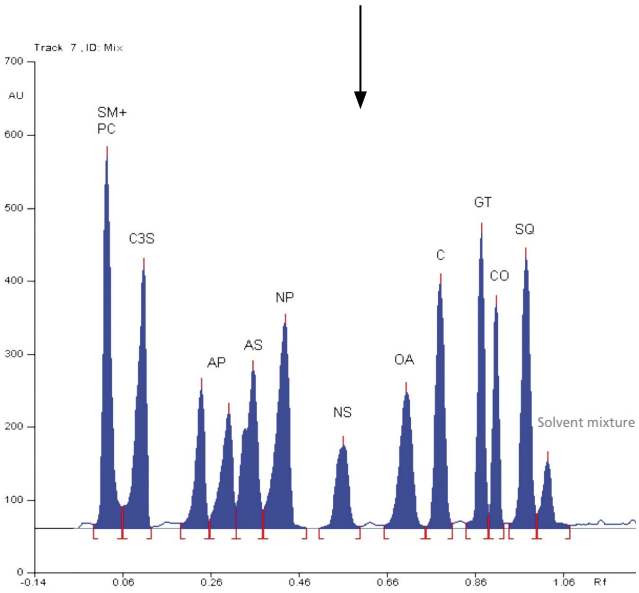
*AMD 2 chromatogram using the 8-step gradient*

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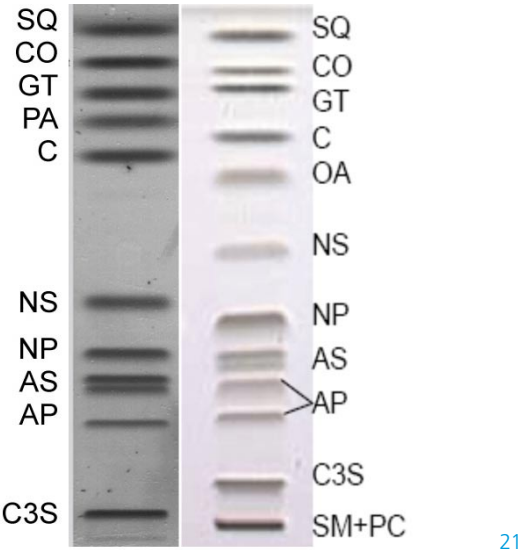
*Densitogram of the standard mixture*

Just phosphatidylcholine and sphingomyeline (at the start position) were not separated by the new gradient. This was also not achieved by Bonté *et al*. [1] with their 26-step gradient with a duration of 6.8 h. Zellmer *et al*. [2] and Farwanah *et al*. [3] did not consider these substances in their methods.

A chromatogram comparison of the 17-step gradient by Farwanah *et al*. [3] and the optimized 8-step gradient shows a comparable resolution despite the reduced number of steps. The gradient time was reduced by one hour (from 2.5 h to 1.5 h) and the derivatization time was reduced from 20 min (at 150 °C) to 8 min (at 170 °C).

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*Chromatogram comparison of the 17-step gradient by Farwanah et al. ([3], left) and the optimized 8-step gradient (right)*

At present this method is in the validation process and *in vivo*-extraction of defined skin zones is optimized to separate and quantify the most important skin lipids.

Further information is available from the authors on request.

[1] F. Bonté *et al.* J Chromatogr B 664 (1995) 311

[2] S. Zellmer *et al.* J Chromatogr B 691 (1997) 321

[3] H. Farwanah *et al.* J Chromatography B 780 (2002) 443

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# Determination of additives in plastic foils


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*Elisabeth Dytkiewitz, Prof. Dr. Wolfgang Schwack*

At the Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany, efficient analytical work is conceptualized and then developed and implemented. In doing so, planar chromatography is often used due to its pragmatism in solving analytical tasks. The following example concerning the analysis of food packaging materials is part of the doctoral thesis of Elisabeth Dytkiewitz.

## Introduction

Plastic foils of polyvinyl chloride (PVC) used for packaging meat, cheese, fresh vegetables, etc., contain plasticizers and other additives in high percentages. Most of them have in common that they are not chemically bonded to the polymer, making them potentially free for migration into the packaged food. According to the European legislation, migrating constituents must not endanger human health [1]. Therefore migrating studies with food simulating solvents must be performed to check that the composition is compliant. An effect directed analysis (EDA) brings the bioactivity of substances into focus. Using EDA for the analysis of migrating additives was the aim of this study.

**After separation by HPTLC, additives present in migrates are solvent-free accessible, making detection with bioluminescent *Vibrio fischeri* bacteria practicable. Therefore many samples can be run in parallel, revealing not only the presence of toxicologically relevant compounds but also information as to their strength of effect. In contrast to common mixed mode cuvette tests, HPTLC-EDA enables the detection of both inhibition and enhancement of luminescence. Detected additives can be identified from the same plate by coupling of HPTLC with mass spectrometry via the TLC-MS Interface. MassWorks software delivers exact masses and molecular formulae even from a mass spectrometer of low resolution. Hence, HPTLC-bioactivity-MS enables rapid analysis of plastic migrate components based on their toxicological effects.**

**Concerning rapid screenings with focus on major components, the TLC-MS Interface is also suitable for the direct extraction of additives from the packaging films followed by online mass spectrometric analysis.**

## Sample preparation

Foil samples (0.8 g) were extracted by 150 mL ethanol (95 % vol) in a screw capped bottle for 4 h at 60 °C. After rotary evaporation of the solvent, the residues were taken up in 2 mL toluene. The solutions were directly used for HPTLC after membrane filtration.

## Layer

HPTLC plates silica gel 60 $F_{254}$, Merck, 20 x 10 cm, prewashed by development with methanol, dried for 15 min in a drying oven at 100 °C.

## Sample application

Bandwise with ATS4, band length 6 mm, track distance 18 mm, distance from lower plate edge 8 mm, distance from the edges min. 20 mm, application volumes of sample solutions 5–10 µL.

## Chromatography

In the ADC2 with isooctane – toluene – diethyl ether – ethyl acetate 8:7:4:1 after chamber saturation for 10 min up to a migration distance of 65 mm. Plate activity was controlled by saturated magnesium chloride solution for 3 min (42 % relative humidity). Plate drying was automatically performed for 30 min.

## Detection with biological activity

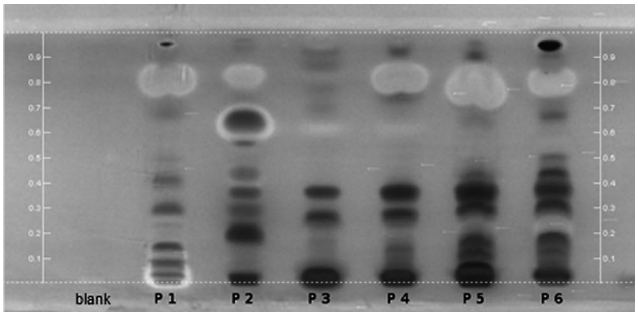
Using the TLC Chromatogram Immersion Device (vertical speed 3 cm/s, immersion time setting 0 s), the plate was dipped in a suspension of *Vibrio fischeri* bacteria (BioLuminex kit, ChromaDex, Boulder, USA) and documented with the Bioluminizer (exposure time 50 s) directly after dipping and after 5 and 10 min.

## Mass spectrometry

TLC-MS Interface (oval elution head 4 x 2 mm) coupled to an Agilent 1100 LC/MSD system, operating in positive ESI mode, zone elution with ethanol (95 %vol) at a flow rate of 0.2 mL/min during 10 s. Exact masses were calculated with MassWorks software (Cerno Bioscience, Danbury, CT, USA).

## Results and discussion

Ethanol (95 %vol) as a fatty food simulating solvent was used for extraction of additives from plastic foils [2]. These migrates were directly used for HPTLC, which was performed by automated standardized development in the ADC 2 to ensure repeatable results. After some modification of the mobile phase [3] a good separation was obtained. Detection with bioluminescent bacteria showed numerous substances with inhibiting effects in the samples. Even in a plastic wrap of polyethylene (PE), luminescence inhibiting substances were detected. Remarkably, some of the zones caused an activating effect on the bacteria's luminescence, a notable advantage given by the previous chromatography. A cuvette test of the whole migrate would not reveal this property.



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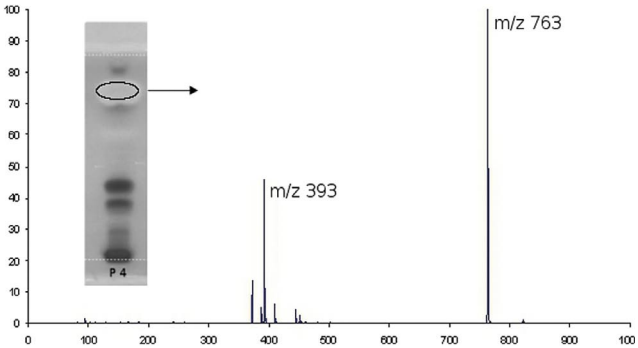
*Separation and detection of 6 packaging foils; inhibition of bioluminescence displayed by dark zones, enhancement of bioluminescence displayed by light zones. Track assignment: blank: ethanol, P1 and P2: commercial PVC cling film, P3: commercial PE cling film, P4: foil of a mushrooms' packaging, P5: packaging foil for meat, and P6: packaging foil for cheese.*

For the identification of EDA detected zones, mass spectra were recorded using the TLC-MS Interface. Ethanol was used to elute the compounds into the ion source. With an elution time of just 10 s, the mass spectrum of the sample zone was achieved immediately. Sodium adducts are caused by the saline bacteria suspension remaining at the HPTLC plate surface. After calculation by MassWorks software, the signals from a low resolution mass spectrometer were improved. Thus, exact masses and molecular formula were obtained enabling the identification of additives.



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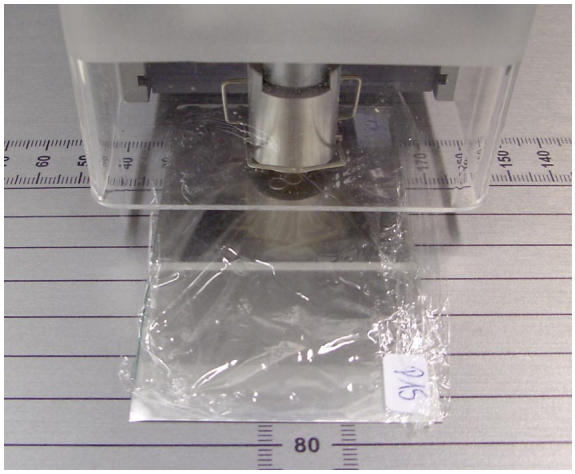
*HPTLC-ESI/MS spectra of the light zone at $hR_F$ 84 of a mushroom packaging foil (P4).*

For a rapid screening of additives extractable from packaging foils, the TLC-MS Interface was directly applied for the transfer of migrates from the foil to the MS, i.e. without chromatography. To accomplish this, the sample foil was placed on the back of a TLC aluminum foil. After tightening the

elution head, the mass spectrum was recorded within 10 s.

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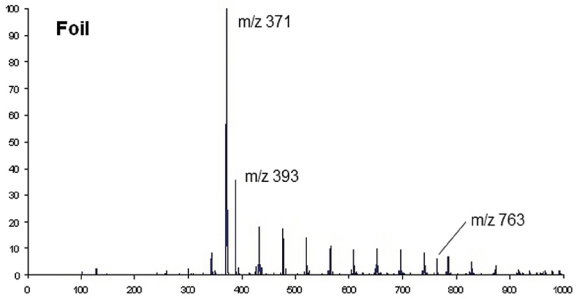
*Direct extraction of a PVC plastic wrap by the TLC-MS Interface. The film was placed planar on the back of a TLC aluminum foil.*

Using this short elution time, the highly soluble and more concentrated additives could easily be identified by mass spectrometry, protonated molecules being generally detected.

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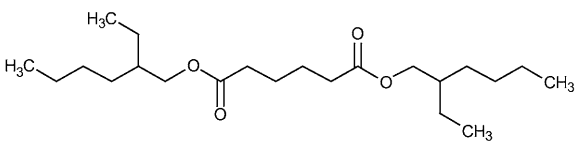
*ESI-MS spectrum of compounds directly extractable from a foil by the TLC-MS Interface (P4)*

Applying mass spectrometry, bis(2-ethylhexyl) adipate could exemplarily be identified with marginal deviations from the theoretical masses.



*Bis(2-ethylhexyl) adipate*

| MS-Signals of | Mass determined | Theoretical mass | Δ (ppm) | Molecular formula | Assigned to |
|---|---|---|---|---|---|
| Plastic foil | 371,3174 | 371,3161 | -3,4071 | $C_{22}H_{43}O_4$ | $[M+H]^+$ |
| HPTLC plate | 393,2985 | 393,2981 | -1,0691 | $C_{22}H_{42}O_4Na$ | $[M+Na]^+$ |
| | 763,6077 | 763,6064 | -1,7164 | $C_{44}H_{84}O_8Na$ | $[2M+Na]^+$ |

Other substances showing inhibiting effects by *Vibrio fischeri* are not present in this spectrum. The low concentration of some substances and the short time of just 10 s for direct foil extraction are the reasons for the reduced elution. Of course, substances eluted into the food simulating solvent could be measured more intensely after a longer contact time simulated by an intermediate flow stop; however, this would also reduce sample throughput.

Direct foil extraction is useful for rapid screening, but the ultimate procedure which produces additional information is chromatography of migrates, obtained after prolonged heat treatment (4 h at 60 °C) and concentration (by a factor of 75). Thus, information is more comprehensive. *Vibrio fischeri* is ideal for detecting other components with toxicological potential, not just major components. It is a specific benefit that from the wealth of these migrating compounds just bio-actives can be detected and identified.

[1] Regulation (EC) No. 1935/2004 as amended on 18.06.2009

[2] Attachment 10 for §11 BedarfsgegenständeVO as amended on 23.09.2009

[3] H. Chen, Y. Wang, R. Zhu, Chinese J Chromatogr A 24 (2006) 69

Further information is available from the author on request.

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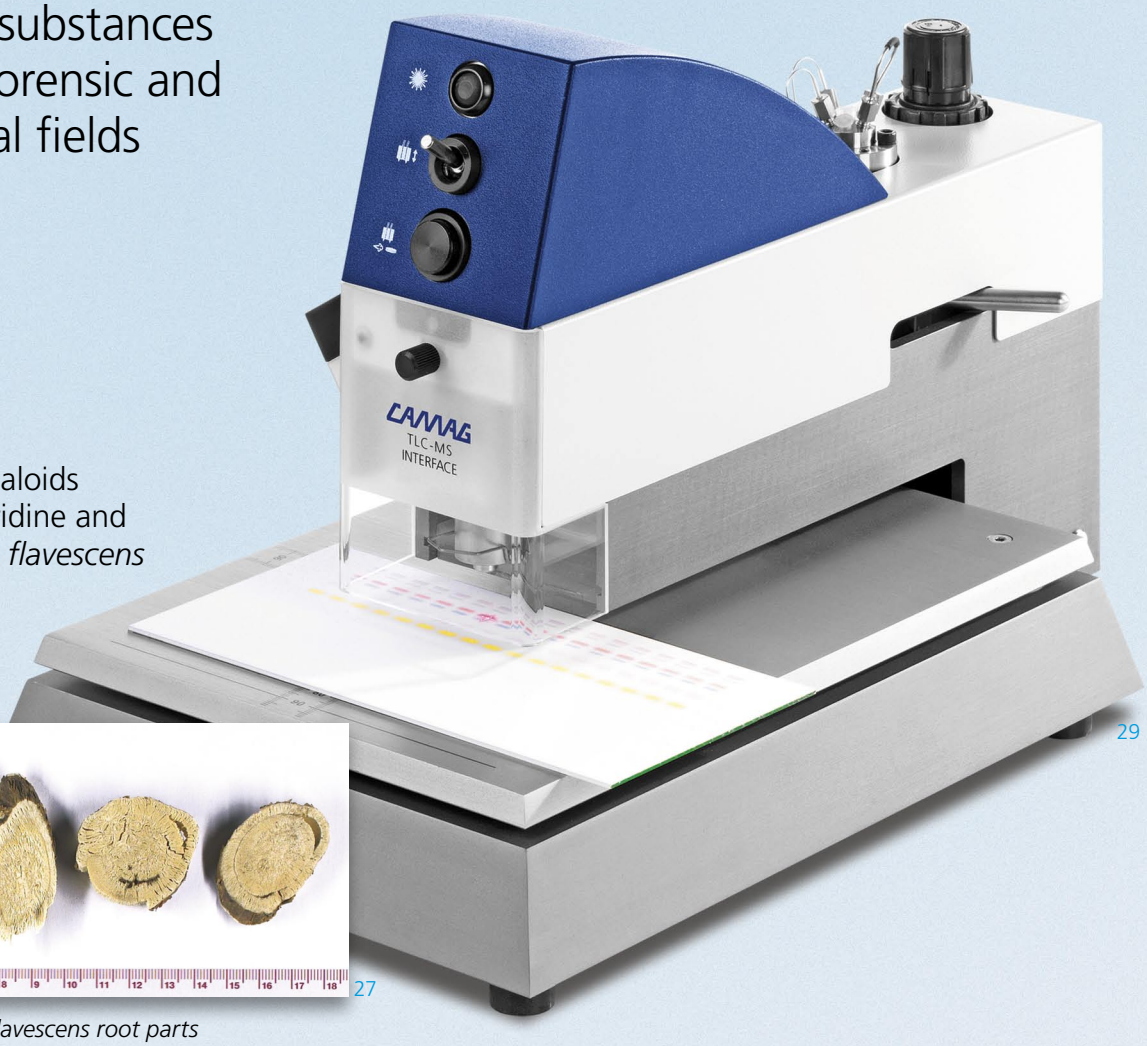
# TLC-MS Interface

World wide used
for identification and elucidation
of unknown substances
in research, forensic and
environmental fields

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Identification of alkaloids
oxymatrine, sophoridine and
matrine in *Sophora flavescens*
extract*



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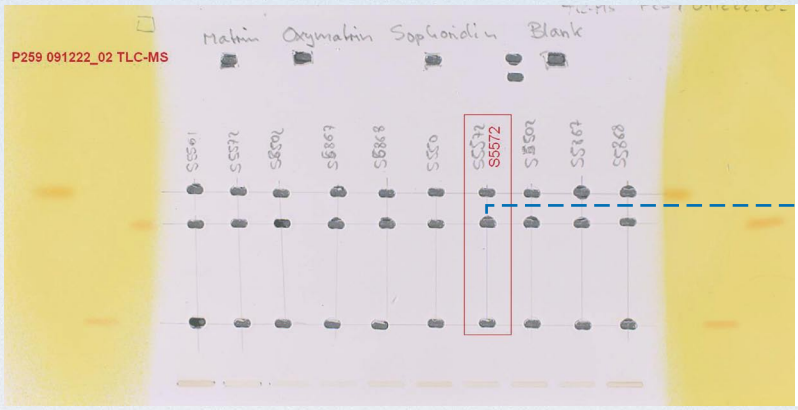
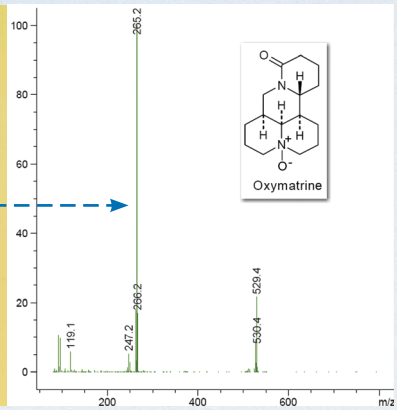
*Cut and milled Sophora flavescens root parts*




*HPTLC plate after extraction of zones with the TLC-MS Interface*

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*Confirmation of oxymatrine, m/z 265 $[M+H]^+$*

Further information at: **www.camag.com/tlc-ms**

*Diploma thesis R. Vizzini

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