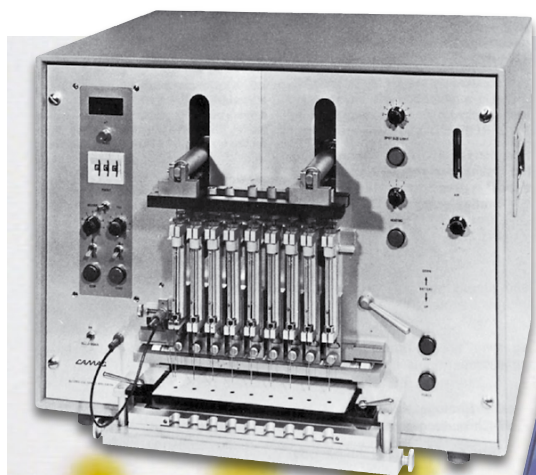


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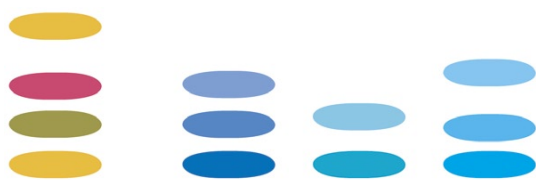


*Automatic Sample Applicator
for conventional TLC plates 1974*



*Automatic TLC Sampler 4
for TLC and HPTLC*

Automatic Sample Application 1974 and today



50 YEARS

CAMAG

101

No. 101, September 2008

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Planar Chromatography
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cbs@camag.com
published by CAMAG Switzerland

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Planar Chromatography in Practice

Identification and quantification of amino acids in peptides

Presented at the
International Symposium for HPTLC,
Helsinki, June 11-13, 2008



From left to right: L. Hamon,
F. Diancourt, L. Roy and R.
Sbaffo-Poasevara

Ipsen is an international specialty pharmaceutical group, which markets more than 20 drugs, employing nearly 4,000 people around the world. It is specialized on primary care and therapeutic areas, like oncology, endocrinology and neuromuscular disorders. At the Institute Henri Beaufour, which is one of the Ipsen research and development centers in Les Ulis near Paris, Mrs. Roseline Sbaffo-Poasevara* is the head of the Analytical Development Laboratory for API. One focus of research is laid on synthetic peptides for pharmaceutical products. These peptides are synthesized from natural and non-natural amino acids or are linked with small molecules. For registration a monograph has to be set up with an identification test of the amino acids after hydrolysis of the peptide. But also during the development, it could be helpful to quantify the free amino acids in the peptide. To accomplish all of this they use different analytical methods and recently they started with HPTLC.

Introduction

Currently identification of amino acids is performed after hydrolysis of the peptide in a strong acidic medium. During acidic hydrolysis some amino acids are partially degraded. Only 7 amino acids are stable. The hydrolyzate is then analyzed by a HPLC method with ninhydrin derivatization. However, the HPLC analysis with the amino acid analyzer is time-consuming and expensive, only to give semi-quantitative results for unstable amino acids. This method is not flexible regarding the common gradient for non-natural amino acids or for small molecules linked to the peptide.

For trace analysis of free amino acids in the peptide, the peptide is not hydrolyzed and a huge amount of peptide has to be loaded onto the chromatographic column, which can have an immense effect on chromatography due to the fact that the peptide elutes during the aqueous gradient. However, with single use HPTLC the peptide fixed at the starting region or in the front does not matter, making HPTLC the technique of choice for this stage. An HPTLC method was established as the limit test for free amino acids in the peptide and for quantification of amino acids after hydrolysis of the peptide.

Standard solutions

Amino acids (Abu, Ala, Arg, Asp, Aib, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Nal, Phe, Pro, Ser, Thr, Trp, Tyr, Val, D-Bal, Apc, Inp) were dissolved in water (1 mg/mL each).

Sample preparation

After 24 h hydrolysis with HCl (6 M, stabilized with 1% phenol) at 110 °C the residue was dissolved in water (0.15 µg/mL).

Layer

HPTLC plates silica gel 60 F₂₅₄, HPTLC plates diol F₂₅₄, and HPTLC aluminum foil cellulose, 20 x 10 cm (foils had to be cut to size, all Merck)

Application

Bandwise with the Automated TLC Sampler ATS4, band length 6 mm, track distance minimal 8 mm, distance from lower edge 8 mm, dosage speed 80 nL/s, heated spray nozzle 50 °C, application volume 0.5 µL for each amino acid standard solution, 1 µL for cysteine solution and 10 µL for the hydrolyzate solution.

Chromatography

In the twin trough chamber or horizontal chamber with either 2-butanol - acetic acid - pyridine - water 15:3:10:12 or 2-butanol - NH₃ - pyridine - water 39:10:34:26.

Note: Generally the layer is faced downwards in the horizontal chamber; however using aluminum foils, the layer can be faced upwards to monitor the solvent front.

Post-chromatographic derivatization

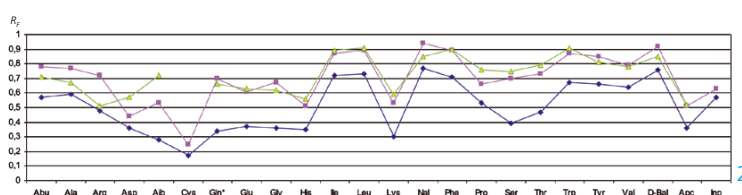
With the Chromatogram Immersion Device III, the plate is dipped into the ninhydrin solution (0.5 % in 2-propanol) with a dipping speed of 4.5 cm/s and 1 s immersion time. As another option ninhydrin can directly be added to the mobile phase at a 0.5 %-level. Both techniques are followed by heating on the TLC Plate Heater at 110 °C for 5 min.

Densitometry

Absorption measurement at 440 nm with TLC Scanner 3 and winCATS software

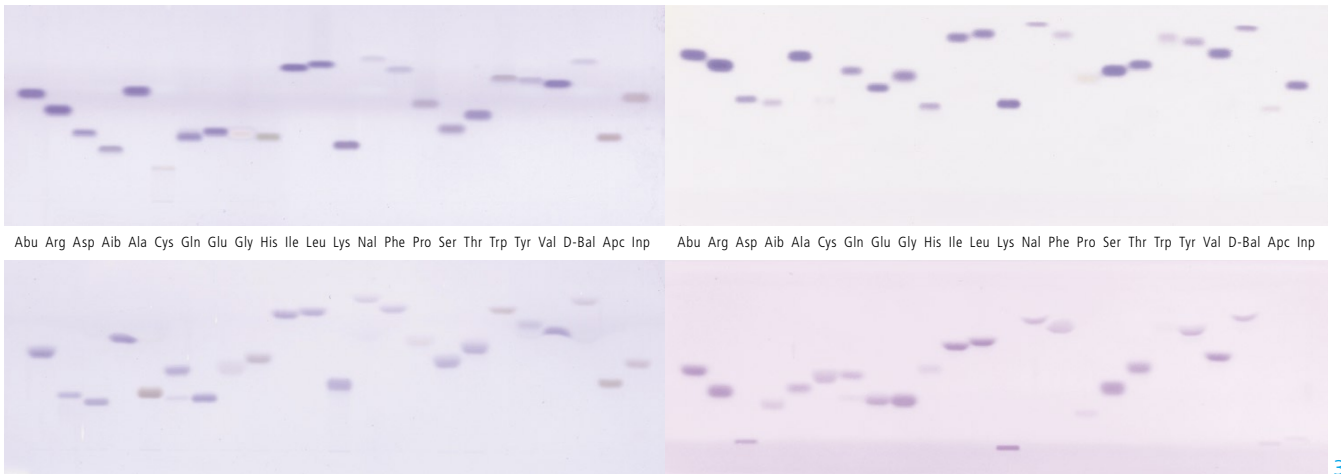
Results and discussion

The method on cellulose with a 2-butanol - acetic acid - pyridine - water mixture [1] was investigated first for analysis of the 24 amino acids of interest. The hR_F -values were more repeatable using the development in the horizontal chamber compared to the twin trough chamber due to the flexibility of positioning the aluminum foil in the vertical development mode. The selectivity and the sensitivity were good despite some critical pairs of amino acids. Thus, besides cellulose, the diol and silica gel phase were investigated as stationary phase (also faced upwards in the horizontal chamber). On the diol phase the amino acids migrated in the hR_F -range of 51-91 whereas the hR_F -range was wider on the silica gel (25-94) and cellulose phase (17-77). Hence, selectivity was found to be better on the cellulose and silica gel phase.



R_F -values of the amino acids on 3 different layers, i.e. cellulose (blue), silica gel (red) and diol phase (green)

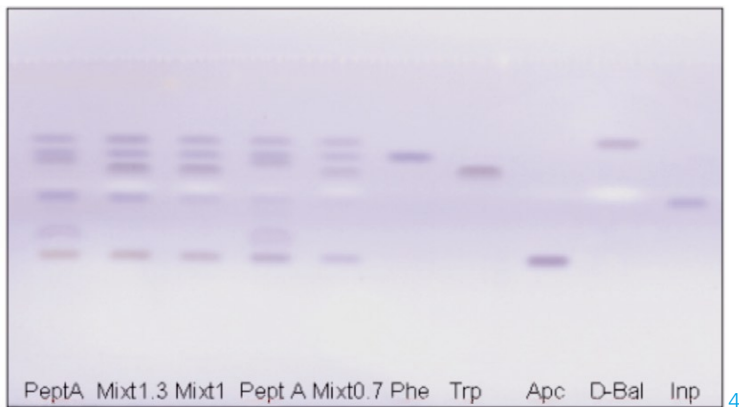
The selection of the chromatographic system depended on the task, i.e. which amino acids have to be separated. A general recommendation could not be given. However, two observations were made: 1) the development in the horizontal mode produced less diffusion of the zones, was more repeatable and allowed a better sensitivity on the silica gel plate compared to the vertical mode, and 2) the ammonia-containing mobile phase produced more diffuse zones compared to the acetic acid-containing mobile phase.



Separation on cellulose (left side) compared to that on silica gel (right side) using acidic (top) or alkaline (below) mobile phases

Based on these initial investigations with standard solutions, a peptide sample was to be analyzed. For this chromatography was chosen on cellulose with the acetic mobile phase. The hydrolyzed peptide containing two natural (Phe and Trp) and three non-natural amino acids (D-Bal, Apc and Inp) was well separated. All five amino acids were identified and quantified to be between 70 and 130% of the theoretical value for non-stable amino acids which can show degradation from 5 to 30%.

The ninhydrin derivatization was most convenient by direct addition of ninhydrin to the mobile phase. Using this mode, the baseline noise was decreased by a factor of 2. The selective derivatization with ninhydrin is advantageous for sample analysis because non-derivatized matrix does not interfere with the detection.



Chromatogram showing the hydrolyzed peptide (PeptA) containing two natural (Phe and Trp) and three non-natural amino acids (D-Bal, Apc and Inp) and standard mixtures in different concentrations as well as the single amino acids

Note: For Trp not identical isomers had been used (D-Trp for sample and L-Trp for standard) which was found out afterwards.

[1] Merck Application for detection of amino acids and peptides

Further information is available from the authors on request.

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Use of reversed phase (RP)-modified pre-coated plates



Michael Schulz, Susanne Minarik

Since 1966 Merck KGaA in Darmstadt has sold pre-coated plates for thin-layer chromatography. 1975 they started to sell HPTLC pre-coated plates. Reversed phases have been available since 1978; middle-polar phases were commercialized later on (1982–1987). Since 2007 Michael Schulz*, head of the laboratory PC-RPL-SIL (Performance & Life Science Chemicals, Research & Development, Silica Synthesis), has been responsible for research and development for thin-layer chromatography. This article was made together with Susanne Minarik.

Introduction

Most of all separations in planar chromatography are performed on pre-coated plates with polar adsorbents like silica gel, aluminum oxide or cellulose. The most commonly used stationary phase is silica gel 60 with a pore size of 6 nm. Although hydrophobic layers (reversed phases) offer several advantages they have not widely been used in planar chromatography.

RP-modified pre-coated plates can be used over a broad range of selectivity for all substance classes. Complex multi-component mobile phases are not necessary. Normally two-component mobile phases, e.g. of methanol and water, are sufficient. By variation of the water content in the mobile phase the retention can systematically be affected. The potential for the formation of artifacts of sensitive substances can be avoided because of the comparatively low surface activity of RP-adsorbents. The in-

fluence of the relative humidity on retention is not significant because of the hydrophobic surface properties and the use of an aqueous mobile phase. Another advantage of RP-layers is the transferability to HPLC methods, mostly performed on RP-18 columns.

In the following the influence of the hydrophobic surface property of two different reversed phases is discussed on the example of steroids separation. HPTLC plates RP-18 F_{254s} are compared with water-wettable HPTLC plates RP-18W F_{254s}.

Standard solutions

Steroids were dissolved in methanol (1.25 mg/mL each).

Layer

HPTLC plates RP-18W F_{254s} and HPTLC plates RP-18 F_{254s}, both 10 × 10 cm (Merck)

Application

Bandwise with Automated TLC Sampler 4 (ATS 4), band length 6 mm, track distance 9 mm, distance from the lower edge 10 mm, application volume 1 µL

Chromatography

Development in the Flat Bottom Chamber 10 × 10 cm with methanol – water 3:2

Post-chromatographic derivatization

Using the TLC/HPTLC-Sprayer the plate is sprayed with perchloric acid (20% in ethanol) and heated on the TLC Plate Heater at 100 °C for 5 min.

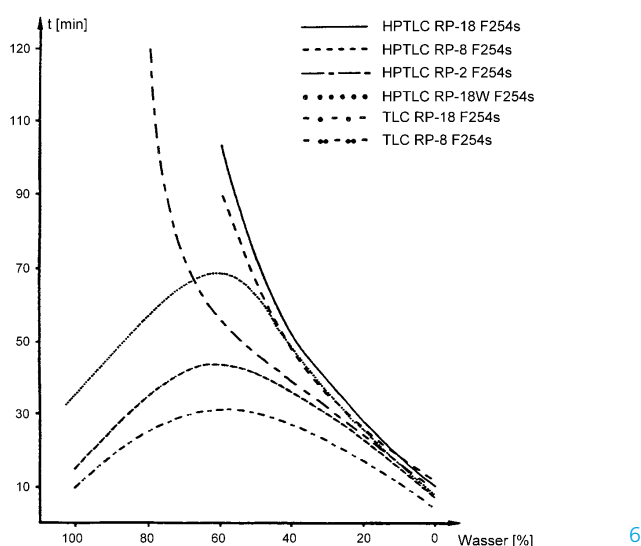
Results and discussion

In conventional thin-layer chromatography the mobile phase migrates because of capillary forces inside the layer. Lipophilic layers like the HPTLC RP-18 layer with a high degree of surface modification slow down or stop migration of the mobile phase depending on its water content. The maximal water content on layers with a high modification degree is between 40–70% depending on the degree of modification, the chain length of the alkyl group, and the composition of the mobile phase.

TLC pre-coated RP plates and water-wettable HPTLC plates RP-18W have a significant lower degree of modification compared to HPTLC plates RP-18. These layers are manufactured via a controlled modification process to modify only a defined amount of the available silanol groups. Chemically bonded alkyl chains support the RP-properties and the rest of the hydroxyl groups support the hydrophilic character of the adsorbent and its wettability by water.

Plate	Degree of surface modification [$\mu\text{mol}/\text{m}^2$]	Merck article
HPTLC RP-2 F _{254s}	3,9	1.13726.0001
HPTLC RP-8 F _{254s}	3,0	1.13725.0001
HPTLC RP-18 F _{254s}	2,6	1.13724.0001
HPTLC RP-18W F _{254s}	0,5	1.13124.0001
TLC RP-2 F ₂₅₄	2,5	1.05747.0001
TLC RP-8 F _{254s}	2,0	1.15388.0001
TLC RP-18 F _{254s}	1,7	1.15389.0001

Thus TLC RP plates and HPTLC plates RP-18W can be used up to a water content of 100% in the mobile phase. The dependence of the water content in the mobile phase acetone – water on migration time is shown for different RP-plates in the following.

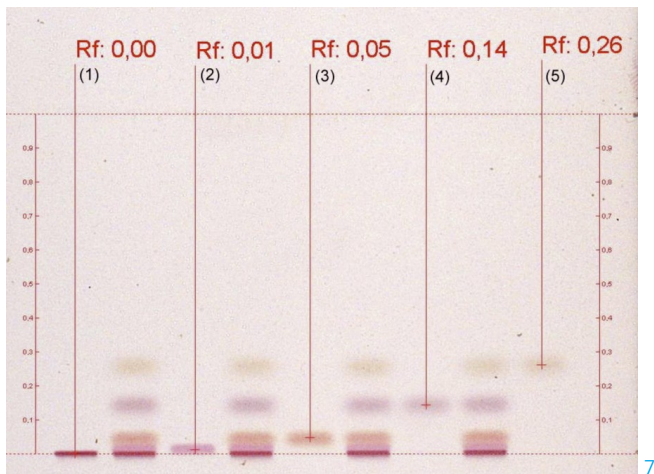


Dependence of the water content in the mobile phase acetone – water on migration time

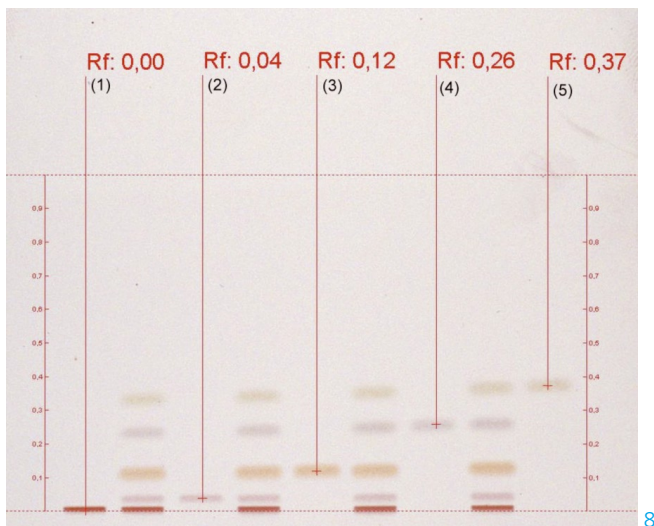
The following separation of steroids illustrates the difference between the HPTLC plate RP-18 F_{254s} and the HPTLC plate RP-18W F_{254s}. Both plates were developed with methanol – water 3:2. The degree of surface modification was extremely different, i.e. 2.6 $\mu\text{mol}/\text{m}^2$ for the HPTLC plate RP-18 F_{254s} compared to just 0.5 $\mu\text{mol}/\text{m}^2$ for the HPTLC plate RP-18W F_{254s}. In spite of the different degree of modification, the difference in retention was low. Free residual silanol groups were blocked by the water of the mobile phase and therefore they had almost no interaction with the sample molecules.

However, the difference in the migration time was huge. The migration time on the hydrophobic HPTLC plate RP-18 F_{254s} took 130 min compared to just 39 min on the water-wettable HPTLC plate RP-18W F_{254s}. This longer migration time led to greater band diffusion. In the case of the highly hydrophobic phases the maximal water content was 40%, whereas the water-wettable HPTLC plates RP-18W F_{254s} can be used with water content up to 100%.

RP-modified pre-coated plates are well suited for solving a lot of different separation problems and offer several advantages compared to pure silica gel layers. However, its degree of surface modification correlates with its wettability by water and thus RP-plates of a high degree of surface modification can only be used up to a defined water content in the mobile phase. If separations require highly aqueous mobile phases water-wettable layers with a low degree of surface modification are recommended. The higher price of such layers is highly compensated by its applicative advantages.



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Separation of steroids, i.e. (1) cholesterol, (2) stanozolol, (3) methyl testosterone, (4) Reichstein's S, and (5) hydrocortisone on a HPTLC plate RP-18 F_{254s} with a migration time of 130 min (top) and on a HPTLC plate RP-18W F_{254s} with a migration time of 39 min (bottom)

Further information is available from the authors on request.

* Michael Schulz, Merck KGaA, PC-RLP-SIL, Frankfurter Str. 250, 64293 Darmstadt, Germany, Tel. +49(0)6151-722830, michael.schulz@merck.de



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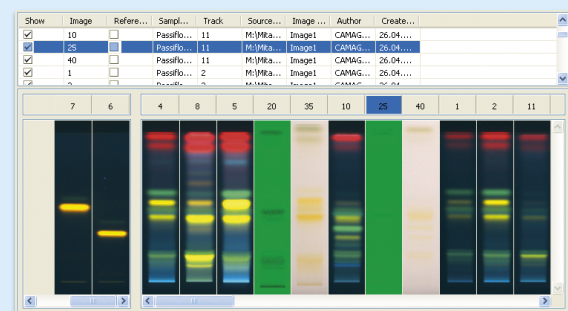
CAMAG TLC Visualizer

This completely new designed system for evaluation, visualization and archiving of planar chromatograms is the successor to CAMAG's DigiStore systems that were configured with the well known CAMAG Reprostar family.

- Image acquisition under UV 254 nm, UV 366 nm and visible light
- Powerful high-resolution 12 bit CCD digital camera with outstanding linearity
- Automatic image optimization and background correction

The TLC Visualizer with winCATS software conforms with cGMP, can be IQ/OQ qualified and is 21 CFR Part 11 compliant.

With the CAMAG TLC Visualizer the quality of the chromatograms is unsurpassed, as is shown by the documented example below (*Passiflora* extracts).



10

We proudly look back at 50 years CAMAG.

Several occasions have provided opportunities to celebrate this milestone.

The first highlight was a reception for our international distributors at the Analytica 2008 in Munich.

In 67 countries we are represented by contractual distributors. Most of these distributors have CAMAG product specialists, who were initially trained at our headquarters in Muttenz and regularly return for refresher courses. We invited these distributors for a reception at our Analytica stand and are very happy that 37 delegates turned up, mostly the bosses. As a result the meeting was a kind of family get together, because most of them knew one another and were all there on the same mission, to support CAMAG costumers to the best of their ability.



Business partners from Asia at the CAMAG Analytica stand

11

International HPTLC Symposium in Helsinki, 11.–13. June 2008

Another occasion for celebration was the International Symposium where many of our customers and their CAMAG distributors got together. During a cruise between Helsinki and Stockholm 40 oral papers and 32 posters on planar chromatography were presented. The CAMAG cocktail reception at the end of the cruise fittingly and appreciatively served as a symposium round-up and as a farewell party.



CAMAG reception on board of the Silja Symphony

12

Summer meeting of the CAMAG managers in Muttenz, 15.–19. August 2008

It has become a tradition that the managers of our daughter companies and our top distributors in China, India and France get together with CAMAG Muttenz management to exchange their experiences and plan strategies for the coming years. This year the meeting began with a convocation in Titisee, Black Forest. Excursions with walking tours around the lakes gave occasion for exchange of ideas and deepened personal relationships, before serious business discussions the following days.



International top distributors celebrating with the CAMAG management

13

**CAMAG LITERATURDIENST
CAMAG BIBLIOGRAPHY SERVICE
PLANAR CHROMATOGRAPHY**

CBS

Liebe Freunde

Mit Ihnen feiern wir 50 Jahre CAMAG – 5 Dekaden im Dienste der Dünnschicht-Chromatographie, der Analysenmethode, die schon mehrfach totgesagt wurde.

Dass die Planar-Chromatographie, wie wir die Methode zur Abgrenzung von der Technik von vor einem halben Jahrhundert bevorzugt nennen, heute leistungsfähiger denn je ist, verdanken wir nicht zuletzt dem ständigen Austausch mit unseren Kunden. Von ihnen erhielten wir immer wieder wertvolle Anregungen, Bedarfshinweise und in vielen Fällen sogar Lösungsvorschläge. Die engagierten und kreativen Mitarbeiter unseres Hauses entwickelten das komfortable und praxiserrechte Instrumentarium, das wir Ihnen heute anbieten können, und worauf wir stolz sind.

Aber wir lehnen uns nicht zurück. Es ist nach wie vor unser Bestreben, für Qualität und Innovation zu sorgen. So können wir bereits instrumentelle Lösungen für den heute sehr aktuellen Bereich der wirkungsbezogenen Detektion anbieten, und wir sind dabei, die Kopplung der Planar-Chromatographie mit der Massenspektrometrie instrumentell online zu realisieren.

Liebe Freunde, begleiten Sie uns weiter auf diesem Weg, den unser Firmengründer Dr. Dieter Jänchen vor 50 Jahren mit grossem Einsatz, Professionalität und Vision vorgegeben hat.

Mit freundlichen Grüssen

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

We are happy to celebrate with you 50 years CAMAG – 5 decades of supporting Thin-Layer Chromatography, the analytical technique that has been declared dead several times.

We are proud that Planar Chromatography, as we preferably call it in order to distinguish it from the technique of half a century ago, is today more efficient than ever. This we owe to a great extent to the perpetual feedback from our customers from whom we have received valuable suggestions, requirements, and often even technical solutions. Based on such input our highly motivated and creative staff members have developed the convenient and practical systems we can offer today.

But, we can not rest on our laurels. We must continue to strive for quality and innovation. Just recently we brought out the instrumental solution for utilizing selective detection of bioactive compounds, and we are in the process of finalizing online coupling of planar chromatography with mass spectrometry.

Dear friends, we ask for your continued loyal support and hope that you will accompany us down the pathway that the founder of our company, Dr. Dieter Jänchen initiated 50 years ago, a pathway based on strong effort, professionalism and vision.

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com



CAMAG

SEPTEMBER
2008

101

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
 - f) Clinico-chemical applications and profiling body fluids
 - g) Herbal and traditional medicines
- 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**

1. Reviews and books

- 101 001 N. ANGELOVA, H. KONG (Kong Hong), R. HEIDEN*, S. YANG (Yang Shih), Y. CHOI (Choi Young), H. KIM (Kim Hye), M. WANG (Wang Mei), T. HANKEMEIER, J. GREFF, G. XU (Xu Guowang), R. VERPOORTE (*Division of Analytical Biosciences, Leyden/Amsterdam Center for Drug Research, Leyden University, 2300 RA Leyden, The Netherlands, heijden@lacdr.leidenuniv.nl): Recent methodology in the phytochemical analysis of Ginseng. *Phytochem. Anal.* 19, 2-16 (2008). Recent developments in the phytochemical analysis of Panax ginseng are described, including different approaches such as the determination of the total saponin content and target compound and group-specific analysis using HPTLC-MS. In metabolite profiling, the paper describes the use of nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry.

food analysis, herbal, quantitative analysis, comparison of methods, review, HPTLC 1a

- 101 002 Teresa KOWALSKA, J. SHERMA (Eds): Thin-layer chromatography in chiral separations and analysis. *Chromatographic Science Series*, Vol. 98, CRC Press, Boca Raton, New York, 2007. XVI + 420 pp.; ISBN 978-0-8493-4369-8. The problem of analysis of enantiomers became acute with the realization that the enantiomers of a drug may have quite different biological activity. TLC for chiral separations is simpler and less expensive than HPLC and enables simultaneous analysis of several samples in a short time. Densitometry and videoscanning enable quantitative analysis with satisfactory accuracy, and - in certain cases - chiral plates can be regenerated. In 15 chapters 24 authors cover all important aspects of the separation and analysis of enantiomers by planar chromatography. In the first and second chapter an overview and general information on chirality is presented; the use of precoated plates, of noncommercial plates, of cellulose- and silica gel-based adsorbents is discussed in the chapters 3 to 5. Chapter 6 summarizes the use of mobile phases containing chiral selectors. Information on the molecular mechanisms and the separation of stable or transient diastereoisomers can be found in chapter 7 and 8, whereas the next chapter is devoted to especially difficult separations using 2D TLC. The next five chapters review applications of TLC in the analysis of enantiomers of important compounds. Extensive literature is cited by the authors.

1a

3. General techniques

- 101 003 L.W. BEZUIDENHOUT*, M.J. BRETT (*University of Alberta, Department of ECE, 2nd Floor, ECERF, Edmonton, AB, Canada T6G 2V4): Ultrathin layer chromatography on nanostructured thin films. *J. Chromatogr. A* 1183 (1-2), 179-185 (2008). Ultrathin layer chromatography (UTLC) with a 10 µm thick monolithic silica sorbent layer provides fast separations with low limits of detection and reduced analyte and solvent volumes. Production of UTLC plates with controllable nanostructure and thickness. The separation characteristics of the layer depend on the nanostructure of the film. Layers made with in-plane anisotropic nanostructures exhibit a decoupling effect, where the analyte spots do not develop in the same direction as the solvent front moves. Discussion of the possibility of application in multi-dimensional TLC by added layer morphology and material selection.

quantitative analysis, qualitative identification

3d

- 101 004 S. ERGÜL*, S. SAVASCI (*Department of Science Education, Faculty of Education, Ondokuz Mayıs University 55200, Atakum Yerleskesi-Samsun, Turkey): Acid modified diatomaceous earth - a sorbent material for thin layer chromatography. *J. Chromatogr. Sci.* 46 (4), 308-315 (2008). Investigation of the usefulness of flux calcinated diatomaceous earth (FCDE-I) (modified from natural diatomaceous earth and characterized by microscopical, physical, and chemical analyses) as a sorbent material for TLC. TLC of sodium diethyldithiocarbamate (DEDTC) and ammonium pyrrolidinedithiocarbamate (PyDTC) as Co or Cu complexes on silica gel and FCDE-I layer individually, and on various FCDE-I and silica gel mixtures. Development with toluene, toluene - cyclohexane 3:1, 1:1, 1:2, 1:3. The best separations were obtained with pure toluene, toluene - cyclohexane 3:1, and 1:1 respectively, using layers of FCDE-I - silica gel 1:3, and 1:1 respective-

ly. Qualitative analysis of mixtures of Cu²⁺ and Co²⁺ cations is possible with the same system.
comparison of methods 3b

- 101 005 Jolanta FLIEGER*, Malgorzata TATARCZAK (*Department of Inorganic and Analytical Chemistry, Medical University of Lublin, 20-081 Lublin, Staszica 6, Poland): Influence of inorganic mobile phase additives on the retention and separation efficiency of selected amino acids in thin-layer chromatography on cellulose layers. *J. Chromatogr. Sci.* 46 (6), 565-573 (2008). Investigation of selected amino acid standards on cellulose layers using organic-aqueous eluent systems modified with neutral and chaotropic salts: chlorides, iodides, nitrates, thiocyanates, perchlorates, and hexafluorophosphates at low concentrations from 10 to 80 mM in the mobile phase. The effect of salts used as mobile phase modifiers was evaluated by comparison of densitograms, peak symmetry coefficients, and theoretical plate numbers. The efficiency of the investigated chromatographic systems depends primarily on the kind of salt and organic solvent in the mobile phase. The best efficiency was obtained by adding ammonium thiocyanate to the mobile phase which contained acetonitrile as an organic modifier.

HPTLC, densitometry, qualitative identification, quantitative analysis 3d, 18a

- 101 006 M. LANCASTER*, D.M. GOODALL, E.T. BERGSTRÖM, S. MCCROSSEN, P. MYERS (*Department of Chemistry, University of York, York YO10 5DD, UK): Quantitative ultraviolet measurements on wetted thin-layer chromatography plates using a charge-coupled device camera. *J. Chromatogr. A* 1182 (2), 219-225 (2008). UV imaging of spots on TLC plates while still wet with solvent. Imaging of spots of benzophenone during and after development by using a charge-coupled device camera. The limit of detection was 5 ng on a wetted plate and 3 ng on a dry plate. The relationship between peak area and sample loading was linear in the low nanogram range over an order of magnitude for both wet and dry modes. It was shown that UV measurements on wet glass plates suffer from low sensitivity; however, increased sensitivity was achieved by using aluminium plates. The separation process can be monitored by measurement of UV absorbance during TLC development on aluminium-backed plates.

quality control, quantitative analysis, qualitative identification, 3f

- 101 007 M. LOPPACHER (CAMAG, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland, matthias.loppacher@camag.com): Controlling best the drying step. *CBS* 100, 9 (2008). The drying step contributes significantly to the overall experimental variability of the TLC system. Comparison of different drying procedures demonstrated that homogenous, rapid and heatless drying by the automatic developing chamber ADC 2 reduced the overall standard deviation by up to a factor of 3 compared to manual use of a hair dryer.

HPTLC, comparison of methods 3d

- 101 008 E. MINCSOVICS (OPLC-NIT Ltd., Andor u. 60, H-1119 Budapest, Hungary; and Corvinus University, Faculty of Horticultural Sciences, Department of Genetics and Plant Breeding, Budapest, Hungary; emil.mincsovics@t-online.hu): Potential of the flowing eluent wall and aliquot on-line detection in the OPLC 50 and 100 systems. *J. Planar Chromatogr.* 21, 97-102 (2008). The flowing-eluent-wall (FEW) procedure is suitable for single and multi-channel OPLC separation by operational segmentation of a non-segmented absorbent bed. OPLC of PTH-amino acids with chloroform - ethyl acetate 9:1 and of glycerol, diethylene glycol, ethylene glycol, 1,4-butanediol, 1,2-propanediol, 1,3-butanediol, 2,3-butanediol, and 1,6-hexanediol on silica gel with dichloromethane - n-dibutyl ether - acetone - acetic acid - water 50:15:20:13:3. Detection by derivatization with chromium(VI) oxide reagent, followed by heating at 120 °C for 5 min. Densitometry at 540 nm.

densitometry 3d

- 101 009 T. TANG (Tang Tie-Xin)*, W. HONG (Hong Wu) (*Center for Medicinal Plants Research, South China Agricultural University, Guangzhou 510642, China): An image analysis system for thin-layer chromatography quantification and its validation. *J. Chromatogr. Sci.* 46 (6), 560-564

(2008). Quantitative TLC is performed with a digital imaging system with simple equipment and software. The method was used for the quantitative assay of cichoric acid in *Echinacea purpurea*. TLC on polyamide phase with chloroform - methanol - formic acid - water 3:6:1:1. Detection by spraying with 3 % aqueous aluminum chloride solution. Images of chromatograms were acquired with a standard digital camera under a UV lamp at 365 nm in a dark room. The three-dimensional grayscale digital image dataset (x, y, gray) was reduced to a two-dimensional dataset (distance, accumulative gray) and then plotted as a curve. For quantification the area under the curve (corresponding to cichoric acid) was integrated.

qualitative identification, HPTLC, densitometry

3f

- 101 010 T. TUZIMSKI (Medical University of Lublin, Faculty of Pharmacy, Department of Physical Chemistry, Staszica 6, 20-081 Lublin, Poland; tomasz.tuzimski@am.lublin.pl): Strategy for separation of complex mixtures by multidimensional planar chromatography. *J. Planar Chromatogr.* 21, 49-54 (2008). Report of a new procedure for separation of complex mixtures by combining different modes of multidimensional planar chromatography on silica gel plates. Initially the complex mixture was separated into five groups of compounds. Mobile phases for separation of these different groups were then optimized by regarding each group as individual separation problem. By use of this new procedure 22 compounds from a complex mixture were separated on 10 x 10 cm TLC and HPTLC plates. TLC and HPTLC of 22 pesticides on silica gel with ethyl acetate - n-heptane 2:3 for TLC and 1:1 for HPTLC in the first direction. After removal of some of the adsorbent layer five further development steps with different eluents followed resulting in the complete separation of the 22 compounds of interest. Detection under UV 254 and 366 nm.

HPTLC, qualitative identification

3g

- 101 011 P.K. ZARZYCKI (Section of Toxicology and Bioanalytics, Department of Environmental Biology, Koszalin University of Technology, Sniadeckich 2, 75-453 Koszalin, Poland): Simple horizontal chamber for thermostated micro-thin-layer chromatography. *J. Chromatogr. A* 1187 (1-2), 250-259 (2008). Description of a simple, fast and robust temperature-controlled system for non-forced-flow micro-planar chromatography. Micro-TLC plates can be developed in horizontal position under controlled temperature and with temperature gradients between - 20 and 80 °C. Saturated or unsaturated chamber conditions are possible and only small amounts of mobile phase are required (0.3 to 1 mL). Plate temperature equilibration is obtained within 5 to 12 min and a typical non-forced flow run can be finished within only 5 to 20 min. On the micro-plate more than 10 spots can be separated in one direction or up to 180 spots per plate for two-dimensional and multi-development runs. Demonstration of fast and efficient separation of a number of analytes including fullerenes, cyclodextrins and steroids as well as complex samples obtained from natural products and pharmaceutical formulations. Thermostated micro-planar chromatography can be applied in retention and quantification studies.

quantitative analysis, qualitative identification

3d

- 101 012 P.K. ZARZYCKI*, M B. ZARZYCKA (*Toxicology and Bioanalysis Section, Department of Environmental Biology, Koszalin University of Technology, Sniadeckich 2, 75-453 Koszalin, Poland; pkzarz@wp.pl): Low cost, efficient TLC sprayer. *J. Planar Chromatogr.* 21, 221-223 (2008). Construction of a simple and inexpensive compressed air-driven spraying device which can handle small and large volumes of TLC derivatization reagents. The device is made from widely available HPLC accessories, including stainless-steel fittings and tubing, and can be used with corrosive liquids with a wide range of viscosity. TLC of fluorescein, methyl red, bromocresol green, phenolphthalein, and crystal violet on RP-18 with methanol - water 7:3. Evaluation under white light.

qualitative identification, quantitative analysis

3e

4. Special techniques

- 101 013 D.J. JANECKI, A.L. NOVOTNY, S.D. WOODWARD, J.M. WISEMAN*, D. NUROK (*Prosolia

Inc., 351 West 10th Street, Indianapolis, IN 46202, USA; wiseman@prosolia.com): A preliminary study of the coupling of desorption electrospray ionization-mass spectrometry with pressurized planar electrochromatography. *J. Planar Chromatogr.* 21, 11-14 (2008). Pressurized planar electrochromatography (PPEC) of 4-cholesten-3-one, 4-androsten-17 β -ol-3-one, 17 α -acetoxy progesterone, androstenedione, 2'-acetonaphthone, benzanilide, 2-nitroaniline, hydrocortisone, and benzamide on spherical RP-18 phase, cut into 3.3 x 12 cm sections. The mobile phase was 55 % aqueous acetonitrile containing 5 mM acetate buffer pH 4.7. The apparatus for PPEC has been described by Nurok et. al., *Anal. Chem.* 78, 2823-2832 (2006). Nine analytes were separated in 2 min. Detection by direct analysis of the TLC plate using DESI coupled to a tandem mass spectrometer.

4e

- 101 014 A. ORINÁK, I. TALIAN, E.V. EFREMOV, F. ARIESE, Renata ORIÁAKOVÁ (*Institute of Chemistry Sciences, Department of Physical Chemistry, University of P. J. Safárik, Moyzesova 11, 041 54 Kosice, Slovak Republic): Diterpenoic acids analysis using a coupled TLC-surface-enhanced Raman spectroscopy system. *Chromatographia* 67 (3-4), 315-319 (2008). Presentation of a hyphenation technique of TLC with surface-based spectral methods which require a homogeneous surface for direct and quantitative analysis on the chromatographic plate after separation. Investigation of the suitability of two different chromatographic substrates and one interface for coupling surface-enhanced Raman spectroscopy (SERS) with TLC, since most chromatographic materials do not produce strong background signals in Raman spectroscopy. Evaluation of a chromatographic thin layer, specially produced for Raman spectroscopy measurements, a monolithic silica thin layer and a typical TLC plate with a modified aluminium backplate foil on one side used as an interface. Test analytes were three biologically active diterpenes (gibberellic acid, abietic acid, and kaurenoic acid) which were applied directly onto the surfaces, followed by the addition of silver colloid and measurements by SERS. The strongest signal (excitation at 514.5 nm) for gibberellic acid was obtained using a Raman treated thin layer where the enhancement factor value was determined to be 102, and several fundamental Raman bands for GA were found at 1622, 1593, 1570, 1542, 1366 and 1236 cm⁻¹. However, no useful SERS signals were observed when the monolithic silica layer was used. Similar SERS spectra on modified aluminium backplate were obtained for abietic acid and gibberellic acid, but no SERS spectrum was obtained for kaurenoic acid.

quality control, quantitative analysis, qualitative identification, comparison of methods 4e

- 101 015 E. TYIHÁK*, E. MINCSOVICS, G. KÁTAY, Z. KIRÁLY-VÉGHÉLY, Á.M. MÓRICZ, P.G. OTT (*Plant Protection Institute, Hungarian Academy of Sciences, Herman Ottó Str. 15, P. O. B. 102, 1525 Budapest, Hungary; etyih@nki.hu): BioArena: An unlimited possibility of biochemical interactions in the adsorbent layer after chromatographic separation. *J. Planar Chromatogr.* 21, 15-20 (2008). TLC and OPLC of polyacetylenes of chamomile, trans-resveratrol, salicylic acid, and ochratoxin A on silica gel (preconditioned at 120 °C for 3 h) with chloroform - methanol 20:1. Detection by bioautography with *Pseudomonas savastanoi* pv. *phaseolicola* race 6 and by immersing the humid plate into MTT solution for 5 s.

herbal, densitometry, quantitative analysis, qualitative identification, biochemistry 4e

7. Phenols

- 101 016 Alina PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Jagiellonska 4, 41-200 Sosnowiec, Poland; alinapyka@wp.pl or apyka@slam.katowice.pl): Application of electrotopological states in QSSR and QSAR analysis of isomeric methylphenols separated by RP-TLC. *J. Planar Chromatogr.* 21, 205-208 (2008). TLC of phenol and its methyl derivatives on RP-2, RP-8, RP-18, cyano phase, and DIOL phase with methanol - water, ethanol - water, n-propanol - water, acetonitrile - water, and acetone - water in different volume proportions (100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, and 65:35), 0.1 M acetic acid and 0.1 M sodium acetate in 30 % methanol (pH 5.0), 0.1 M potassium dihydrogen phosphate in 30 % methanol (pH 7.0), and 1 M ammonia in 30 % methanol (pH 11.3) in a saturated chamber. Best

results were achieved on RP-2 with 0.1 M acetic acid and 0.1 M sodium acetate in 30 % methanol, 0.1 M potassium dihydrogen phosphate in 30 % methanol, and 1 M ammonia in 30 % methanol. Detection by spraying with 2 % methanolic iron(III) chloride solution or with a solution of titanium tetrachloride in concentrated hydrochloric acid (20 %) followed by heating at 120 °C for 30 s or 2 min.

qualitative identification

7

- 101 017 A. SCHUBERT, D.F. PEREIRA, F.F. ZANIN, S.H. ALVES, R.C.R. BECK, Margareth LINDE ATHAYDE* (*Departamento de Farmácia Industrial, Prédio 26, sala 1115, Campus Camobi, Universidade Federal de Santa Maria, RS, Brasil. CEP 97105-900; marga@ccs.ufsm.br): Comparison of antioxidant activities and total polyphenolic and methylxanthine contents between the unripe fruit and leaves of *Ilex paraguariensis* A. St. Hil. *Pharmazie* 62, 876-880 (2007). TLC of matessaponin 1 and matessaponin 2 on silica gel with chloroform - ethanol - water 12:8:1 and 8:8:1. Detection by spraying with anisaldehyde sulfuric acid reagent followed by heating at 100 °C.
- food analysis, qualitative identification

7

8. Substances containing heterocyclic oxygen

- 101 018 T. HOFMANN*, L. ALBERT, T. RÉTFALVI, E. VISI-RAJCZI, G. BROLLY (*University of West Hungary, Department of Chemistry, Ady Endre u. 5, 9400 Sopron, Hungary; hofmann@emk.nyme.hu): TLC analysis of the in-vitro reaction of beech (*Fagus sylvatica* L.) wood enzyme extract with catechins. *J. Planar Chromatogr.* 21, 83-88 (2008). TLC of (+)-catechin and (-)-epicatechin on silica gel with diisopropyl ether - formic acid 9:1 in an unsaturated twin-trough chamber. Detection by spraying with vanillin-sulfuric acid reagent followed by heating at 120 °C for 5 min. Quantitative determination by absorbance measurement at 490 nm.
- herbal, densitometry, qualitative identification, quantitative analysis

8b

- 101 019 Liselotte KRENN*, M. STEITZ, C. SCHLICHT, H. KURTH, F. GAEDCKE (*Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; liselotte.krenn@univie.ac.at): Anthocyanin- and proanthocyanidin-rich extracts of berries in food supplements - analysis with problems. *Pharmazie* 62, 803-812 (2007). TLC of anthocyanins in aronia, blueberry and lingonberry extracts and the respective preparations (e.g. capsules, lozenges, granulate) on silica gel with ethyl acetate - water - anhydrous formic acid 10:3:2 and 10:4:1. Detection in white light prior and after spraying with iron(III)chloride solution or with vanillin phosphoric acid reagent.
- food analysis, cosmetics, review, qualitative identification

8a

11. Organic acids and lipids

- 101 020 A.R. ADEGBOLA*, E.O. DARE, A.A. LASISI (*Department of Chemistry, University of Agriculture, P. O. Box 28, UNAAB post office, Abeokuta, Nigeria): Spray reagents for the visualization and detection of sesame oil unsaponifiables on thin-layer chromatograms. *Chromatographia* 68 (1-2), 151-153 (2008). Anisaldehyde and crotonaldehyde reagent are suitable as spray reagents for the detection of major components of sesame oil unsaponifiables. Derivatization with anisaldehyde reagent lead to chromatograms with coloured zones characteristic for lignans and sterols in *Sesamum indicum*. By derivatization with crotonaldehyde four of the eleven unsaponifiable matters could be detected as more stable and brightl coloured zones. The results obtained could serve as a guide in the monitoring of oil's stability, adulteration and contaminations.
- pharmaceutical research, quality control, postchromatographic derivatization, quantitative analysis

11

- 101 021 K. LING, C. CHEUNG, S. CHENG, L. CHENG, S. LI (Li SongLing), P. NICHOLS, R. WARD, A. GRAHAM, P. BUT* (*Food and Drug Authentication Laboratory, Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China, paulbut@cuhk.edu.hk):

Rapid detection of oilfish and escolar in fish steaks: a tool to prevent keriorrhea episodes. *Food Chem.* 110, 538-546 (2008). TLC of wax esters of oilfish (*Ruvettus pretiosus*) and escolar (*Lepidocybium flavobrunneum*) on silica gel with xylene. Detection by spraying with 40 % sulfuric acid in ethanol - anisaldehyde 9:1, followed by heating at 100 °C for 2 min or until color is observed. The R_f value of wax esters was 60. The method was compared with DNA sequencing and GC-MS. The TLC method is inexpensive, provides a reliable result within 30 min, and is suitable for screening of numerous samples.

food analysis, qualitative identification

11a

- 101 022 J.D. VASTA, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, USA; friedb@lafayette.edu): HPTLC analysis of neutral lipids in the urine of humans and BALB/c mice. *J. Planar Chromatogr.* 21, 39-42 (2008). HPTLC of neutral lipids and ubiquinone on silica gel with petroleum ether - diethyl ether - acetic acid 80:20:1. Detection with phosphomolybdic acid reagent. Quantitative determination by densitometry at 610 nm. Specific detection reagents were used to confirm the identity of particular lipid classes. The studies confirmed the presence of free sterols, free fatty acids, methyl esters, hydrocarbons and ubiquinone, and triacylglycerols.

clinical chemistry research, HPTLC, densitometry, quantitative analysis

11c

13. Steroids

- 101 023 K. SHANKER*, S.C. SINGH, S. PANT, P. SRIVASTAVA, A.K. YADAV, R. PANDEY, R.K. VERMA, M.M. GUPTA (*Analytical Chemistry Division, Central Institute of Medicinal and Aromatic Plants, Lucknow, 226015, India): Quantitative TLC analysis of sterol (24beta-ethylcholesta-5,22 E,25-triene-3beta-ol) in *Agnimantha* (*Clerodendrum phlomidis* Linn). *Chromatographia* 67 (3-4), 269-274 (2008). Presentation of a quantitative method using silica gel HPTLC plates, automated bandwise sample application, and automated visible mode densitometry for the determination of 24beta-ethylcholesta-5,22E,25-triene-3beta-ol (ECTO) in the aerial part of *Clerodendrum phlomidis*, which was used as a chemical marker for the standardization of *C. phlomidis* plant extracts. HPTLC on silica gel with chloroform - methanol 197:3. Detection by derivatization with anisaldehyde reagent. Quantitative determination by absorbance measurement at 650 nm. Linearity was between 150 and 400 ng/band with good correlation ($r^2 = 0.996$).

pharmaceutical research, quality control, traditional medicine, HPTLC, densitometry, quantitative analysis, qualitative identification

13

14. Steroid glycosides, saponins and other terpenoid glycosides

- 101 024 S.A. SASMAKOV, ZH.M. PUTIEVA, Ulrike LINDEQUIST* (*Institute of Pharmacy, Ernst Moritz Arndt University Greifswald, 17487 Greifswald, Germany, lindequi@uni-greifswald.de): New pomolic acid triterpene glycosides from *Zygophyllum eichwaldii*. *Pharmazie* 62, 957-959 (2007). TLC of sugars (after acid hydrolysis) on silica gel or RP-18 after impregnation with 0.3 M sodium dihydrogen phosphate, with n-butanol - methanol - water 5:3:1. Detection by spraying with o-toluidine salicylate.

herbal, qualitative identification

14

17. Amines, amides and related nitrogen compounds

- 101 025 M. BLEICHERT, H.-S. ECKHARDT, K.-F. KLEIN, B. SPANGENBERG* (*University of Applied Sciences Offenburg, Badstrasse 24, 77652 Offenburg, Germany; Spangenberg@FH-Offenburg.de): A simple and reliable method for quantification of glucosamine in nutritional supplements. *J. Planar Chromatogr.* 21, 55-59 (2008). HPTLC of glucosamine on amino phase with methanol - ethanol - 25 % ammonia 1:1:1 in a saturated chamber. Detection by heating at 160 °C for 10 min. A Tidas TLC 2010 system was used for direct spectrophotometry of the HPTLC plates. Averaged glucosamine densitograms in the wavelength range 305 to 330 nm were obtained from the spectra.

food analysis, quality control, HPTLC, densitometry, quantitative analysis 17a

18. Amino acids and peptides, chemical structure of proteins

101 005 Jolanta FLIEGER et al., see section 3d

101 026 H. MÖHRLE*, J. BERLITZ (*Institut für Pharmazeutische und Medizinische Chemie, Universitätsstrasse 1, 40225 Düsseldorf; h.moehrle@uni-duesseldorf.de): Oxidation von Nicotin und Chelatbildner durch Quecksilber(II)-Verbindungen (Oxidation of nicotine and chelating agent by mercury(II)-compounds). Pharmazie 62, 7-13 (2008). TLC of nicotine on silica gel with chloroform - ethanol - ammonia 80:20:1. Detection by spraying with Dragendorff reagent. TLC of iminodiacetic acid on silica gel with n-propanol - water 7:2 or with n-butanol - acetic acid - water 4:1:1. Detection by spraying with ninhydrin reagent.

toxicology, qualitative identification 18a

22. Alkaloids

101 027 V. MIRAKOR, V. VAIDYA*, S. MENON, P. CHAMPANERKER, A. LAUD (*Therapeutic Drug Monitoring Laboratory, 194, Scheme No. 6, Road No. 15, Sion Koliwada, Sion (East), Mumbai 400-022, India; tdmlab@vsnl.net, vaidya_vikas@yahoo.com): HPTLC method for determination of colchicine in a pharmaceutical formulation. J. Planar Chromatogr. 21, 187-189 (2008). HPTLC of colchicine on silica gel with ethyl acetate - acetonitrile - water - formic acid 16:2:1:1 in a twin-trough chamber saturated for 10 min. Quantification by densitometry at 358 nm.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 22

101 028 N. MUKARRAMOV, KH. SHAKIDOYATOV* (*Institute of the Chemistry of Plant Substances, Academy of Sciences, Kh. Abdullaev Str. 77, Tashkent, Uzbekistan, shakidoyatov@rambler.ru): Product control: bromination and oxidation of the alkaloid deoxyepanine. CBS 100, 6-7 (2008). HPTLC of the alkaloid deoxyepanine (from Peganum harmala) and bromination reaction products on silica gel over 60 mm with chloroform - methanol - acetone - cyclohexane 5:1:5:5 for Merck phases and 4:1:4:4 for Whatman phases. Quantitative determination by absorbance measurement at 254 nm.

herbal, densitometry, HPTLC, quantitative analysis 22

101 029 Anna PETRUCZYNIK*, Monika WAKSMUNDZKA-HAJNOS, T. PLECH, T. TUZIMSKI, M.L. HAJNOS, G. JÓZWIAK, Maria GADZIKOWSKA, Anna ROMPALA (*Department of Inorganic and Analytical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): TLC of alkaloids on cyanopropyl bonded stationary phases. Part II. Connection with RP18 and silica plates. J. Chromatogr. Sci. 46 (4), 291-297 (2008). Optimization of the one-dimensional TLC of alkaloid standards on cyano phase, RP-18W, and silica gel with various eluents containing silanol blockers (besides diluent and modifier), such as diethyl amine or ammonia. Separation of alkaloid mixtures with the most selective system (e.g. methanol - water 4:1 in the first direction and methanol - acetone - diisopropylether - diethylamine 15:15:69:1 in the second direction) with an adsorbent gradient method. Alkaloids or plant extracts of Chelidonium majus, Fumaria officinalis, or Glaucium flavum were chromatographed in the first system, the plates were connected with the plate pre-coated with various adsorbents, and partly separated fractions were transferred to the second layer and developed in a second system. Cyano - silica - RP-18W and cyano - silica - silica were used as the connected layers. The alkaloids were identified based on the R_f values of standards, and by comparison of UV spectra obtained by densitometry with a diode array detector.

pharmaceutical research, quantitative analysis, qualitative identification, densitometry, comparison of methods 22

101 030 Jarmila SKARKOVA*, V. OSTRY, J. RUPRICH (*National Institute of Public Health, Centre

for Hygiene of Food Chains, Palackeho 3a, 612 42, Brno, Czech Republic; skarkova@chpr.szu.cz): Instrumental HPTLC determination of alpha-solanine and alpha-chaconine in peeled potato tubers. *J. Planar Chromatogr.* 21, 113-117 (2008). HPTLC of alpha-solanine and alpha-chaconine on silica gel with chloroform - methanol - water - aqueous ammonia 140:60:10:1. Detection by dipping in cerium(IV) sulfate derivatization reagent. Quantitative determination by absorbance measurement at 505 nm.

herbal, food analysis, HPTLC, densitometry, quantitative analysis

22

- 101 031 Monika WAKSMUNDZKA-HAJNOS*, G. W. JÓZWIAK (*Department of Inorganic Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland; monika.hajnos@am.lublin.pl): Special modes of development in preparative-layer chromatography of extracts of alkaloids from *Fumaria officinalis*. *J. Planar Chromatogr.* 21, 61-66 (2008). Investigation of overloading, methods of sampling, effect of layer thickness on band resolution, and the effect of derivatization on the location of the separated band. Another objective of the work was the investigation of the use of unidimensional multiple development (UMD) and incremental multiple development (IMD) for resolution of the alkaloids from *Fumaria officinalis* extracts. TLC of medium polarity alkaloids and strongly polar alkaloids on silica gel with acetic acid - propanol - water 1:4:5 and with acetic acid - water - methanol - dichloromethane 2:1:8:29 (UMD technique), and with acetic acid - propanol - dichloromethane 1:4:5 (IMD technique). Detection by derivatization with Dragendorff reagent. Quantification by densitometry at 520 nm.

herbal, quality control, preparative TLC, densitometry

22

23. Other substances containing heterocyclic nitrogen

- 101 032 Marta STEFANIAK (Department of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland; m_stefaniak@op.pl) : Effect of temperature on the R_F of porphine and its metal complexes in normal and reversed-phase TLC. *J. Planar Chromatogr.* 21, 201-204 (2008). TLC of porphine and its complexes with Cu(II), Ni(II), and Zn(II) on silica gel with chloroform - n-hexane 5:5, 3:2, and 7:3; and on RP-18 with methanol, methanol - water 9:1, ethanol, and ethanol - water 9:1 at 5+/- 2, 15+/- 2, 25+/- 2, 35+/- 2, 45+/- 2, and 55+/- 2°C. Detection under white light. An increased temperature results in increased R_F values.

comparison of methods

23a

25. Organic phosphorus compounds

- 101 033 S. FUJIWARA*, A. YAMANAKA, Y. YAMADA, K. HIROOKA, H. HIGASHIBATA, W. FUKUDA, J. NAKAYAMA, T. IMANAKA, E. FUKUSAKI (*Department of Bioscience, Nanobiotechnology Research Center, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda 699-1337, Japan, fujiwara-s@kwansei.ac.jp): Efficient synthesis of trans-polyisoprene compounds using two thermostable enzymes in an organic-aqueous dual-liquid phase system. *Biochem. Biophys. Res. Commun.* 365, 118-123 (2008). TLC of prenyl alcohols (farnesol and geranylgeraniol) after enzymatic hydrolysis of trans-isoprenyl diphosphates on RP-18 with acetone - water 9:1. Detection with iodine vapour. Quantitative determination by measuring the absolute radioactivity of the spots.

pharmaceutical research, quantitative analysis, qualitative identification, radioscanning

25

27. Vitamins and various growth regulators

- 101 034 T. HODISAN, Dorina CASONI*, M. S. BELDEAN-GALEA, C. CIMPOIU (*Faculty of Chemistry and Chemical Engineering, Babes Bolyai University, 11 Arany Janos, 400686 Cluj-Napoca, Romania; casoni_dorina@yahoo.com): Identification and quantification of tocopherols in vegetable oil by thin-layer chromatography. *J. Planar Chromatogr.* 21, 213-215 (2008). TLC of alpha-, gamma-, and delta-tocopherol in sunflower, olive, corn, soy, and almond oil on silica gel with chloroform. Drying at room temperature. Detection by spraying with a 1:1 mixture of 0.5 % bipyridyl in methanol and 0.2 % iron(III) chloride in methanol. Densitometric evaluation with a

flatbed scanner.

food analysis, qualitative identification

27

28. Antibiotics, Mycotoxins

- 101 035 A. KWIECIEN, J. KRZEK*, L. BINIEK (*Jagiellonian University, Collegium Medicum, Department of Inorganic and Analytical Chemistry, Medyczna 9 str., 30-688 Kraków, Poland; jan-krzek@cm-uj.krakow.pl): TLC-densitometric determination of azithromycin in pharmaceutical preparations. *J. Planar Chromatogr.* 21, 177-181 (2008). TLC of azithromycin on silica gel with chloroform - ethanol - ammonia 30:70:1. Detection by spraying with sulfuric acid - ethanol 1:4 followed by heating at 120 °C for 5 min. Quantification by densitometry at 483 nm.

pharmaceutical research, quality control, densitometry, quantitative analysis 28

- 101 036 S.R. NAIK*, S.K. DESAI, R.K. NANDA, M.S. NARAYANAN (*Sinhgad Institute of Pharmaceutical Sciences, S. No. 309/310, Kusgaon (BK), Off Mumbai Pune Expressway, Lonavala, Pune 410401, India; sipslonavala@yahoo.co.in): Fermentation, isolation, purification, and biological activity of SJA-95, a heptaene polyene macrolide antibiotic produced by the *Streptomyces* sp. strain S24. *Arzneim.-Forsch./Drug Res.* 56, 171-179 (2007). TLC of SJA-95 and mycosamine on silica gel with butanol - acetic acid - water - tetrahydrofuran 6:2:2:1 and butanol - acetic acid - water - dioxane 6:2:2:1. Detection under UV 254 nm and under white light after spraying with ninhydrin reagent.

pharmaceutical research, preparative TLC, qualitative identification 28a

- 101 038 Z.Z. RAYKOV, K. VASSILEV, G. GRIGOROVA, A. LYAPOVA, A. ALEXIEV*, G. PETROV (*Faculty of Chemistry, University of Sofia, 1 J. Bourchier Boul., 1164 Sofia, Bulgaria; alexiev@chem.uni-sofia.bg): Spin-labeled rifamycin: biological activity. *Pharmazie* 63, 61-66 (2008). TLC of 3-[(2,2,6,6-tetramethylpiperidine-4-ylimino)methyl]rifamycin and 3-[(2,2,6,6-tetramethyl-1-oxyl-piperidine-4-ylimino)methyl]rifamycin on silica gel with chloroform - methanol 4:1. Detection under white light.

pharmaceutical research, qualitative identification 28a

- 101 039 R. VOLK (Department of Pharmaceutical Biology, Pharmaceutical Institute, University of Kiel, Kiel, Gutenbergstrabe 76, 24118 Kiel, Germany, volk@pharmazie.uni-kiel.de): A newly developed assay for the quantitative determination of antimicrobial (anticyanobacterial) activity of both hydrophilic and lipophilic test compounds without any restriction. *Microbiol. Res.* 163, 161-167 (2008). Test solutions containing 0.25 to 64 µg of norharmane, quinine, and tetracycline (as bases and hydrochloride salts) were applied as 10 mm bands on silica gel plates. After coating the plate with a concentrated suspension of the living cyanobacterial test organism (spraying or dipping), it was kept moist in a TLC chamber at 27 °C for 1 to 2 days under continuous illumination (25 - 30 µmol photon/m²s). Cytotoxic concentrations of a test compound resulted in an easily recognizable regional decolourisation of the test organism.

pharmaceutical research, environmental, qualitative identification, densitometry 28a

29. Pesticides and other agrochemicals

- 101 040 V.R. CHANDEGAONKAR, D.B. SHINDE, D.V. MANE* (*Department of Chemistry, Chhatrapati Shivaji College, Omurga, (MS)-413606, India; manedv.2007@rediffmail.com): A new chromogenic spray reagent for detection and identification of monocrotophos. *J. Planar Chromatogr.* 21, 199-200 (2008). TLC of monocrotophos (after alkaline hydrolysis) and other organophosphorus insecticides (malathion, parathion, fenthion, dimethoate, and phorate), organochlorine insecticides (endosulfan and HCH), carbamate insecticides (Baygon and carbaryl), synthetic pyrethroid insecticides (cypermethrin and deltamethrin) on silica gel with chloroform - acetone 7:3 in a saturated chamber. Detection by spraying first with 5 % sodium hydroxide then with 1 % vanillin reagent (1 % vanillin in acetone) followed by heating at 100 °C for 10 min. The detection

limit for monocrotophos was 500 ng.

toxicology, agricultural, qualitative identification

29b

- 101 041 R. MAVLE, H. KATKAR, B. DAUNDKAR, M. MALVE, R. KRISHNAMURTHY* (*Directorate of Forensic Science Laboratories, Home Dept, State of Maharashtra, Mumbai 400098, India, dfsl.mah@gmail.com): Thin layer chromatographic technique for detection and identification of endosulfan insecticide with m-dinitrobenzene reagent. *J. Planar Chromatogr.* 21, 197-198 (2008). TLC of endosulfan on silica gel with n-hexane - acetone 4:1 in a saturated twin-trough chamber. Detection by spraying with 5 % aqueous sodium hydroxide solution followed by heating for 5 min. The air-cooled plate was then sprayed with a 2 % solution of m-dinitrobenzene in dimethyl sulfoxide. The limit of detection was 5 µg.

toxicology, agricultural, qualitative identification

29a

30. Synthetic and natural dyes

- 101 042 F. SOPONAR*, A.C. MOT, C. SÂRBU (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, Arany Janos Street 11, 400028 Cluj Napoca, Romania): Quantitative determination of some food dyes using digital processing of images obtained by thin-layer chromatography. *J. Chromatogr. A* 1188 (2), 295-300 (2008). Determination of food dyes (tartrazine, azorubine and sunset yellow) in different products by HPTLC combined with image processing of scanned chromatograms, on 3-aminopropyl modified silica gel with isopropanol - diethyl ether - ammonia 2:2:1. Quantification by using digital processing of images with special-purpose software. The limit of detection was between 5 and 9 ng/spot and the limit of quantification was between 10 and 18 ng/spot. Recovery was between 96.4 and 102.7 %.

quality control, food analysis, HPTLC, quantitative analysis, qualitative identification 30

- 101 043 T. TUZIMSKI*, A. WOZNIAK (*Medical University of Lublin, Faculty of Pharmacy, Chair of Chemistry, Department of Physical Chemistry, Staszica 6, 20-081 Lublin, Poland; tomasz.tuzimski@am.lublin.pl): Application of solid-phase extraction and planar chromatography with diode-array detection to the qualitative and quantitative analysis of dyes in beverages. *J. Planar Chromatogr.* 21, 89-96 (2008). TLC of dyes in normal-phase systems on silica gel, diol phase, cyano phase, and amino phase, and in reversed-phase systems on cyano phase, diol phase, amino phase, and RP-18. RP chromatography with different mobile phase modifiers (THF, dioxan, methanol, acetonitrile, and acetone) at different concentrations, containing different amines, cationic and anionic ion-pair reagents, buffers, and ammonia, again at different concentrations. Based on the results the best system was selected: HPTLC of tartrazine, sunset yellow FCF, allura red AC, ponceau 4R, brilliant blue FCF, indigotine, brilliant black PN, quinoline yellow, patent blue V, brilliant green BS, azorubin, and brown HT on RP-18 with acetate buffer pH 3.5 containing 15-25 % modifier and 0.025 M propylamine or diethylamine. Detection in white light and under UV 254 and 366 nm. Quantification by diode array densitometry in the range of 191 to 1033 nm.

food analysis, quality control, HPTLC, quantitative analysis, qualitative identification 30a

32. Pharmaceutical and biomedical applications

- 101 044 O.B. ABDEL-HALIM, A.M. MARZOUK*, R. MOTHANA, N. AWADH (*Department of Pharmacognosy, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt; amarzouk2003@yahoo.co.uk): A new tyrosine inhibitor from *Crinum yemense* as potential treatment for hyperpigmentation. *Pharmazie* 63, 405-407 (2008). TLC of (+)-haemanthamine on silica gel or on RP-18. Detection by spraying with Dragendorff reagent or with 1 % cerium sulfate - sulfuric acid, followed by heating.

herbal, pharmaceutical research, qualitative identification

32e

- 101 045 A. ABOU-DONIA*, S. TOAIMA, H. HAMMODA, E. SHAWKY (*Department of Pharma-

cognosy, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt, shawkyeman@yahoo.com): New rapid validated HPTLC method for the determination of galanthamine in Amaryllidaceae plant extracts. *Phytochem. Anal.* 19, 353-358 (2008). HPTLC of galanthamine in the bulbs of *Narcissus* cv. on silica gel with chloroform - methanol 9:1 with chamber saturation for 30 min. Quantitative determination by absorbance measurement at 288 nm. The *hRf* value was 72 and selectivity regarding matrix was given. Linearity was in the range of 0.25 and 7.5 µg/spot. The limits of detection and quantification were 47 and 142 ng/spot, respectively. Recovery was 97.8 %. No significant intra- and interday variation was observed. The method proved to be precise, accurate, and rapid in comparison with a reference HPLC method.

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

- 101 046 S.P. AGARWAL*, A. ALI, S. AHUJA (*Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi 110062, India): HPTLC determination of artesunate as bulk drug and in pharmaceutical formulations. *Ind. J. Pharm. Sci.* 69 (6), 841-844 (2007). HPTLC of artesunate in bulk and pharmaceutical formulations on silica gel aluminium foil with toluene - ethyl acetate - acetic acid 10:40:1. Detection by spraying with vanillin reagent (1 % vanillin and 5 % sulphuric acid in ethanol). The pink zone for artesunate was stable for more than a day. Quantitative determination by densitometry in absorbance mode at 520 nm. The *hRf* value for artesunate was 44. Linearity was between 100 and 600 ng/spot. Recovery (by standard addition) was 98.9 to 99.9 % both from tablets and injections.

pharmaceutical research, quality control, HPTLC, densitometry 32a

- 101 047 S. AHMAD*, M. RIZWAN, Rabea PARVEEN, M. MUJEEB, M. AQUIL (*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Hamdard University, New Delhi, 110062, India, sahmad_jh@yahoo.co.in): A validated stability-indicating TLC method for determination of forskolin in crude drug and pharmaceutical dosage form. *Chromatographia* 67 (5-6), 441-447 (2008). HPTLC of forskolin on silica gel aluminium foil with benzene - methanol 9:1. Quantitative determination by densitometry in the absorbance mode at 545 nm after spraying with anisaldehyde sulphuric acid reagent. The method was validated: linearity was between 100 and 1000 ng/spot ($r = 0.994$) and the limits of detection and quantification were 8 and 27 ng/spot respectively. Application of the proposed method for determination of forskolin in *Coleus forskohlii* root and in capsule dosage forms, which showed 0.18 and 0.57 % w/w of forskolin, which was subjected to acid and alkali hydrolysis, oxidation, photodegradation and heat degradation. The method shows good repeatability, selectivity and accuracy, and effectively separates forskolin from components of *C. forskohlii* root, from excipients of capsule as well as the degradation products of forskolin. It can be used for routine analysis and as a stability-indicating method.

quality control, doping, pharmaceutical research, quantitative analysis, qualitative identification, postchromatographic derivatization, densitometry, HPTLC 32c

- 101 048 Boglárka BAGÓCSI*, Z. VÉGH, K. FERENCZI-FODOR (*Gedeon Richter, H-1475 Budapest, 10, Box 27, Hungary; b.bagocsi@richter.hu): Optimization of the visualization of steroids separated by OPLC. *J. Planar Chromatogr.* 21, 107-112 (2008). Optimization and comparison of the acidic visualization methods most often used for steroids. OPLC of ethinyl estradiol, 'dienolether' (3-methoxyestra-2,5(10)-dien-17b-ol), norethisterone, norethisterone acetate, norethisterone enanthate, nandrolone, and nandrolone decanoate on HPTLC silica gel with cyclohexanone - ethyl acetate - chloroform 1:1:1. Detection with sulfuric acid at three different concentrations, phosphomolybdic acid, and phosphoric acid with different heating temperatures for different times. Evaluation under UV 366 nm (sulfuric acid, phosphoric acid) and in white light (phosphomolybdic acid). It was found that derivatization at higher temperatures for shorter periods usually results in greater sensitivity, although heating for longer periods at lower temperatures leads to a more stable and robust result. Evaluation by videodensitometry.

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

- 101 049 B. BENEDEK, B. WENIGER, I. PAREJO, J. BASTIDA, G. J. ARANGO, A. LOBSTEIN, C. CODINA* (*Grup de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Catalonia, Spain; carlescodina@ub.edu): Antioxidant activity of isoflavones and biflavones isolated from *Godoya antioquiensis*. *Arzneim.-Forsch./Drug Res.* 56, 661-664 (2006). TLC of two biflavones, ochnaflavone and 2'',3''-dihydroochnaflavone and two isoflavones, 5,7,4'-trihydroxy-3',5'-dimethoxyisoflavone (piscigenin) and 5,4'-dihydroxy-7,3',5'-trimethoxyisoflavone on silica gel with dichloromethane - methanol 9:1. Detection by spraying with methanolic DPPH (1,1-diphenyl-2-picryl hydrazyl) solution (20 g/L).
herbal, pharmaceutical research, qualitative identification 32e
- 101 050 A. BIJEV*, D. YANEVA, A. BOCHEVA, G. STOEV (*Department Organic synthesis and fuels, University of Chemical Technology and Metallurgy, 8 Kl. Ohridski Blvd., 1756 Sofia, Bulgaria; a.bijev@uctm.edu): Ligand-based design, synthesis and primary in vivo screening of pyrrole derivatives as potential tricyclic anti-inflammatory agents. *Arzneim.-Forsch./Drug Res.* 56, 753-759 (2006). TLC of twelve new 5-aryl-1H-pyrrole analogs of celecoxib on silica gel with chloroform - ethanol 20:3 and ethanol - chloroform - toluene - 0.5 M ammonium acetate 6:6:6:1. Detection under UV 254 nm.
pharmaceutical research, qualitative identification 32a
- 101 051 I.A. DARWISH*, H.F. ASKAL, A.S. KHEDR, R.M. MAHMOUD (*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt): Stability-indicating thin-layer chromatographic method for quantitative determination of ribavirin. *J. Chromatogr. Sci.* 46 (1), 4-9 (2008). Presentation of a simple and accurate stability-indicating method for the quantitative determination of ribavirin in its bulk and capsule forms by TLC on silica gel aluminium layer with chloroform - methanol - acetic acid 4:1:1. Detection by spraying with anisaldehyde reagent. Ribavirin is found to undergo degradation under all stress conditions, and the degradation products are well resolved from the pure drug with significantly different R_f values. Linearity was between 5 and 40 µg/spot (r = 0.9980). The limit of detection and of quantification was 1 and 5 µg/spot, respectively. Application of the proposed TLC method for the determination of ribavirin in pure form and in capsules, with good accuracy and precision. The results obtained by the proposed TLC method are comparable with those obtained by the official method.
pharmaceutical research, quality control, quantitative analysis, qualitative identification 32c
- 101 052 Á.Z. DÁVID*, E. MINCSOVICS, K. PÁPAI, K. LUDÁNYI, I. ANTAL, I. KLEBOVICH (*Semmelweis University, Department of Pharmaceutics, Högyes Endre Street 7, 1092 Budapest, Hungary; dadam@gyok.sote.hu): OPLC comparison of methods for aqueous extraction of *Sennaefolium* and *Tilia flos* plant samples. *J. Planar Chromatogr.* 21, 119-123 (2008). TLC of aqueous *Senna* and *Tilia* extracts with sennoside A and B as standards on silica gel after immersion in acetonitrile - water 17:3, with 2-propanol - ethyl acetate - water 9:9:7 (for *Senna* extracts) and 2-butanone - ethyl acetate - formic acid - water 3:5:1:1. Detection under UV 254 nm. Although quantitative analysis was not performed, peak areas served for comparative evaluation.
herbal, traditional medicine, qualitative identification, densitometry 32e
- 101 053 K. DHALWAL, V. SHINDE*, Y. BIRADAR, K. MAHADIK (*Department of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane-Kothrud, Pune 411038, Maharashtra, India, vaibhavshinde2@rediffmail.com): Simultaneous quantification of bergenin, catechin, and gallic acid from *Bergenia ciliata* and *Bergenia ligulata* by using thin-layer chromatography. *J. Food Comp. Anal.* 21, 496-500 (2008). HPTLC of bergenin (1), catechin (2), and gallic acid (3) from *Bergenia ciliata* and *Bergenia ligulata* on silica gel with toluene - ethyl acetate - formic acid 4:6:1. Quantitative determination by absorbance measurement at 280 nm. The hR_f values of (1), (2), and (3) were 29, 54, and 60, respectively. Selectivity regarding matrix was given. Linearity was between 160 and 800 ng/spot for (1), 160 and 480 ng/spot for (2), and 160 and 560 ng/spot for (3). The limits of detection and quantification were 120 and 160 ng for (1), 80

and 120 ng for (2), and 40 and 80 ng for (3), respectively. Recovery was 99.3 % for (1), 98.6 % for (2), and 99.2 % for (3). The intermediate/interday/intra-day precision (n=6) was 0.04 % and 0.07 % for (1), 0.07 % and 0.06 % for (2), and 0.02 % and 0.11 % for (3).

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

101 054 M. DHANANJAY*, B. SHASHIKANT, T. MADHUKAR (*Department of Pharmaceutical Science, R. T. M. Nagpur University, Nagpur 440033 (MS.) India): High performance thin layer chromatographic estimation of itraconazole in capsules. *Ind. J. Pharm. Res.* 6 (4), 205-207 (2007). HPTLC of itraconazole on silica gel with toluene - acetone - triethylamine 30:30:1. Detection at 270 nm. The *R_f* value of itraconazole was 62. Linearity was between 0.2 and 0.6 µg. The market formulation contained itraconazole amounts of 99.6 % via peak height and 100.2 via peak area, respectively. Recovery (by standard addition) was 99.4 % and 100.3 % respectively.

pharmaceutical research, quality control, HPTLC, densitometry 32a

101 055 P.C. DHANDHUKIA*, V.R. THAKKAR (*BRD School of Biosciences, Sardar Patel Maidan, Vadtal Road, Satellite Campus, P.O. Box 39, Sardar Patel University, Vallabh Vidyanagar 388 120 Gujarat, India): Separation and quantitation of jasmonic acid using HPTLC. *J. Chromatogr. Sci.* 46 (4), 320-324 (2008). HPTLC of jasmonic acid (extracted from *Lasiodiplodia theobromae* with ethyl acetate) on silica gel with isopropanol - ammonia - water 10:1:1. Quantification by densitometry at 295 nm in absorbance mode. The limit of detection and quantification was 1 µg and 80 µg, respectively. The method is useful for high sample throughput, e.g. for routine analysis of jasmonic acid in perfumery industries.

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

101 056 N. DHAVALE*, S. GANDHI, S. SABNIS, K. BOTHARA (*Department of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Kennedy Road, Near R.T.O, Pune, 411001, India): Simultaneous HPTLC determination of escitalopram oxalate and clonazepam in combined tablets. *Chromatographia* 67 (5-6), 487-490 (2008). Simultaneous determination of escitalopram oxalate and clonazepam in a combined tablet dosage form by HPTLC on silica gel aluminum plates with toluene - ethyl acetate - triethylamine 14:7:6. Quantification by densitometry at 258 nm.

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

101 057 L. DHOOGHE*, C. VAN MIERT, H. JANSEN, A. VLIETINCK, L. PIETERS (*Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; Liene.Dhooghe@ua.ac.be): A new decomposition product of dihydroartemisinin. *Pharmazie* 62, 900-901 (2007). TLC of dihydroartemisinin and the degradation products 2-(3-oxobutyl)-3-methyl-6-(2-propanol)-cyclohexanon and 2-(3-oxobutyl)-3-methyl-6-ethyl-cyclohexanon on silica gel with chloroform - methanol 19:1. Detection by spraying with vanillin reagent (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol).

herbal, qualitative identification 32e

101 058 S.V. DHUMAL*, S.R. KULKARNI (*Department of Pharmacognosy and Phytochemistry, The Bombay College of Pharmacy, Sunder Nagar, Kalina, Santacruz (E), Mumbai 400098, India): Antibacterial and wound healing activity of roots of *Sesamum indicum*. *Indian Drugs* 44 (12), 937-944 (2007). HPTLC of methanolic and ethyl acetate extracts of *Sesamum indicum* root on silica gel with ethyl acetate - n-hexane 1:9. Densitometric evaluation at 549 nm. The red zones were isolated by preparative TLC and identified by IR as 1,4 naphthoquinone derivatives.

herbal, HPTLC, densitometry, comparison of methods 32a

101 059 V. DIGHE, O. DHOTRE*, G. PAREKH, A. GURSALE (*Department of Chemistry, Ramnarain

Ruia College, Matunga (East), Mumbai 400 019, India; atishgrsl@yahoo.co.in): Quantification of dopamine in *Portulaca oleracea* Linn. by HPTLC. *J. Planar Chromatogr.* 21, 183-186 (2008). HPTLC of dopamine on silica gel with n-butanol - acetic acid - water 7:2:1. Detection and quantification by densitometry at 280 nm.

herbal, HPTLC, densitometry, quantitative analysis

32e

101 060 Izabela FECKA*, S. TUREK (*Department of Pharmacognosy, Wroclaw Medical University, 50-367 Wroclaw, Poland, Izabela@farmgn.am.wroc.pl): Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoran by chromatographic techniques. *Food Chem.* 108, 1039-1053 (2008). HPTLC of luteolin-7-O-beta-glucuronide (1), lithospermic acid (2), and rosmarinic acid (3) in Lamiaceous species (*Thymi herba*, *Serpylli herba*, and *Majoranae herba*) on amino phase for (1) and silica gel for (1), (2), and (3) with acetone - formic acid 17:3 and diisopropyl ether - acetone - formic acid - water 5:3:1:1, respectively. Evaluation at 365 nm before and after spraying with 2 % methanolic aluminium chloride, natural product/polyethylene glycol reagent or in visible light after treatment with bis-diazotized sulfanilamide (yellow, orange, red, and brown bands).

herbal, HPTLC, qualitative identification

32e

101 061 Agnes GASPAS, Sophie LECLERE*, Veronique MARIGNIER (*Bioeurope, Route de Oullins, F-28260 Anet, France, sophie.leclere@solabia.fr): Quantification of beta-ecdysone in a Brazilian ginseng juice (*Pfaffia glomerata*). *CBS* 100, 10-12 (2008). HPTLC of beta ecdysone in *Pfaffia glomerata* extract on silica gel over 50 mm with the lower phase of chloroform - methanol - water 7:5:2 after chamber saturation for 15 min. Detection by dipping in anisaldehyde reagent (0.5 mL anisaldehyde, 10 mL acetic acid, 5 mL sulfuric acid, and 85 mL methanol) followed by heating at 120 °C for 20 min. Quantitative determination by absorbance measurement at 432 nm. Polynomial calibration in the range of 0.04 to 0.2 µg/band (sdv 1.6 %). The evaluated extracts contained 0.8 to 1.2 % of beta ecdysone.

herbal, quality control, quantitative analysis, HPTLC, densitometry

32e

101 062 C. GOPU, S. AHER, H. MEHTA, A. PARADKAR, K. MAHADIK* (*Poona College of Pharmacy, Bharati Vidyapeeth University, 411030 Pune, India, krmahadik@rediffmail.com): Simultaneous determination of cinnamaldehyde, eugenol and piperine by HPTLC densitometric method. *Phytochem. Anal.* 19, 116-121 (2008). HPTLC of cinnamaldehyde (1) in the shoots of *Cinnamomum zeylanicum*, eugenol (2) in the flower buds of *Eugenia caryophyllus* and piperine (3) in the fruits of *Piper nigrum* on silica gel with petroleum ether - dichloromethane - formic acid 20:40:1. Quantitative determination by absorbance measurement at 290 nm. The hRf values were 47, 61, and 12 for (1), (2), and (3), respectively. Linearity was between 54 and 735 ng/spot for (1), 533 and 8531 ng/spot for (2), and 50 and 300 ng/spot for (3). The limits of detection and quantification were 12 and 21 ng/spot for (1), 240 and 426 ng/spot for (2), and 18 and 40 ng/spot for (3). Recovery was 99 % for each substance. No significant intra- and interday variation was observed. The method proved to be rapid and useful in comparison with GC and HPLC methods.

herbal, HPTLC, quantitative analysis, comparison of methods

32e

101 063 Erzsébet HÁSNAGY-RADNAI*, K. PINTYE-HÓDI, S. CZIGLE, T. MARTINEK, G. JANICSÁK, I. MÁTHÉ, I. ERÖS (*Institute of Pharmacognosy, University of Szeged, Eötvös 6, 6720 Szeged, Hungary; haznagy.radnai@pharm.u-szeged.hu): Chromatographic determination of iridoids in *Stachys recta*, and investigation of inorganic elements by X-ray fluorescence spectroscopy. *J. Planar Chromatogr.* 21, 27-32 (2008). TLC of extracts of *Stachys recta* and harpagide, acetylharpagide, harpagoside, ajugoside, and aucubin on silica gel with chloroform - methanol - water 25:10:1. Visualization by spraying with Ehrlich's reagent (1 % solution of dimethylamino-benzaldehyde in concentrated hydrochloric acid) followed by heating at 105 °C for 5 min. Densitometric evaluation at 540 nm.

herbal, densitometry, quantitative analysis, qualitative identification

32e

- 101 064 U. HAURI, Vera BAUMGARTNER. CH. HOHL* (*Kantonales Laboratorium Basel-Stadt, Non Food, P.O. Box, 4012 Basel, Switzerland, christopher. hohl@bs.ch): Bio-activity based analysis of irradiated sunscreens using HPTLC and in situ detection with *Vibrio fischeri*. *CBS* 100, 2-5 (2008). HPTLC of photodegraded UV filters and sunscreen on silica gel LiChrospher prewashed with methanol. AMD 2 development of UV filter standards photodegradation products with diisopropylether - n-hexane in 6 steps over 50 mm without preconditioning, and of sunscreen samples photodegradation products with t-butylmethylether - n-hexane in 7 steps over 50 mm with preconditioning, followed by drying at 120 °C for 30 min. Detection at 254 and 366 nm, followed by biodetection via dipping the plate in a *Vibrio fischeri* solution for 1 s and evaluation with the Bioluminizer (exposure time 55 s). Densitometric evaluation by multi-wavelength scan at 200-400 nm.
- food analysis, quality, control, toxicology, cosmetics, HPTLC, densitometry, quantitative analysis, qualitative identification, AMD 32f
- 101 065 J. HUMLJAN*, S. STARCEVIC, V. CAR, P. STEFANIC ANDERLUH, D. KOCJAN, B. JENKO, U. URLEB (*Drug Discovery Department, Lek Pharmaceuticals, Verovskova 57, 1526 Ljubljana, Slovenia; jan.humljan@sandoz.com): Optimization of UDP-N-acetylmuramic acid synthesis. *Pharmazie* 63, 102-106 (2008). TLC of benzyl 2-acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-alpha-D-glucopyranoside 1',4-lactone, 2-acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-alpha-D-glucopyranoside 1',4-lactone, and 2-acetamido-6-O-acetyl-3-O-[(R)-1-carboxyethyl]-2-deoxy-alpha-D-glucopyranoside-1-alpha-(diphenyl phosphate) 1',4-lactone on silica gel with ethyl acetate - acetone 2:1. TLC of triethylammonium muramyl phosphate on silica gel with isopropanol - water - 25 % ammonia 6:1:3. Evaluation under UV light and by heating the plate at 250 °C.
- pharmaceutical research, qualitative identification 32a
- 101 066 V. KUMAR, K. MUKHERJEE, S. KUMAR, M. MAL, P. MUKHERJEE* (*School of Natural Products Studies, Department of Pharmaceutical Technology, Jadavpur University, 700075 Kolkata, India, pknatprod@yahoo.co.in): Validation of HPTLC method for the analysis of taraxerol in *Clitoria ternatea*. *Phytochem. Anal.* 19, 244-250 (2007). HPTLC of taraxerol in *Clitoria ternatea* on silica gel with hexane - ethyl acetate 4:1. Detection by spraying with anisaldehyde reagent. Quantitative determination by absorbance measurement at 420 nm. The R_f value of taraxerol was 53 and selectivity regarding the matrix was given. Linearity was between 100 and 1200 ng of taraxerol. Recovery values were between 99.6 and 99.7 %. The limit of detection and quantification for taraxerol was 31 and 105 ng/spot, respectively.
- traditional medicine, herbal, HPTLC, qualitative identification 32e
- 101 067 S.G. LATE, A.K. BANGA* (*Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30431, USA; banga_ak@mercer.edu) : Thermal and non-thermal methods to evaluate compatibility of granisetron hydrochloride with tablet excipients. *Pharmazie* 63, 453-458 (2008). Comparison of the R_f value of granisetron hydrochloride alone with the R_f value of granisetron hydrochloride of different drug excipient systems, e. g. beta-cyclodextrin, 2-hydroxypropyl-beta-cyclodextrin, mannitol, and magnesium stearate. No significant change in the R_f value with the different excipients was observed. After TLC separation of compounds, plates were placed into an iodine chamber to identify the spots.
- pharmaceutical research, qualitative identification 32b
- 101 068 Y. LIU* (Liu Yonghong), N. MURAKAMI, S. ZHANG, T. XU (*Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510-301, China; yonghongliu@scsio.ac.cn): Structure-activity relationships of 1'-acetoxychavicol acetate homologues as new nuclear export signal inhibitors. *Pharmazie* 63, 659-662 (2008). HPTLC and TLC of 1'-acetoxychavicol acetate on silica gel and RP-18.

Detection under UV 254 and 365 nm, and after spraying with 1 % cerium sulfate/10 % sulfuric acid, and anisaldehyde reagent (5 mL acetic acid, 25 mL concentrated sulfuric acid, 425 mL ethanol, and 25 mL water).

herbal, pharmaceutical research, HPTLC, qualitative identification 32e

- 101 069 Sheryl M. VERBITSKI*, G.T. GOURDIN, L.M. IKENOUYE, J.D. McCHESNEY (*ChromaDex Analytics, Inc., 2830 Wilderness Pl, Boulder, CO 80301, USA): Detection of *Actaea racemosa* adulteration by thin-layer chromatography and combined thin-layer chromatography-bioluminescence. *J. AOAC Int.* 91, 268-275 (2008). HPTLC of actein, 23-epi-26-deoxyactein, cimiracemose, cimifugoside, caffeine, and tyrosine as standards on silica gel (prewashed with methanol) with toluene - ethyl formate - formic acid 5:3:2 in a saturated chamber. Detection under UV 254 and 365 nm, under white light after spraying with anisaldehyde reagent, and via the Bioluminex assay. For the Bioluminex assay the developed and dried HPTLC plate was coated with a buffered solution of luminescent *Vibrio fischeri*. Documentation of luminescent and inhibited zones with a CCD camera.

pharmaceutical research, traditional medicine, herbal, qualitative identification, HPTLC, quantitative analysis 32e

- 101 070 B.H. MEHTA, S.B. MORGE* (*Department of Chemistry, University of Mumbai, Vidaynagari, Santacruz [E] Mumbai 400098, India; s_morge@yahoo.com): HPTLC-densitometric analysis of candesartan cilexetil and hydrochlorothiazide in tablets. *J. Planar Chromatogr.* 21, 173-176 (2008). HPTLC of candesartan cilexetil and hydrochlorothiazide on silica gel with acetone - chloroform - ethyl acetate - methanol 6:6:6:1 at 25 +/- 5°C and a relative humidity of 50-60 % in a saturated twin trough separation chamber. Quantification by densitometry at 280 nm.

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

- 101 071 D.B. MESHARAM*, S.B. BAGADE, M.R. TAJNE (*University Department of Pharmaceutical Sciences, RTM Nagpur University, Nagpur 440033, Maharashtra, India; dbmeshram@yahoo.com): A simple TLC method for analysis of fluconazole in pharmaceutical dosage forms. *J. Planar Chromatogr.* 21, 191-195 (2008). HPTLC of fluconazole and clotrimazole (as internal standard) on silica gel with toluene - chloroform - methanol 6:15:2. Quantification by densitometry at 210 nm.

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

- 101 072 H.P. MHASKE*, V.V. VAIDYA, S.N. MENON, S. SHAILAJAN, N.A. GOMES (*Therapeutic Drug Monitoring Laboratory, 194 Scheme No. 6, Road No. 15, Sion Koliwada, Sion (East), Mumbai 400 022, India; vaidya_vikas@yahoo.com, tdmlab@vsnl.net): Quantification of puerarin in *Pueraria tuberosa* DC by HPTLC. *J. Planar Chromatogr.* 21, 217-219 (2008). HPTLC of puerarin on silica gel, prewashed with methanol, with ethyl acetate - methanol - distilled water 8:1:1 in a twin-trough chamber saturated for 5 min. Quantification by densitometry at 366 nm.

traditional medicine, herbal, radioscanning, HPTLC 32e

- 101 073 R.K. PATEL, A.M. PRAJAPATI* (*Ganpat University, S. K. Patel College of Pharmaceutical Education and Research, Kherava 382 711, Mehsana, Gujarat, India; arun_time@yahoo.co.in): Development and validation of a visible absorption densitometry method for quantitation of conessine in *Holarrhena antidysenterica* (Kurchi). *J. AOAC Int.* 91, 339-343 (2008). HPTLC of conessine on silica gel (prewashed with methanol) with toluene - ethyl acetate - dimethylamine 13:5:2 in a saturated twin-trough chamber. Detection by spraying with Dragendorff reagent, followed by spraying with a 10 % solution of aqueous sodium nitrite. Quantitative determination by densitometry at 520 nm. Linearity was between 10 and 60 ng/spot. The limit of detection was 3 ng/spot.

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

- 101 074 R.K. PATEL*, A.M. PRAJAPATI, M.M. PATEL (*S. K. Patel College of Pharmaceutical Education and Research, Ganapat University, Kherava 382711, Mehsana, Gujarat, India): High-performance thin-layer chromatographic method for quantification of atisine from *Aconitum heterophyllum* Roth. *Indian Drugs* 45 (3), 233-225 (2008). HPTLC of atisine in *Aconitum heterophyllum* on silica gel with toluene - ethyl acetate - diethylamine 7:2:1. Densitometric evaluation at 274 nm. Detection by spraying with Dragendorff reagent followed by spraying with 10 % sodium nitrate. Densitometric evaluation of reddish brown zones at 520 nm. Linearity of atisine was between 10 and 60 ng/spot.
pharmaceutical research, herbal, HPTLC, densitometry, quantitative analysis 32a
- 101 075 B.H. PATEL*, B.N. SUHAGIA, M.M. PATEL, J.R. PATEL (*Department of Pharmaceutical Chemistry, S. K. Patel College of Pharmaceutical Education and Research, Ganpat Vidyanagar, Kherva, Mehsana 382711, Gujarat, India): HPTLC determination of rabeprazole and domperidone in capsules and its validation. *J. Chromatogr. Sci.* 46 (4), 304-307 (2008). HPTLC rabeprazole and domperidone in pure powder and in capsule formulations on silica gel aluminium layers with ethyl acetate - methanol - benzene - acetonitrile 3:2:3:2. Quantification by absorbance measurement at 287 nm. Linearity was between 400 and 1200 ng/spot for rabeprazole and 600 and 1800 ng/spot for domperidone. Recovery was 99.8 % for rabeprazole and 99.4 % for domperidone. This method is simple, precise, and sensitive, and it is suitable for the simultaneous determination of rabeprazole and domperidone.
quality control, pharmaceutical research, HPTLC, quantitative analysis, densitometry, qualitative identification 32c
- 101 076 B.H. PATEL*, B.N. SUHAGIA, M.M. PATEL, J.R. PATEL (*S.K. Patel College of Pharmaceutical Education and Research, Ganpat Vidyanagar, Kherva, Mehsana, Gujarat, India): High-performance liquid chromatography and thin-layer chromatography for the simultaneous quantitation of rabeprazole and mosapride in pharmaceutical products. *J. Chromatogr. Sci.* 46 (1), 10-14 (2008). Quantitative determination of rabeprazole and mosapride in their combined pharmaceutical dosage forms by TLC on silica gel with ethyl acetate - methanol - benzene 4:1:5. Quantification by densitometry at 276 nm. Linearity was between 400 and 1200 ng/spot for rabeprazole and between 300 and 900 ng/spot for mosapride, respectively. The limit of detection was 132 ng/spot for rabeprazole and 98 ng/spot for mosapride.
pharmaceutical research, quality control, densitometry, quantitative analysis, qualitative identification, comparison of methods 32c
- 101 077 O. POZHARITSKAYA, S. IVANOVA, A. SHIKOV*, V. MAKAROV (*Interregional Center „Adaptogen“, 47/5 Piskarevskiy pr., St. Petersburg, 195067, Russia, alexs79@mail.ru): Separation and free radical-scavenging activity of major curcuminoids of *Curcuma longa* using HPTLC-DPPH method. *Phytochem. Anal.* 19, 236-243 (2008). HPTLC of curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin (3) in the rhizome of *Curcuma longa* L. on silica gel with toluene - acetic acid 4:1 for curcuminoid separation and n-hexane - ethyl acetate - acetic acid 16:5:1 for quantification of total curcuminoid content. Quantitative determination by absorbance measurement at 254 nm. To determine the free radical-scavenging activity of individual compounds, the plate was dipped into a 0.5 mM solution of 1,1-diphenyl-2-picrylhydrazyl in methanol for 5 s, dried in darkness at room temperature and heated at 60 °C for 30 s. Quantitative determination by absorbance measurement at 517 nm as negative peaks. The hRf values were 50, and 25 for (1) and (3), respectively. Linearity was between 80 and 250 ng/spot for (1), 40 and 280 ng/spot for (2), and 85 and 270 ng/spot for (3). The limits of detection and quantification were 20 and 60 ng/spot for (1), 10 and 30 ng/spot for (2), and 25 and 75 ng/spot for (3). Recoveries were 99.0 %, 96.9 %, and 95.8 %, (reference value 80 %), respectively. The intermediate/interday/intra-day precision for (1), (2), and (3) was 3.15 %, 3.00 %, and 2.85 %, (n=6), respectively. For the free radical-scavenging activity, linearity was between 70 and 400 ng for (1), 50 and 550 ng for (2), and 60 and 250 ng for (3). The limits of detection and quantification were 40 and 70 ng for (1), 25 and 50 ng for (2), and 45 and 60 ng for (3). The intermediate/interday/intra-day precision

for (1), (2), and (3) was 6.50 %, 2.30 %, and 1.50 % (n=6), respectively.

herbal, HPTLC, quantitative analysis, postchromatographic derivatization 32e

- 101 078 Alina PYKA*, W. KLIMCZOK (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Jagielonska 4, 41-200 Sosnowiec, Poland; apyka@slam.katowice.pl): Influence of impregnation of silica gel and Kieselguhr with copper(II) sulphate (VI) on profile change of the spectrodensitograms and the R_f values of nicotinic acid and its derivatives. *J. Liq. Chromatogr. Relat. Technol.* 31, 526-542 (2008). TLC of nicotinic acid, nicotinamide, ethyl, isopropyl, butyl, hexyl, benzyl nicotinate, N-methylnicotinamide, N,N-diethylnicotinamide, 3-pyridinecarbaldehyde, 3-pyridinecarbonitrile, 3-pyridylmethanol, and methyl 3-pyridyl ketone on silica gel and kieselguhr impregnated with 2.5 % and 5 % aqueous solutions of copper(II)sulfate, with acetone - n-hexane in different volume compositions. Detection by spraying with Rhodamine B solution. Quantification by densitometry. The number of absorption bands of the compounds investigated depends on impregnation of the sorbent and the visualization agent.

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

- 101 079 I. QIAN (Qian Iguang-Sheng)*, Q. WANG (Wang Qing), K.S. LEUNG (Leung Sze-Yin Kelvin), Y. QIN (Qin Yong), Z. ZHAO (Zhao Zhongzhen), Z. Jiang (Jiang Zhi-Hong) (*School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China): Quality assessment of rhizoma et radix notopterygii by HPTLC and HPLC fingerprinting and HPLC quantitative analysis. *J. of Pharm. Biomed. Anal.* 44 (3), 812-817 (2007). HPTLC of extracts of *Notopterygium incisum* Ting ex H.T. Chang (or *Notopterygium forbesii* Boiss) root and rhizome, and marker compounds isoimperatorin, notopteron and bergapten. The authentication of rhizoma et radix *Notopterygium* was achieved by comparing the colors and R_f values of the bands in TLC fingerprints with those of the marker compounds. The HPLC fingerprints of 16 batches of herbal samples from different regions of China showed similar chromatographic patterns. Five peaks were selected as characteristic peaks, and three of these were identified by using LC-MS-MS techniques.

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

- 101 080 G. RAHUL*, B. LEENA, V. ASFAK, D. MRINALINI (*Department of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Kennedy Road, Near R.T.O, Pune, 411001, India): A validated high performance thin layer chromatographic method for simultaneous estimation of ofloxacin and satranidazole in pharmaceutical dosage form. *J. Pharm. Res.* 6 (4), 233-235 (2007). HPTLC of ofloxacin and satranidazole on silica gel with n-butanol - ethanol 5:5 containing 4 % ammonia. Densitometric analysis at 320 nm. The hR_f value of ofloxacin and satranidazole was 54 and 83 respectively. Linearity was between 200 and 1000 ng/spot for ofloxacin and between 300 and 1500 ng/spot for satranidazole. Recovery was 99.1 - 99.8 % for both compounds.

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

- 101 081 M. RAJBHANDARI, TH. SCHOEPKE, R. MENDEL, Ulrike LINDEQUIST* (*Institute of Pharmacy, Ernst-Moritz-Arndt-University Greifswald, Friedrich-Ludwig-Jahn-Strasse 17, 17487 Greifswald, Germany; lindequi@uni-greifswald.de) : Antibacterial and antiviral naphthazarins from *Maharanga bicolor*. *Pharmazie* 63, 633-635 (2008). TLC of deoxyalkannin, alkannin-1'-beta-hydroxy-isovalerate, and alkannin-1'-beta-acetoxy-isovalerate on silica gel with hexane - ethyl acetate - ethanol 20:1:1. Detection under UV 254 nm.

herbal, pharmaceutical research, qualitative identification 32e

- 101 082 M. RAM*, S. KHAN, P. JHA, M.A. KHAN, U. KIRAN, S. JAVED, M.Z. ABDIN (*Centre for Transgenic Plant Development, Department of Biotechnology, Jamia Hamdard, New Delhi, 110062, India): Rapid TLC method for estimation of mevalonic acid in the leaves of medicinal plants. *Chromatographi* 68 (1-2), 129-133 (2008). Description of a simple, rapid, specific and

sensitive method for the quantitative estimation of mevalonic acid in leaves of *Artemisia annua*, *Psorelia corylifolia*, *Vinca rosea*, *Withania somnifera* and *Barleria proinites*. TLC of leaf extracts on silica gel with benzene - acetone 3:2 which involved conversion of mevalonic acid to its lactone, mevalonolactone. Detection by treatment with anisaldehyde reagent. Quantitative determination of mevalonolactone by absorbance measurement at 600 nm. Linearity was between 100 and 500 ng per spot. Recovery (by standard addition) was higher than 98 % for mevalonolactone. The limit of detection was 50 ng per spot.

quality control, pharmaceutical research, traditional medicine, quantitative analysis, qualitative identification, densitometry 32e

- 101 083 A. RATHI*, N. SRIVASTAVA, S. KHATOON, A.K.S. RAWAT (*Department of Pharmacognosy and Ethnopharmacology, National Botanical Research Institute, Lucknow, 226 001, India): TLC determination of strychnine and brucine of *Strychnos nux vomica* in Ayurveda and homeopathy drugs. *Chromatographia* 67 (7-8), 607-613 (2008). Quantitative estimation of the two major alkaloids strychnine and brucine in *Strychnos nux vomica* by TLC on silica gel with chloroform - ethyl acetate - diethyl amine 1:17:2. The R_f values for strychnine and brucine were 55 and 42 and selectivity regarding matrix was given. Recovery was between 93.1 and 99.8 % for strychnine and between 96.9 and 99.5 % for brucine. The limit of detection and quantification for strychnine was 2 and 8 ng, respectively, and for brucine 2 and 9 ng, respectively.

quality control, pharmaceutical research, traditional medicine, quantitative analysis, qualitative identification 32c

- 101 084 E. REICH*, Anne SCHIBLI, Alison DEBATT (*CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland; eike.reich@camag.com): Validation of HPTLC methods for the identification of botanicals in a cGMP environment. *J. AOAC Int.* 91, 13-20 (2008). Comprehensive proposal for the validation of qualitative HPTLC methods. The steps of the validation process (method selection and optimization, stability, specificity, precision, and robustness) are illustrated with examples of identification methods: green tea leaf, ginseng root, eleuthero root, echinacea root, black cohosh rhizome, licorice root, kava root, milk thistle aerial parts, feverfew aerial parts, and ginger root. The validation protocol is a key element for structuring, managing and documenting the validation process. HPTLC is suitable for reliable identification of botanicals because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. Reproducibility is improved if suitable instrumentation is used, a standardized HPTLC methodology is implemented, and methods have been validated.

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

- 101 085 J. RICHTER*, J. JIRMAN, J. HAVLÍČEK, R. HRDINA (*University of Pardubice, Faculty of Chemical Technology, Department of Technology of Organic Compounds, TP Doubravice, Studentská 95, 532 10 Pardubice, Czech Republic; Jindrich.richter@zentiva.cz): Pioglitazone impurities. *Pharmazie* 63, 580-584 (2008). TLC of 5-{4-[2-(5-ethyl-6-{4-[5-ethylpyridin-2-yl]ethoxy}phenyl)pyridin-2-yl)-ethoxy]benzyl}-1,3-thiazolidine-2,4-dione (pioglitazone), 5-{4-[2-(5-ethyl-4-{4-[2-[5-ethylpyridin-2-yl]ethoxy]phenyl)pyridin-2-yl)-ethoxy]benzyl}-1,3-thiazolidine-2,4-dione, and 5-{6,4'-bis-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}pyridin-2-yl)-ethoxy]biphenyl-3-ylmethyl}-1,3-thiazolidine-2,4-dione on silica gel with chloroform - methanol - ammonia 60:30:1. Detection under UV 254 nm.

pharmaceutical research, qualitative identification 32a

- 101 086 N.J. SHAH*, B.N. SUHAGIA, R.R. SHAH, N.M. PATEL (*Shri B. M. Shah College of Pharmaceutical Education and Research, Modaso 383315, India): Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form. *Ind. J. Pharm. Sci.* 69 (6), 834-836 (2007). HPTLC of olmesartan medoxomil and hydrochlorothiazide simultaneously in combined dosage forms on silica gel with acetonitrile - chloroform - acetic acid 14:4:1. Detection at 254 nm. Linearity was between 500 and 750 ng/spot for olmesartan medoxomil and 100 and 600 ng/spot for hydrochlorothiazide.

The limit of detection and the limit of quantification for olmesartan medoxomil was 170 and 500 ng/spot respectively, and for hydrochlorothiazide 30 and 100 ng/spot, respectively. The proposed method can be used to determine the drug content of marketed tablet formulation.

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

- 101 087 B. SPARZAK, Mirosława KRAUZE-BARANOWSKA*, L. POBŁOCKA-OLECH (*Medical University of Gdansk, Department of Pharmacognosy with the Medicinal Plants Garden, Hallera 107, 80-416 Gdansk, Poland; krauze@amg.gda.pl): HPTLC determination of catechins in in-vitro cultures of two species of the genus *Phyllanthus*. *J. Planar Chromatogr.* 21, 103-106 (2008). HPTLC of (-)-epicatechin, (+)-catechin, (-)-epigallocatechin, (-)-gallocatechin, (-)-catechin gallate, and (-)-epicatechin gallate on RP-18 at room temperature and 75 % relative humidity in an automated development chamber with methanol - water - formic acid 30:70:6. Detection by spraying with diazotized sulfanilic acid. Quantification by densitometry at 440 nm.

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

- 101 088 V. SRIVASTAVA, M. SINGH, R. MALASONI, K. SHANKER, R. VERMA, M. GUPTA*, A. GUPTA, S. KHANUJA (*Analytical Chemistry Division, Central Institute of Medicinal and Aromatic Plants, 226 015 Lucknow, India, guptammg@rediffmail.com): Separation and quantification of lignans in *Phyllanthus* species by a simple chiral densitometric method. *J. Sep. Sci.* 31, 47-55 (2008). HPTLC of phyllanthin (1), hypophyllanthin (2), niranthin (3), and nirtetralin (4) in the leaves of four *Phyllanthus* species, *P. amarus*, *P. maderaspatensis*, *P. urinaria*, and *P. virgatus*, on a chiral TLC plate with n-hexane - acetone - 1,4-dioxane 18:2:1. Detection by dipping in vanillin reagent (1 g vanillin, 5 mL sulphuric acid, 95 mL ethanol) followed by heating at 110 °C for 15 min. Quantitative determination by absorbance measurement at 620 nm. The hRf values were 30, 36, 41, and 48 for (1) to (4), respectively. Linearity was between 100 and 500 ng/band and the recoveries for (1) to (4) were 99.9, 100.5, 99.2, 98.7 %, respectively. The limit of detection and quantification was 26 and 88, 45 and 136, 53 and 176, and 57 and 187 ng/band for (1) to (4), respectively. No significant intra- and interday variation was observed.

herbal, quantitative analysis, HPTLC 32e

- 101 089 J.V. SUSHEEL*, M. LEKHA, T.K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramkrishna Institute of Paramedical Sciences, Coimbatore 641044, India): High performance thin layer chromatographic estimation of lansoprazole and domperidone in tablets. *Ind. J. Pharm. Sci.* 69 (5), 684-686 (2007). HPTLC of lansoprazole and domperidone in tablet formulations on silica gel with n-butanol - acetic acid - water 186:5:10. Densitometric analysis in absorbance mode at 288 nm. The calibration covered the range of 100 to 500 ng/spot for both substances. The hRf value of lansoprazole and domperidone was 78 and 21, respectively. The limit of detection and quantification for lansoprazole was 10 and 40 ng/spot and for domperidone 30 and 65 ng/spot, respectively. The method is suitable for routine quality control.

pharmaceutical research, quality control, HPTLC, densitometry 32a

- 101 090 N.G. TAYADE*, M.S. NAGARSENKER (*Department of Pharmaceutics, Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai 400098, India): Validated HPTLC method of analysis for artemether and its formulations. *J. Pharm. Biomed. Anal.* 43 (3), 839-844 (2007). HPTLC of artemether both as a bulk drug and in pharmaceutical formulations on silica gel aluminum plates with toluene - ethyl acetate - formic acid 80:20:3. Quantification of artemether by densitometry at 565 nm. Linearity was between 200 and 1000 ng/spot. The limit of detection and quantification was 65 and 197 ng/spot, respectively. The method was successfully applied in the analysis of lipid based parenteral formulations and marketed oral solid dosage formulation.

quality control, HPTLC, densitometry, quantitative analysis, qualitative identification 32c

- 101 091 M. TOMCZYK*, D. DROZDOWSKA, A. BIELAWSKA, K. BIELAWSKI, J. GUDEJ (*Depart-

ment of Pharmacognosy, Faculty of Pharmacy, Medical University of Bialystok, ul. Mickiewicza 2a, 15-230 Bialystok, Poland; tomczyk@amb.edu.pl): Human DNA topoisomerase inhibitors from *Potentilla argentea* and their cytotoxic effect against MCF-7. *Pharmazie* 63, 389-393 (2008). TLC of methyl brevifolincarboxylate and kaempferol 3-O-beta-D-(6"-E-p-coumaroyl)-glucopyranoside tiliroside on silica gel with ethyl acetate - methanol - water 18:1:1. Detection under UV light.

pharmaceutical research, qualitative identification 32e

- 101 092 Nada U. PERISIC-JANJIC*, S.O. PODUNAVAC-KUZMANOVIC (*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia; nadap@uns.ns.ac.yu): RPTLC study of QSRR and QSAR for some benzimidazole derivatives. *J. Planar Chromatogr.* 21, 135-141 (2008). Quantitative structure-retention relationships and quantitative structure-activity relationships have been used to study the chromatographic behavior and the antibacterial activity of different substituted benzimidazole derivatives. HPTLC of eleven benzimidazole derivatives on RP-18 with methanol - phosphate buffer (pH 7 resp. pH 8). Evaluation under UV 254 nm.

pharmaceutical research, qualitative identification, HPTLC 32a

- 101 093 Irina URAKOVA, Olga POZHARITSKAYA, A. SHIKOV*, Vera KOSMAN, Valery MAKAROV (*Interregional Center „Adaptogen“, 47/5 Piskarevskiy pr., St. Petersburg, 195067, Russia, alexs79@mail.ru): Comparison of high performance TLC and HPLC for separation and quantification of chlorogenic acid in green coffee bean extracts. *J. Sep. Sci.* 31, 237-241 (2008). HPTLC of chlorogenic acid in green coffee beans on silica gel with ethyl acetate - dichloromethane - formic acid - acetic acid - water 100:25:10:10:11. Quantitative determination by absorbance measurement at 330 nm. The R_f value was 27 and selectivity regarding matrix was given. Linearity was between 550 and 1750 ng/spot. The limits of detection and quantification were 80 and 250 ng, respectively. Recovery was 97.1 %. The intermediate/interday/intra-day precision was between 1.06 % and 1.35 % (n=6). The data showed no statistically significant differences for HPTLC and HPLC methods.

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

- 101 094 N. VADERA*, G. SUBRAMANIAN, P. MUSMADE (*Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka 576104, India): Stability-indicating HPTLC determination of imatinib mesylate in bulk drug and pharmaceutical dosage form. *J. Pharm. Biomed. Anal.* 43 (2), 722-726 (2007). HPTLC of imatinib mesylate both as a bulk drug and in formulations on silica gel aluminium plates with chloroform - methanol 3:2. Quantitative determination by absorbance measurement at 276 nm. Linearity was between 100 and 1000 ng per spot. The limit of detection and quantification was 10 and 30 ng, respectively. The method is repeatable, selective and accurate and can be used for stability control.

quality control, pharmaceutical research, doping, HPTLC, quantitative analysis, qualitative identification, densitometry 32c

- 101 095 G. VÖLGYI, K. DEÁK, J. VÁMOS, K. VALKÓ, Krisztina TAKÁCS-NOVÁK* (*Semmelweis University, Department of Pharmaceutical Chemistry, 1092 Budapest, Högyes E. u. 9, Hungary; novkri@hogyes.sote.hu): RPTLC determination of log P of structurally diverse neutral compounds. *J. Planar Chromatogr.* 21, 143-149 (2008). RPTLC method for parallel estimation of the lipophilicity of chemically diverse neutral compounds or weak acids and bases. TLC of 28 drugs on RP-18 prewashed with methanol and heated at 160 °C for 1 h, with mixtures of water with methanol, ethanol, 1-propanol, acetone, acetonitrile, and 1,4-dioxane in a saturated chamber. Acetone - water 9:11 and 3:2 were best suited. Densitometric evaluation at 200 and 254 nm.

pharmaceutical research, qualitative identification 32a

- 101 096 Valeria WIDMER, E. REICH*, Alison DEBATT (*CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland; Eike.Reich@camag.com): Validated HPTLC method for identification of *Hoodia gordonii*. *J. Planar Chromatogr.* 21, 21-26 (2008). HPTLC of extracts of *Hoodia gordonii* with fructose and beta-sitosterol as standards on silica gel with chloroform - methanol - water 70:30:3 in an automatic developing chamber fitted with a twin-trough chamber. The chamber was saturated for 20 min with mobile phase and relative humidity was controlled (33 %). Detection by dipping in anisaldehyde reagent, followed by heating at 100 °C for 3 min. Documentation and evaluation before derivatization under UV 366 nm and after derivatization under white light. The method was validated. It is specific and allows discrimination of *Hoodia gordonii* from *Hoodia currorii*, *Hoodia parviflora*, and the common adulterant prickly pear cactus (*Opuntia ficus-indica*). The sample is stable in solution and on the plate for at least 3 h, as well as during chromatography (2D test). After derivatization the chromatogram is stable for at least 1 hour. Precision (repeatability, intermediate precision, and reproducibility) was good and the method is robust. The method is sensitive to changes in relative humidity. If relative humidity exceeds 47% the plate must be conditioned to 33% RH to ensure proper separation.

herbal, traditional medicine, quality control, HPTLC, densitometry, qualitative identification
32e

33. Inorganic substances

- 101 097 Vukosava ZIVKOVIC-RADOVANOVIC*, Gordana VUCKOVIC (*Faculty of Chemistry, University of Belgrade, P.O. Box 158, 11001 Belgrade, Serbia) : Use of different salt solutions in salting-out TLC of Co(III) complexes on silica gel. *Chromatographia* 67 (3-4), 259-267 (2008). Investigation of saturated aqueous solutions of 28 different salts used as potential mobile phases for salting-out TLC, on silica gel, of a series of four mixed bis-aminocarboxylato cobalt(III) complexes. Confirmation of three alkali metal chlorides, and four alkaline earth metal chlorides, for linear dependences previously established on different adsorbents with (NH₄)₂SO₄ solutions by linear regression analysis of chromatographic data obtained for fifteen mixed aminocarboxylato Co(III) complexes (four series) with solutions of ammonium chloride. Comparison of separation achieved with different salts. Li⁺, Mg²⁺, and Ca²⁺ chlorides are most suitable.

comparison of methods
33a

35. Other technical products and complex mixtures

- 101 098 A. MOHAMMAD*, S.A. BHAWANI (*Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering & Technology, Aligarh Muslim University, Aligarh 202 002, India): Silica thin-layer chromatographic studies of surfactants with mixed aqueous-organic eluents containing thiourea: simultaneous separation of co-existing cetyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and polyoxyethylene (20) sorbitan monolaurate. *J. Chromatogr. Sci.* 46 (4), 298-303 (2008). HPTLC of three surfactants, cetyltrimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DTAB), and polyoxyethylene (20) sorbitan monolaurate (Tween 20), on silica gel with 5 % aqueous thiourea - acetone - methanol 3:1:1. Evaluation of the effect of the carbon chain length of alcohols (methanol, ethanol, n-propanol, and n-butanol) on the mobility of these surfactants. The comparative study was performed with sulfur- (thiourea) and oxygen- (urea) containing compounds in the eluent. Investigation of the interference on the resolution of the mixture of CTAB, DTAB, and Tween 20, due to presence of metal cations as impurities.

quality control, HPTLC, quantitative analysis, qualitative identification
35a

37. Environmental analysis

- 101 099 A. ALPMANN, Gertrud MORLOCK* (*Institute for Food Chemistry, University of Hohenheim, 70593 Stuttgart, Germany, gmorlock@uni-hohenheim.de) : Rapid and sensitive determination of acrylamide in drinking water by planar chromatography and fluorescence detection after derivatization with dansulfinic acid. *J. Sep. Sci.* 31, 71-77 (2008). HPTLC of acrylamide in drinking water on silica gel, derivatization in situ with 5-dimethylaminonaphthalene-1-sulfinic acid (1.6

µg/µL in methanol), followed by heating at 120 °C for 1 hour and developed with ethyl acetate. For fluorescence enhancement, the plate was dipped into a solution of 25 % polypropylene glycol in n-hexane and dried immediately. Quantitative determination by fluorescence at 366/>400 nm. Verification was based on HPTLC-ESI/MS, HPTLC-direct analysis in real time (DART)-TOF/MS and NMR. The *R_f* value of acrylamide (as 3-dansylpropanamide) was 69. Linearity was between 0.1 and 0.4 µg/L. Within-run precision and the mean between-run precision (n=3) were 4.6 and 11.0 %. The limit of detection and quantification for acrylamide was 0.025 and 0.083 µg/L, respectively. Recovery (by standard addition) was 96.4 %. The method showed comparable result with HPLC-MS/MS.

environmental, quality control, agricultural, HPTLC, quantitative analysis, comparison of methods 37c

101 100 A. ALPMANN, Gertrud Morlock* (*Institute of Food Chemistry, University of Hohenheim, 70593 Stuttgart, Germany, gmorlock@uni-hohenheim.de): Determination of acrylamide in drinking water. CBS 99, 14-15 (2007); see 101 099.

101 101 S. GHOSAL (Natreon-Inc., CL-18A, Sector II, Salt Lake City, Kolkata 700091, India): The signatures of energy-transducing organic molecules in meteorites. Sci. Cult. 74 (1-2), 22-30 (2008). Bio-organic molecules such as oxygenated dibenzo-a-pyrones, their aminoacyl conjugates, and polyprenylbenzo-quinones have earlier been reported as transducers of energy in animals and human cells and were now found in four samples of meteorites. HPTLC on silica gel 60 with 1) n-butanol - acetone - acetic acid - water 7:7:2:4 for amino acids, followed by detection with ninhydrine reagent and densitometric evaluation at 610 nm; 2) n-butanol - acetic acid - water 3:1:2 for sugars, followed by detection with p-anisidine reagent and densitometric evaluation at 380 nm; and 3) chloroform - methanol 9:1 for dibenzopyrones, followed by densitometric evaluation at 240 nm and 360 nm.

environmental, HPTLC, densitometry, postchromatographic derivatization, qualitative identification 37a

101 102 R. VOLK (Department of Pharmaceutical Biology, Pharmaceutical Institute, University of Kiel, Kiel, Gutenbergstrabe 76, 24118 Kiel, Germany, volk@pharmazie.uni-kiel.de) : Screening of microalgae for species excreting norharmane, a manifold biologically active indole alkaloid. Microbiol. Res. 163, 307-313 (2008). HPTLC of norharmane from cyanobacterial culture medium extracts on silica gel with ethyl acetate - methanol - water 200:33:27 or ethyl acetate - formic acid - water 20:2:1. The *R_f* values of norharmane were 65 and 20, respectively. Evaluation under UV 254 nm without further derivatization. The screening of microalgal culture medium was performed by HPTLC and HPLC.

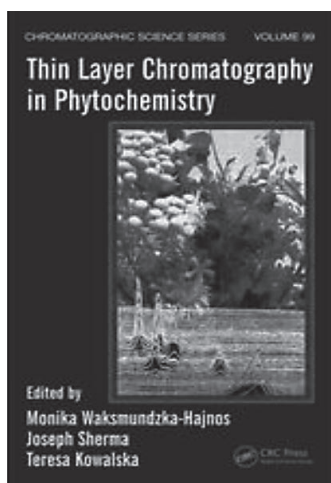
pharmaceutical research, environmental, HPTLC, qualitative identification 37c

38. Chiral separation

101 103 R. BHUSHAN*, C. AGARWAL (*Department of Chemistry, Indian Institute of Technology, Roorkee 247667, India; rbushfey@iitr.ernet.in): Direct resolution of six beta blockers into their enantiomers on silica plates impregnated with L-Asp and L-Glu. J. Planar Chromatogr. 21, 129-134 (2008). TLC of racemic metoprolol, propranolol, carvedilol, bisoprolol, salbutamol, and labetalol on silica gel impregnated with optically pure L-Glu with acetonitrile - methanol - water - dichloromethane 10:3:1:2 or 10:3:3:2 (for propranolol), and on silica gel impregnated with L-Asp, with acetonitrile - methanol - water - dichloromethane 5:3:1:3, or acetonitrile - methanol - water - glacial acetic acid 50:10:5:7. Detection with iodine vapor; the detection limits were 0.23, 0.1, 0.27, 0.25, 0.2, and 0.2 µg for each enantiomer, respectively.

pharmaceutical research, quality control, qualitative identification 38

- 101 104 Katalin NEMÁK*, E. FOGASSY, A. BÉNYEI, I. HERMECZ (*Chinoin, Tó u. 1-5, 1045 Budapest, Hungary; katalin.nemak@sanofi-aventis.com): The role of TLC in investigation of diastereomeric salt formation by a group of pipercoloxylidides. *J. Planar Chromatogr.* 21, 125-128 (2008). TLC of a racemic mixture of diastereomeric salts formed by pipercoloxylidides (pipercoloxylidide, mepivacaine, N-ethylpipercoloxylidide, ropivacaine, bupivacaine) with O,O'-dibenzoyl-2R,3R-tartaric acid on silica gel with 1-butanol - glacial acetic acid - water 12:3:5 after chamber saturation for 2 h at 25 °C. TLC was also performed using 2-propanol, ethanol, and methanol instead of 1-butanol. Detection by spraying with 1 N potassium permanganate solution and Dragendorff reagent. Evaluation under UV light.
pharmaceutical research, qualitative identification 38
- 101 105 Beata POLAK*, A. HALKA, T.H. DZIDO (*Department of Physical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland; beata.polak@am.lublin.pl): Pressurized planar electrochromatographic separation of the enantiomers of tryptophan and valine. *J. Planar Chromatogr.* 21, 33-37 (2008). Pressurized planar electrochromatography (PPEC) and TLC of tryptophan and valine on chiral plates (with D-4-hydroxyproline as the chiral selector) prewashed with methanol, with acetonitrile - methanol - water - 1.25 mM citric acid and disodium phosphate buffer 7:1:1:1. Detection by spraying with a solution of ninhydrin in acetone, followed by heating at 110 °C for 5 min.
qualitative identification 38
- 101 106 M. SAJEWICZ, D. KRONENBACH, M. GONTARSKA, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland; kowalska@uranos.cto.us.edu.pl): TLC and polarimetric investigation of the oscillatory in-vitro chiral inversion of L-alanine. *J. Planar Chromatogr.* 21, 43-47 (2008). TLC and polarimetry was used to investigate the tendency of L-alanine to undergo oscillatory in-vitro chiral inversion when dissolved in neutral, acidic, and basic solvents. The influences of temperature and sample mixing were investigated as well. It was confirmed that L-alanine undergoes chiral inversion. TLC of L-alanine on silica gel, prewashed with methanol - water 9:1, and impregnated twice with aqueous copper sulfate solution and L-proline in water - methanol 9:1. Development with 2-propanol - acetonitrile - water 6:2:3 at 22 °C. Detection by dipping in freshly prepared 0.2 % methanolic ninhydrin solution, followed by heating at 100-110 °C for 10 min. Quantification by densitometry at 540 nm.
quantitative analysis, densitometry 38



Thin Layer Chromatography in Phytochemistry

Chromatographic Science Series, Volume 99

Edited by Monika Waksmundzka-Hajnos,
Joseph Sherma and Teresa Kowalska.

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Thin Layer Chromatography in Phytochemistry fills a lack in the scientific literature reporting planar chromatography; indeed the whole chain of plant analysis and of botany in the characterization by way of the extraction is approached. Renowned scientists working with laboratories around the world demonstrate the applicability of planar chromatography to a remarkable diversity of fields, including plant genetics, drug discovery, phytotherapy products, nutraceuticals, and toxicology.

The book is organised in two parts comprising a total of 29 chapters. Part I starts with a chapter introducing the book followed by chapters on plant material; herbal medicines and nutritional supplements; chemosystematics; biological activity of primary and secondary metabolites; plant chemosystematics; generalities about sorbents and precoated layer for analysis; chambers, sample application, and chromatogram development, HPTLC, AMD, OPLC; an interesting chapter about derivatization, detection, and quantification; and one about the recent biodetection methods.

Part II contains 19 chapters reporting methods for the TLC separation, identification, and quantification of particular classes of metabolites in a great variety of plant samples from primary metabolites, carbohydrates, lipids, amino acids, peptides and proteins, followed by secondary metabolites, phenols, coumarins, flavonoides, lignans, terpenes and terpenoids, steroids, quinones, and alkaloids, and an original and complete chapter about polyacetylene compounds.

One of the bigger interests of this book resides in the sum of the bibliography references. What is on the other hand a pity is the fact that the illustrations are in black-and-white; today's books on TLC with illustrations in color bring a great deal of information to the reader. Nevertheless, this book is of big interest for the scientists working in the field of vegetable pharmacognosy, laboratories of control, and industries of cosmetics and flavors. This book will serve as a supplement to the following works: *Pharmacognosy* by Bruneton, *Analysis of medicinal plants* by Reich and Schibli, and *Plant drug analysis* by Wagner and Blast.

Professor Dr. Jacques Pothier
Department of Pharmacognosy
Faculty of Pharmacy
Tours, France

First Class Service for our customers – CAMAG training programs worldwide



CAMAG Courses

Regularly CAMAG offers in Muttenz primary and continuing training courses for customers and product specialists of our distributors. These courses inform about theoretical and practical aspects of modern Thin-Layer Chromatography, method development and handling of instruments and software. In the laboratory participants have the opportunity to perform experiments, which are of practical importance.

CAMAG offers courses of similar content also at customer's site and in collaboration with the distributors in Europe, USA, Far East and South America.

Courses on planar chromatography for industry are also available on demand. Since the year 2000 about 80 of such trainings have been conducted alone in Germany.

Joint courses with USP

Last year the US FDA released cGMPs for the dietary supplement industry. While large companies already have to be in compliance with these regulations, medium and small companies still have one or two years for implementation. HPTLC in its modern form is one of the most suitable analytical tools for reliable identification of botanical materials, detection of adulteration and assessment of overall quality. The USP includes about 40 monographs on medicinal plants and plant extracts. They feature TLC methods for identification. Currently these monographs are under revision and will be complemented by images of TLC and HPTLC results. With this project the USP is taking a leading role in setting standards of quality also for botanical dietary supplements.

CAMAG has many years of experience in developing and validating HPTLC methods for identification of medicinal plants and actively promoted a standardized HPTLC methodology. Now USP and CAMAG jointly took the initiative to help small and medium size companies with the implementation of GMPs by offering dedicated courses. These courses provide comprehensive information about a standardized TLC/HPTLC methodology and the scientific basis for a successful application of existing methods as well as the development and validation of new HPTLC methods.

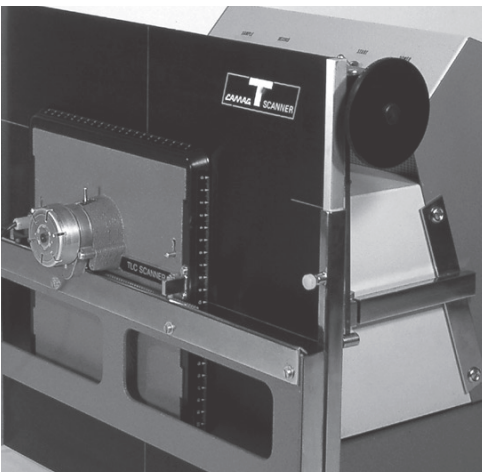
At the end of April 2008 two USP courses - TLC for Dietary Supplements: Botanicals – were held in Wilmington, NC. Participants were able to take advantage of the fully equipped CAMAG Laboratory for gaining practical experience. Further USP course are scheduled for October 30. (Salt Lake City, UT) and November 5. and 6. (Irvine, CA).

5 Dekaden CAMAG

Die Entwicklung eines Unternehmens zum Marktführer Planar-Chromatographie anhand der Sortimentsentwicklung



Als im Dezember 1958 Dr. Dieter Jänchen eine Firma unter dem Namen »CAMAG **C**hemie-Erzeugnisse und **A**dsorptionstechnik (**M**uttENZ) **AG**« in das Handelsregister eintragen liess, ahnte wohl niemand, dass sich daraus einmal die Nummer 1 auf dem Gebiet der Planar-Chromatographie (weltweit) entwickeln würde. Vorausgegangen war der Erwerb einer Liegenschaft in MuttENZ bei Basel, was sich indirekt im Firmennamen widerspiegelt.



CAMAG T-Scanner 1964



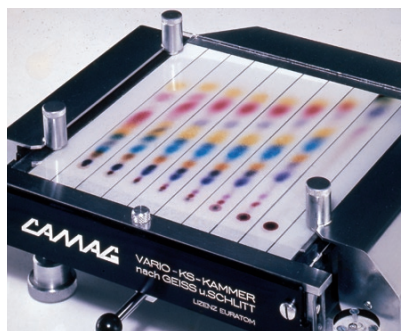
TLC-Scanner 3

Die erste Dekade 1958–1968

Der ursprüngliche Firmenzweck war die Herstellung von Aluminiumoxid für die Chromatographie, das in verschiedenen Varianten (basisch, neutral, sauer und in verschiedenen Aktivitätsstufen nach Brockmann) angeboten wurde. Die Anwendungen reichten von präparativer Säulenchromatographie im Labormassstab bis zum industriellen Einsatz, überwiegend zur Entfernung unerwünschter Nebenprodukte aus Mutterlaugen. Noch heute partizipiert Aluminiumoxid mit annähernd 10% am CAMAG Umsatz.

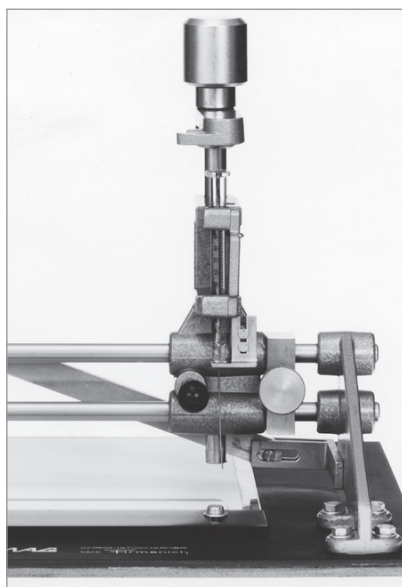
Die Gründung der CAMAG fiel zeitlich zusammen mit der raschen Verbreitung der Dünnschicht-Chromatographie (DC), was das Verdienst von Egon Stahl war, der sie in Europa bekannt machte und ihre Standardisierung einführte. CAMAG nahm sich der neuen Methode an und hatte bereits 1962 ein umfangreiches Sortiment für alle damals benutzten Arbeitsschritte: Platten-Selbstbereitung, Probenauftragen, Chromatogrammentwick-

lung, Derivatisierung durch Sprühen und UV-Inspektion.



Bemerkenswert war die Vario-KS-Kammer nach Geiss und Schlitt (Euratom), die eine systematische Optimierung verschiedener Parameter der Chromatogrammentwicklung erlaubte. Die Vario-KS-Kammern mussten wir übrigens meist zweimal »verkaufen«, einmal dem Entscheidungsträger, damit er das Geld freigab, und dann noch einmal dem Laborpersonal, damit sie auch eingesetzt wurde.

Bereits 1964 brachte CAMAG ihren ersten Scanner für die fotografische Direktauswertung von DC-Platten heraus, zeitgleich mit dem von Hellmut Jork entwickelten Auswertegerät von Zeiss, das auf dem Spektralfotometer PMQ-II basierte. Der CAMAG T-Scanner war ein Turner Fluorometer, das mittels eines vertikal angeordneten Kreuz-Messtischs – heute würde man Interface sagen – für die DC-Auswertung angepasst wurde. Übrigens brachte CAMAG auch bald darauf ein entsprechendes Interface für das Zeiss PMQ-II heraus, den Z-Scanner.



Chromatocharger zum strichförmigen Probenauftragen, in erster Linie für die präparative Dünnschicht-Chromatographie

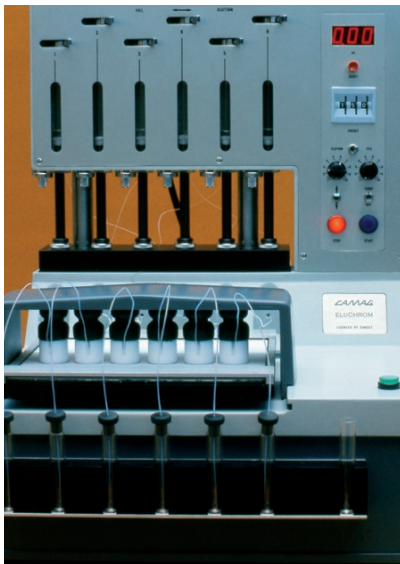
Eine sehr weitsichtige Entscheidung war der Entschluss, DC-Kunden bzw. Interessenten kostenlos die einschlägige Literatur nahe zu bringen. Hierzu wurde 1965 der »CAMAG Bibliography Service« (CBS) ins Leben gerufen, der die aktuellen DC-Publikationen in Form von Kurzreferaten zugänglich machte. In diesem Jahr erschien bereits CBS-Heft 100, in dem Werdegang dieser Firmenzeitschrift und Internetzugriff auf mehr als 8000 Publikationen beschrieben sind (<http://www.camag.com>).

In die erste Dekade der CAMAG Geschichte fällt auch die Einführung des CAMAG Hochspannungselektrophorese-Systems (HVE). Desse Kernstück war die Elektrophorese-Zelle, die auf einem einfachen, aber überraschend wirksamen Prinzip der Wärmeübertragung beruhte. Annähernd 30 Jahre lang war das CAMAG HVE-System ein wichtiges Standbein des Unternehmens. Wir nahmen es erst Anfang der 80er Jahre aus dem Programm. Zum einen hatte die HPLC die meisten der zuvor mit HVE gelösten Aufgaben übernommen, und zum anderen hätten die inzwischen enorm verschärften Sicherheitsbestimmungen eine völlige Neukonstruktion erfordert. Es sei aber bemerkt, dass mit einem CAMAG HVE-System kein einziger Unfall vorgekommen ist.

Das zweite Jahrzehnt 1969–1978

In diesen Zeitraum fällt die systematische Instrumentalisierung aller Arbeitsschritte der DC. Das wurde unumgänglich, als Merck 1975 die HPTLC-Trennschichten auf den Markt brachte. CAMAG wurde zum Vorreiter der Geräteentwicklung und damit zum anerkannten Marktführer der Dünnschicht-Chromatographie.

Anfang dieser Dekade war sich die Fachwelt noch nicht einig, ob eine Quantifizierung durch fotometrische Direktauswertung oder durch Elution der Trennzonen mit nachfolgender Fotometrie in Lösung die genaueren Resultate ergibt. Mit dem CAMAG Eluchrom konnten 6 Substanzzonen simultan mit 1.5 mL Lösungsmittel ohne Abkratzen der Schicht von der Platte eluiert werden.



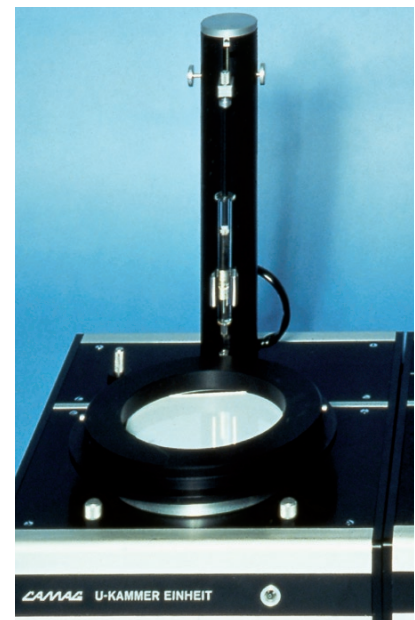
Eluchrom

Mit dem Linomat (I) wurde der Grundstein gelegt für eine Familie von Auftragegeräten, die die Sprühtechnik zum Aufbringen strichförmiger Substanzzonen benutzt. Durch strichförmiges Auftragen wird die Trennleistung deutlich verbessert. Wurden die ersten zwei Generationen Linomat noch vorwiegend für präparative Aufgaben eingesetzt, so war der Linomat III, den CAMAG 1976 auf den Markt brachte, ein Gerät mit entscheidend verbesserter Reproduzierbarkeit der Volumendosierung, wie es für die quantitative DC/HPTLC benötigt wurde.



Linomat III

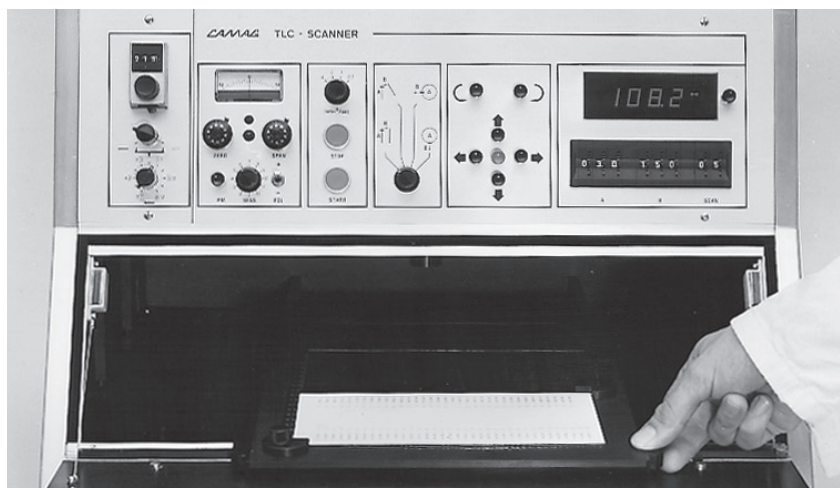
Seit 1973 beschickten wir die DC-Kurse, die Rudolf E. Kaiser an seinem Institut für Chromatographie in Bad Dürkheim hielt, mit Geräten, gelegentlich auch mit Gastvorträgen. An einem dieser Kurse 1975 stellte Merck das neue Plattenmaterial vor, und Kaiser hatte auch dafür gleich eine revolutionäre Entwicklungstechnik erdacht, die zirkulare. Aus einem von ihm gebauten Funktionsprototypen entwickelten wir innerhalb von zwei Monaten ein verkaufsfähiges Gerät, die U-Kammer. Die dafür benötigte hochpräzise Fließmittel-Dosiervorrichtung konnten wir so zu sagen aus der Schublade ziehen; es war der Dosierturm für unseren Linomat III, dessen Entwicklung kurz vor dem Abschluss stand.



CAMAG U-Kammer-Chromatograph, die zweite Generation U-Kammer

Die neuen Schichten erforderten besondere Sorgfalt bei der Probenauftragung, unabhängig davon, ob zirkular oder normal (linear) chromatographiert werden sollte. Dafür entwickelte CAMAG den Nanomat (I), mit dem die Probendosierung aus 100 bzw. 200 nL PtIr-Kapillaren erfolgte, die magnetisch auf die Schicht abgesenkt wurden. Als alternative Dosiervorrichtung hatten wir den Nano-Applikator (Kombination einer Spritze mit einer Mikrometerschraube), der das Auftragen variabler Volumina von 50–230 nL ermöglichte. Er konnte mit dem Nanomat positioniert werden.

1976 wurde – eigens für HPTLC-Schichten – eine neue Kammer mit horizontaler Plattenanordnung herausgebracht. Aus dieser »Linear-Entwicklungskammer« entstand später die Horizontal-Entwicklungskammer, die noch heute im Sortiment ist. Diese Kammer erlaubt die Chromatogrammentwicklung von beiden Seiten zur Mitte, wodurch die Probenanzahl verdoppelt wird. Sie benötigt nur wenige mL Lösungsmittel, macht die Trennung schnell und kostengünstig und übertrifft in dieser Hinsicht HPLC und GC.

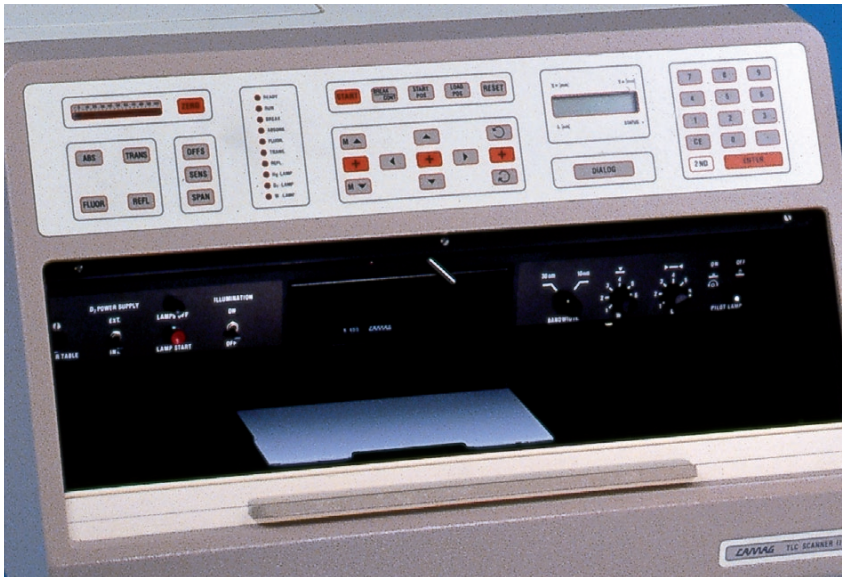


CAMAG TLC-Scanner I

Die Krönung dieses Jahrzehnts war der Abschluss der Entwicklung des ersten CAMAG TLC-Scanners, den wir 1978 auf den Markt brachten. Der Spektralbereich betrug 200–800 nm, er war eingerichtet für Absorptions- und Fluoreszenz-Messungen, und zwar in Remission und Transmission, letzteres weil wir ihn universell nutzbar machen wollten, sowohl für die Auswertung von Dünnschicht-Chromatogrammen als auch von Elektropherogrammen. Der Scanner war auch eingerichtet für die Aus-

wertung von zirkular entwickelten Chromatogrammen. Es gab ihn in zwei Versionen, einer mit Filterrad ausgerüsteten und einer (teureren) mit Monochromator. Letztere bauten wir, einerseits um dem konkurrierenden Zeiss KM 3 nicht nachzustehen. Andererseits glaubten wir, der Markt würde sich für die preiswertere Filter-Version entscheiden; eine eklatante Fehleinschätzung! Der CAMAG TLC-Scanner I trug wesentlich dazu bei, der quantitativen DC zum Durchbruch zu verhelfen.

Die Jahre 1979–1988



TLC-Scanner II, der 1983 auf den Markt kam

Als wir 1980 unseren Neubau in der Sonnenmattstrasse in Muttenz bezogen, war der TLC-Scanner (I) zu unserem erfolgreichsten Gerät im Sortiment geworden. Wir hatten zunächst eine 10er-Serie gebaut und sahen vor, einige weitere Kleinserien aufzulegen, bevor es von einem Nachfolgemodell abgelöst werden sollte. Tatsächlich verkauften wir dann aber mehr als 500 Stück, bevor wir 1983 unseren TLC-Scanner II herausbrachten.

Der Scanner wurde überwiegend in Verbindung mit einem Integrator, teils auch nur mit einem Analogschreiber betrieben. Schon 1976 hatte Siegfried Ebel mit seiner Gruppe in Marburg, später Würzburg, den Zeiss Scanner KM3 für den Betrieb mit einem Computer ausgerüstet. Kaum war der CAMAG Scanner auf dem Markt, entwickelte Ebel auch für diesen eine Computersteuerung. Ab 1980 verkaufte CAMAG die Ebel'sche Software, später entwickelten wir unsere eigene.

Probenaufträgen war nun der Arbeitsschritt, den es zu automatisieren galt. Die europäischen Wettbewerber auf dem DC-Markt hatten dafür verschiedene Lösungen. Alle beruhten darauf, dass parallele Dosiervorrichtungen simultan mehrere Proben auftragen. Desaga benutzte eine Peristaltik-Pumpe, Shandon und CAMAG parallel betriebene Spritzen. Das CAMAG Gerät (s. Abbildung auf der Titelseite dieses CBS) hatte die Besonderheit, dass die Ausdehnung der Auftragszone kontrollierbar war.

Es zeigte sich, dass das Prinzip der Parallelauftragung für die miniaturisierten Dimensionen der HPTLC nicht geeignet war, und so entwickelten wir den CAMAG DC-Probenautomat I. Er benutzte ein einziges Dosiermodul, mit dem die aus einem Rack zugeführten Proben sequentiell auf eine Platte aufgetragen wurden. Das Gerät war softwaregesteuert und eignete sich sowohl für konventionelle als auch für HPTLC-Platten.

Für den TLC-Scanner II entwickelten wir zunächst unsere Software 86 und später CATS (**C**AMAG **T**LC **S**oftware). Mit dieser Software und diversen Auswerte-Optionen wurde ein Konzept zur Verfügung gestellt, mit dem über viele Jahre auf dem Markt ein hohes Mass an Akzeptanz erreicht werden konnte.

Klaus Burger, Bayer Dormagen, hatte das vom Perry, Glunz et al. in den 70er Jahren vorgestellte Verfahren Programmed Multiple Development zu einer mehrstufigen Gradienten-Entwicklung vervollkommen. Wir konstruierten ein marktfähiges System daraus, das wir auf dem International Symposium on HPTLC in Würzburg (1983) unter der Bezeichnung AMD (**A**utomated **M**ultiple **D**evelopment) der Öffentlichkeit vorstellten. Das Prinzip eröffnete der DC völlig neue bisher nicht bekannte Trennleistungen und Anwendungsbereiche.

Die Aufzählung dieser Geräteentwicklungen soll nicht vergessen machen, dass wir auch eine ganze Reihe weniger spektakulärer Neuheiten herausbrachten, u. a. Linomat IV, das Fotodokumentationssystem Reprostar II und diverse Weiterentwicklungen unseres Sortiments.

Der Zeitabschnitt 1989–1998

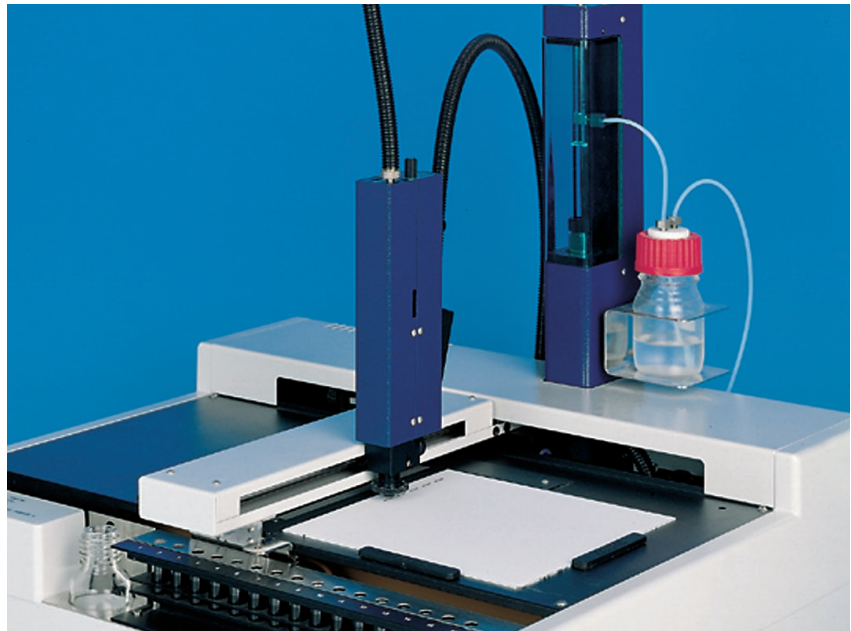
Unsere Software CATS wurde durch eine beträchtliche Anzahl benutzerfreundlicher Ergänzungen und Optionen erweitert, u. a. diverse Kalibrierfunktionen, Bahnoptimierung, Untergrundsubtraktion, Spektrenaufnahme, Spektrenbibliothek, um nur einige zu nennen. CATS unterstützte auch die für viele Pharma-Kunden wichtige Berechnungsmöglichkeit der Ergebnisse in mg/Tablette, Content Uniformity Test (CUT), und die Korrelationsberechnung der entsprechenden Spektren für Identitäts- und Reinheitsprüfungen.

Mit der Geräte- und Software-Entwicklung trug CAMAG den Anforderungen von cGMP (current Good Manufacturing Practice) Rechnung.

Ab 1991 wurde bei CAMAG ein Qualitätssicherungssystem aufgebaut, auf dessen Grundlage das Unternehmen 1995 im ersten Anlauf die ISO 9001-Zertifizierung schaffte und sie seitdem regelmässig wieder erlangte.

Mit dem Probenautomat III (ATS III) wurde 1990 ein softwaregesteuertes Gerät auf den Markt gebracht, das punkt- und strichförmiges Auftragen im nL- und μ L-Bereich ermöglichte. Anfänglich wurde es stand-alone betrieben. Später wurde die ATS 3 Software in CATS eingebunden, so dass die entsprechenden Parameter nicht nochmals für den Scanner eingegeben werden mussten. Der ATS III half vielen Kunden weltweit, die Planar-Chromatographie für ihre Massenanalytik einzusetzen. Das Gerät hielt sich 10 Jahre im CAMAG Sortiment, bis es 2000 vom ATS 4 abgelöst wurde.

Auch das etwas weniger anspruchsvolle Probenauftragegerät, Linomat IV, wurde anfangs dieser Dekade ein-



DC-Probenautomat III

geführt und blieb bis 2001 im Programm. Das Gerät war Mikroprozessor gesteuert, die Auftrageparameter wurden über ein Keyboard eingegeben.

1992 wurde mit der Automatischen Entwicklungskammer ADC (**A**utomatic **D**eveloping **C**hamber) auch dieser Arbeitsschritt automatisiert und damit für den Benutzer komfortabel gestaltet.

Übrigens, seit dem Erscheinen von JPC (Journal of Planar Chromatography) und in Anbetracht der durchgängigen Instrumentalisierung benutzen wir vorzugsweise den Begriff Planar-Chromatographie.

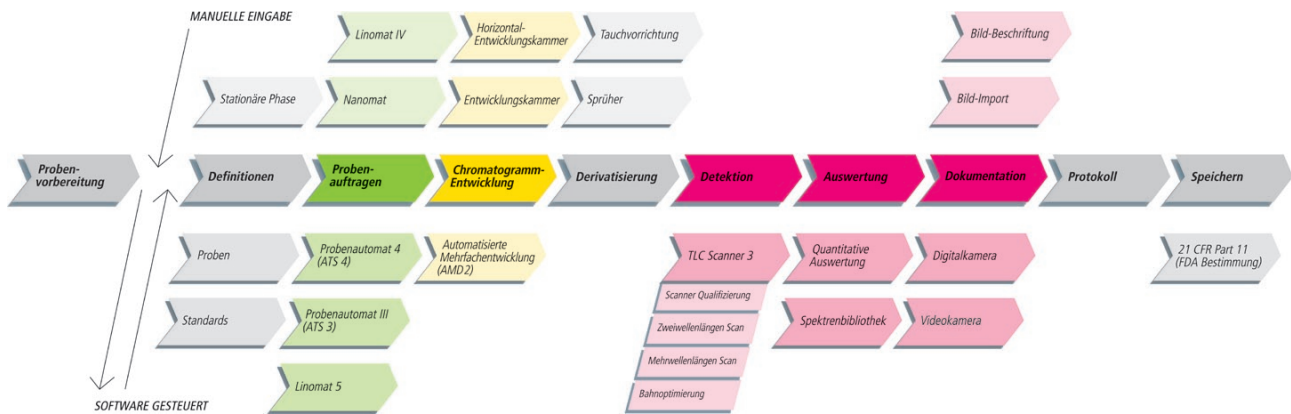
1994 brachten wir den TLC-Scanner 3 auf den Markt. Das Gerät zeichnet sich aus u. a. durch seinen Spektralbereich von 190 bis 800 nm, Messgeschwindigkeit bis 100 mm/s, Schrittauflösung wählbar zwischen 25 und 200 μ m, schnelle Spektrenaufnahme bis 100 nm/s bei einer Oversampling-Rate von 40/nm. Es

ist noch heute das leistungsfähigste Densitometer für die Planar-Chromatographie.

Trotz aller Fortschritte der klassischen Densitometrie und der Vorteile der spektralen Auflösung zeichnete sich ab, dass viele Anwender der Planar-Chromatographie sich der elektronischen Bilderfassung entweder alternativ oder komplementär bedienen wollten. Deshalb erweiterte CAMAG ihr Dokumentationssystem mit Video-Store zur Dokumentation und Archivierung und mit VideoScan zur Quantifizierung.

Zu erwähnen ist noch AMD 2, die wir 1997 herausbrachten. Bei diesem System werden die Entwicklungsstrecken nicht mehr über Zeitinkremente gesteuert, sondern ebenso wie bei der ADC direkt über einen CCD-Sensor. AMD ist nach wie vor für viele Anwender die einzige Möglichkeit, matrixbelastete Vielkomponenten-Gemische mittels Planar-Chromatographie hoch auflösend zu trennen.

1999 bis heute



Bereits Mitte der 90er Jahre wurde die Entwicklung unseres völlig neuen Software-Konzepts winCATS – Planar Chromatography Manager gestartet. Wie der Name sagt, werden mit dieser Software alle grösseren CAMAG Geräte gesteuert und alle anfallenden Daten und Ergebnisse gemäss cGMP gesichert. winCATS ist 21 CFR 21 Part 11 compliant und hinsichtlich Konzept, Umfang und Qualität weltweit einmalig. Text- und Graphikangebote können in einem vom Kunden selbst zu bestimmenden Umfang dokumentiert werden. Für sämtliche Einzelgeräte wurden SOP's (Standard Operation Procedures) erarbeitet, auf deren Grundlage heute speziell geschulte CAMAG Spezialisten auf Kundenwunsch IQ/OQ (Installation Qualification/Operation Qualification)-Zertifizierungen durchführen.

Die erste Version winCATS kam im Jahr 2000 heraus, anlässlich der Markteinführung des DC-Probenautomat 4 (ATS 4) (Abbildung siehe Titelseite dieses CBS). Präzision und Robustheit des Probenauftragens im nL- und µL-Bereich wurden weiter gesteigert. Ausser der Punkt- und Strichauftragung ist die Auftragung matrix- und wasserhaltiger Proben in Form eines Rechtecks möglich.

2001 folgte ein weiterentwickeltes Gerät der Linomat-Familie, Linomat 5, der sowohl über winCATS als auch stand-alone betrieben werden kann.

Mit dem weltweiten Siegeszug der Digitalkamera stellte auch CAMAG seit 2000 ihr Dokumentationssystem um. Seit 2005 kombiniert Digi-Store 2 die Beleuchtungseinheit Reprostar 3 mit einer hochauflösenden und hochlinearen 12 bit CCD-(Industrie) Kamera mit schneller Datenübertragung via FireWire. Das System vereint Funktionen zur Belichtungsoptimierung und zur Hintergrundkorrektur mit hervorragender Farbtreue.

2002 wurde das bei Bayer entwickelte Prinzip der Biolumineszenz-Detektion zum Aktivitäts- und Toxizitäts-Screening mit den marinen Bakterien *Vibrio fischeri* (Kit Bioluminex™ von ChromaDex) in das Lieferprogramm aufgenommen und 2006 mit dem CAMAG BioLuminizer optimiert. Eine gekühlte 16 bit CCD-Kamera mit hoher Auflösung und Quanteneffizienz gestattet den kostengünstigen und schnellen Nachweis von Probestandteilen mit biologischer Aktivität.

Seit 2005 steht die ADC 2 für Platten 20x10 und 10x10 zur Verfügung. Der vollautomatische Ablauf gewährleistet die Vorkonditionierung und Aktivitätseinstellung der Schicht, die Überwachung der Entwicklungsstrecke mittels CCD-Sensor sowie die Kontrolle der Trocknungsbedingungen. Damit wird eine in der Planar-Chromatographie bisher nicht erreichte Konstanz der R_F -Werte innerhalb einer Platte sowie von Platte zu Platte erzielt. Die ADC 2 kann sowohl über winCATS als auch stand-alone betrieben werden.

Mit dem Einsatz hochwertiger Digitalkameras zur Bilddokumentation und Quantifizierung wuchsen auch die Ansprüche an die Beleuchtungseinheit. Dem trägt der seit 2008 angebotene CAMAG TLC Visualizer Rechnung.



Fazit



Mit der Entwicklung und Herstellung von Hard- und Software für die Planar-Chromatographie hat sich CAMAG in den 50 Jahren ihres Bestehens auf dem Weltmarkt eine Position erobert, die zu Recht den Slogan erlaubt: Weltweit führend in der Planar-Chromatographie.

Die Stärken der Planar-Chromatographie - Flexibilität, Schnelligkeit, Kosteneffizienz - machen sie nach wie vor zu einer attraktiven Analysenmethode. Ihre vielfältigen Einsatzmöglichkeiten werden beispielhaft in CAMAG Applikationsschriften, in JPC (Journal of Planar Chromatography) sowie in den häufig von CAMAG initiierten Publikationen in CBS und auf den HPTLC Symposien dargestellt. Manches analytische Problem wurde im CAMAG Applikationslabor gelöst. Dies erleichterte

vielen Kunden die Kaufentscheidung und damit ihre Zuwendung zur Planar-Chromatographie. Auf der Basis der erreichten soliden Marktposition haben wir der Forschung und Entwicklung, einer qualitätsgerechten Produktion sowie einem kundenfreundlichen Service stets grosse Aufmerksamkeit gewidmet.

Unsere zahlreichen Innovationen bis in die jüngste Gegenwart belegen die Kreativität unseres Unternehmens und zeigen, dass CAMAG auch im 21. Jahrhundert gut aufgestellt ist. Für ihre Treue möchten wir allen unseren Kunden sehr herzlich danken.



Weltweit führend in der Planar-Chromatographie

Fast identification of unknown impurities by HPTLC/MS

Presented at the
International Symposium for HPTLC,
Helsinki, June 11–13, 2008



From left to right: G. Goncalves, A. Carminati and M. Nimod

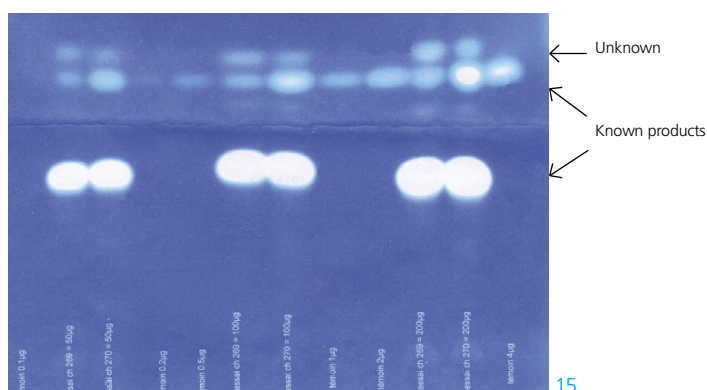
The Analytical Research and Development Department (SRDA) of Sanofi-Aventis located in Sisteron (Southern France) belongs to Industrial Affairs. The analytical chemists Muriel Nimod, Gilbert Goncalves and Alain Carminati* are in charge of the development and validation of analytical methods to characterize new active ingredients for new drug applications. Among other methods they use HPTLC coupled to mass spectrometry (MS) for fast identification of impurities formed during chemical reactions.

Introduction

During an in-process control, an impurity was detected by HPTLC and the whole production process was stopped for identification of the unknown substance.

The usual way of identifying impurities is performed by HPLC/MS coupling. But in this case, the impurity was detected by HPTLC and it would have been time-consuming to correlate the HPTLC peaks with the HPLC peaks or to scrape off the unknown, followed by elution and HPLC/MS analysis.

Hence the only way to solve this problem in time was to identify the impurity directly on the HPTLC plate. Therefore an extraction-based interface, called ChromeXtractor, was used. The interface was plug-and-play integrated into a given HPLC/MS system and within a minute the unknown substance was extracted into the MS.



In-process control by HPTLC revealed an unknown impurity

Sample preparation

The sample was dissolved in methanol (5 mg/mL).

Layer

HPTLC aluminum foils silica gel 60 F₂₅₄ (Merck), 20 × 10 cm, prewashed by development with the mobile phase

Application

Bandwise with Automatic TLC Sampler 4 (ATS 4), band length 6 mm, track distance 8 mm, distance from the lower edge 10 mm

Chromatography

In the twin-trough chamber 20×10 cm with n-butanol – water – acetic acid 3:1:1

Post-chromatographic derivatization

Using the TLC/HPTLC-Sprayer the plate was sprayed with the sulfuric acid reagent (10% in methanol) and heated on the TLC Plate Heater at 120 °C for 15 min.

Coupling with MS

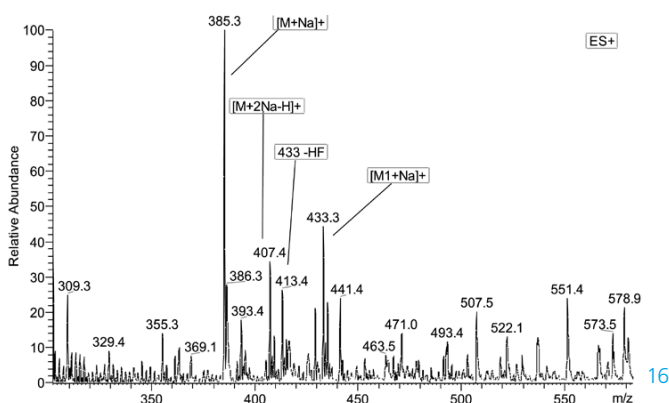
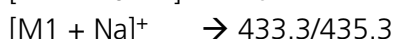
Under UV 366 nm several zones of the fluorescent unknown were marked. For extraction of the unknown from the silica gel a mixture of methanol – formic acid (0.1% in water) 9:1 was used at a flow rate of 0.3 mL/min delivered by the HPLC pump. The zone of the unknown was positioned beneath the extraction head of the ChromeXtractor (Chrom-An, Holzhausen, Germany) and tightened.

Note: The upgraded interface will be available from CAMAG in 2009.

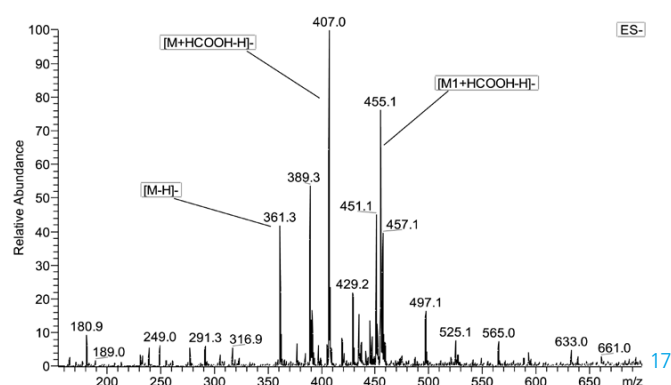
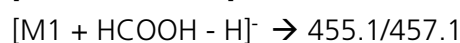
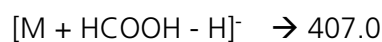
By switching the interface valve, the zone was directly extracted into the MS. The Finnigan LTQ LC-MS (Thermo Scientific) equipped with an electrospray ionization (ESI) interface was used in the positive and negative ion mode and MS/MS and MS/MS/MS studies were performed on the linear ion trap. The MS parameters were as follows: ionization voltage 4.0 kV, capillary voltage 27 V, capillary temperature 300 °C, drying gas 5 AU, nebulizing gas 10 AU.

Results and discussion

The following cluster ions were recorded in the positive ESI mode:

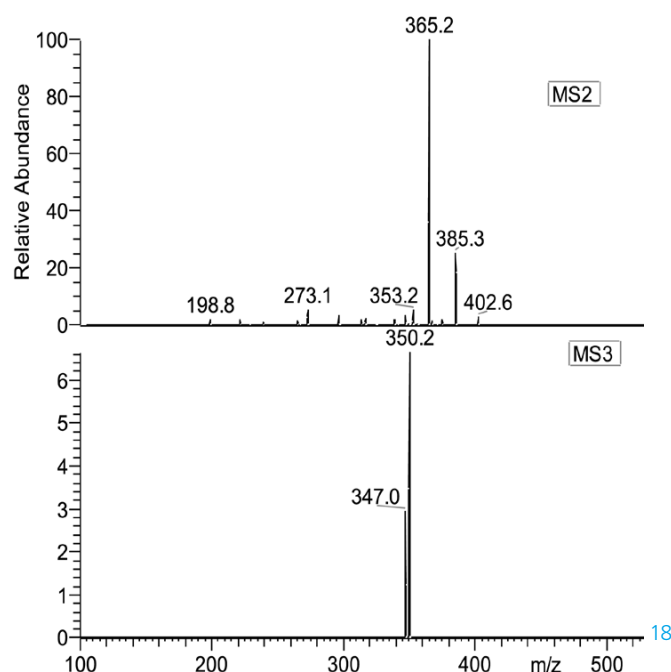


Further on the cluster ions were recorded in the negative ESI mode with a higher signal intensity:

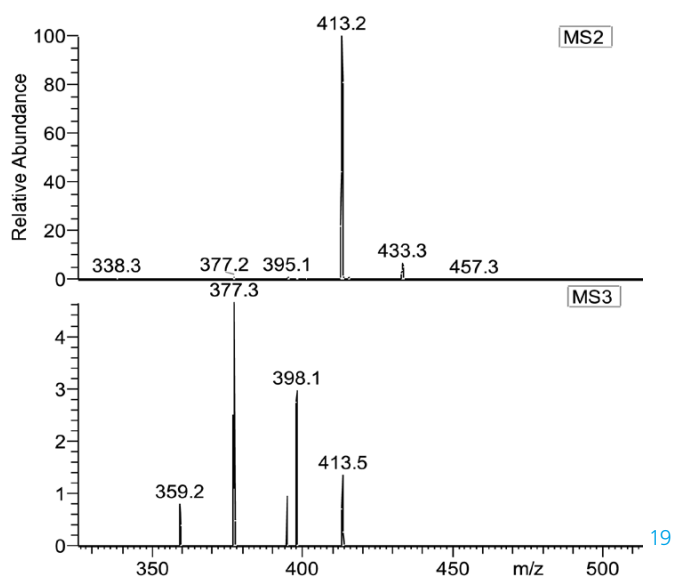


So far the positive and negative ESI spectra revealed that two unknowns at m/z 385 and 433 were co-eluted.

For identification of the unknowns, MSⁿ was performed. The MS² spectra of the first substance ion at m/z 385 led to [M + Na - HF]⁺ → 365 and the MS³ spectra to [M + Na - HF - CH₃]⁺ → 350.



The MS² spectra of the second substance ion at m/z 433 led to $[M + Na - HF]^+ \rightarrow 413$ and the MS³ spectra to:



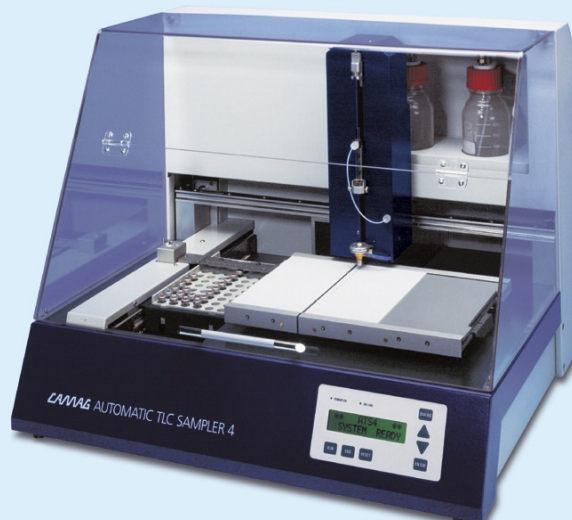
The unknown zone which was detected by HPTLC could finally be identified to be two impurities, i.e. an aldehyde derivative ($R-CHO$, $M = 362$ g/mol) and a chlorinated ketone derivative ($R-CO-CH_2Cl$, $M = 410$ g/mol).

The methodology of coupling HPTLC and MS was very helpful because the identification of the new process impurities was performed in very short time. Within a minute the mass spectrum of the impurities was obtained by direct extraction into the MS. The tedious development of an HPLC/MS method, which was the usual way of identification and structure elucidation of impurities in HPTLC, was not necessary anymore.

This procedure can be used for routine in-process control.

Further information is available from the authors on request.

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CAMAG Automatic TLC Sampler 4 (ATS 4)

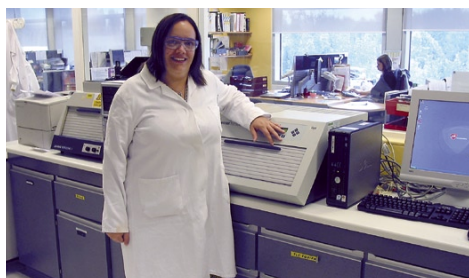
For the HPTLC/MS method as well as in all other contributions of this CBS issue, the ATS 4 is used for fully automatic application of substances onto the HPTLC plate. Samples can be applied as bands using the spray-on technique or as spots by contact transfer. Sample application in form of rectangles allows the application of large volumes.

The ATS 4 is easy to operate and controlled by winCATS. It applies substances under a cover to protect the object from environmental factors. It features a self-adjusting object support to accommodate objects of various thickness. The spray nozzle does not require adjustments. Aside of the standard rack also a special rack holding 96-well-plates can be installed. Automatic sample application increases precision and robustness during routine analysis in a GLP/GMP environment.

ATS 4 is available as option with a heated spray nozzle, especially useful for the application of aqueous solutions (see p. 3).

Use of HPTLC as a problem solving technique in pharmaceutical analysis

Presented at the
International Symposium for HPTLC,
Helsinki, June 11–13, 2008



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Clare McKinlay

Clare McKinlay* is an analytical scientist in the Chemical Development department of GlaxoSmithKline (GSK) in Stevenage near London. In her work on the development of new chemical entities, she has been using planar chromatography to investigate various findings encountered at different project stages, varying from candidates selection stage to new drug application and launch. As a result of some successes, Ms. McKinlay has been involved in increasing the awareness of the benefits of the technique in the department.

Introduction

HPLC and GC are the commonly used chromatographic techniques in the analysis of starting materials, process intermediates and active pharmaceutical ingredients (API) in R&D at GSK. HPTLC capabilities are in place, but are not used routinely. However this technique has proven to be an ideal solution for various problems and can complement other chromatographic techniques.

Problems from several projects have arisen where these more favored chromatographic techniques, HPLC and GC, have been unable to provide the project team with the appropriate level of understanding. HPTLC has been used to investigate these problems and has shown to be a very useful tool. This paper will focus on two examples of this problem solving ability of planar chromatography and will summarize the reasons why it was successful.

Example 1: Investigation of a mass imbalance discovery in light degraded samples of API

During a drug substance stability study a mass imbalance was discovered in light degraded samples. It was observed as the low assay result by HPLC could not be accounted for by the sum of all the impurity determinations. HPTLC was investigated to see if any new impurities could be detected to explain the observation.

Layer

HPTLC plates silica gel 60 F₂₅₄ (Merck), 20 × 10 cm, prewashed by development with methanol then dried on TLC plate heater at 80 °C for 15 min.

Sample preparation

Samples were prepared at a concentration of 15 mg/mL in ethyl acetate.

Application

Samples were applied bandwise at a loading of 2 µL using the Automatic TLC Sampler.

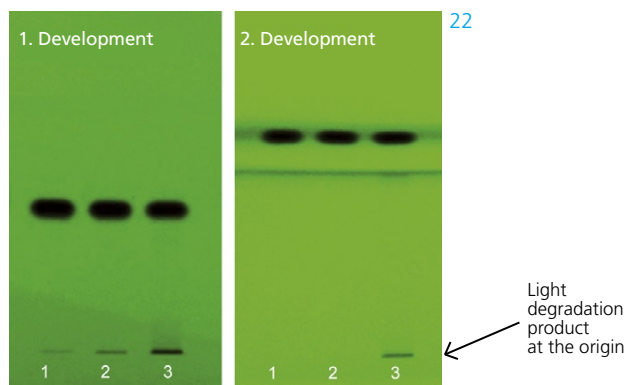
Chromatography

Plates were developed in the Horizontal Developing Chamber with a mobile phase of ethyl acetate – heptane, 9:1 to a migration distance of 50 mm from the lower plate edge. The plates were dried on the TLC plate heater and then eluted with a secondary solvent of tetrahydrofuran to elute synthetic oligomers from the origin. After the second development only the new light degradants remained at the origin.

Results and discussion

Plates were scanned at 240 nm with the TLC Scanner 3 and images taken under illumination at 254 nm to show the difference between stability and control samples.

Since HPTLC made it possible to see everything on the plate, it was clear that there were additional, new impurities present in the degraded sample which were not in the control sample. This method was shown to be sensitive enough to detect the



HPTLC plate (section) after initial chromatography (left) and after second development (right); 1) control track, 2) sample after 72 hours, 3) after 21 days exposure

impurity in samples which had a 2% mass imbalance and provided very useful information to the project team. Further impurity identification work was performed on these samples by preparative TLC and mass spectrometry.

Note: The upgraded extraction interface, available at CAMAG in 2009, coupled to a high resolution mass spectrometer allows the identification of an unknown in a fraction of the time (by extraction of the unknown directly from the analytical plate).

Example 2: Investigation of color differences in samples of an API

During the development of another project, differences in color between batches of an API were observed. No differences in HPLC impurity profile were seen and all other analyses gave consistent results. HPTLC was used to try to identify any new impurities in the yellow colored samples. The advantage of HPTLC having the ability to see everything on the plate was utilized here.

Layer

HPTLC plates NH₂ F₂₅₄ (Merck), 10 × 10 cm, pre-washed by development with methanol then dried on TLC plate heater at 80 °C for 15 min.

Sample preparation

Samples were prepared at a concentration of 2 mg/mL in methanol.

Application

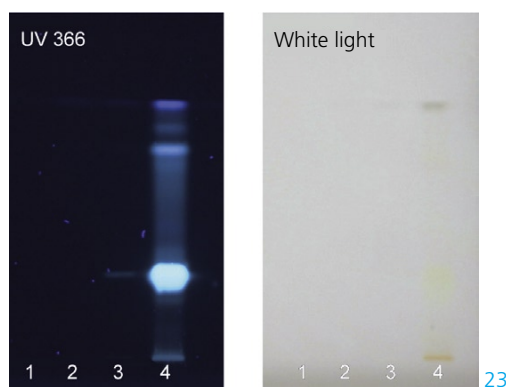
Samples were applied bandwise at a loading of 4 µL using the Automatic TLC Sampler.

Chromatography

Plates were developed in the Horizontal Developing Chamber with a mobile phase of methanol to a migration distance of 60 mm from the lower plate edge.

Results and discussion

Samples of varying colors were analyzed along with a highly colored reaction liquor sample. HPTLC clearly showed that a yellow colored band was present in the colored API which was not seen in the less colored API. This explained the differences in color and a new impurity of interest was found. The impurity also showed a native blue fluorescence at 366/>400 nm.



HPTLC plate (section) of blank (track 1), white API (track 2), yellow API (track 3), and colored extract (track 4) under 366 nm (left) and white light (right)

This impurity was extracted from the plate for further investigation by mass spectrometry to identify this unknown impurity. The method developed for HPTLC was also transferred to solid phase extraction cartridges for further preparation work.

Conclusion

The examples presented above all show how HPTLC can be used as a problem solving tool and can complement HPLC or GC for the analysis of certain compounds. The information gained from using the technique has been key to progress on several of the projects. Clearly, it has been proven that HPTLC deserves its place in modern pharmaceutical laboratories.

Further information is available on request from the author.

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Validation of HPTLC methods for the identification of botanicals



Dr. E. Reich*, Head of the CAMAG laboratory in Muttenz

Introduction

There is a great need for appropriate testing methods since the current Good Manufacturing Practices (cGMPs) for Dietary Supplements have become effective in the United States. The analysis of botanicals is challenging because plant based materials are complex mixtures of compounds which exhibit natural variability. Identification methods must be specific to distinguish the presence of wrong species.

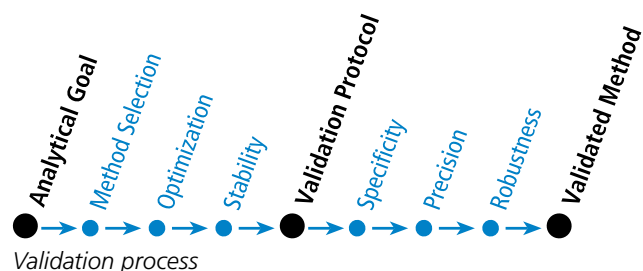
HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. To fully take advantage of this unique feature inherent to HPTLC, reproducible results and images must be ensured.

Reproducibility is improved if suitable instrumentation is used, a standardized HPTLC methodology is implemented, and methods have been developed and validated according to the following concept [1].

The validation process and its elements

Based on a clearly defined analytical goal the validation process starts with the selection/optimization or with the development of a method [2]. Following a series of stability evaluating experiments a validation protocol is elaborated. Data obtained in the validation experiments are evaluated and compared with the acceptance criteria of the validation protocol.

If all criteria are met the method can be regarded as valid.



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Method selection

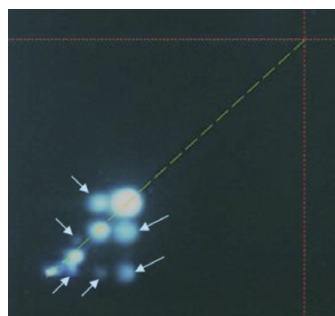
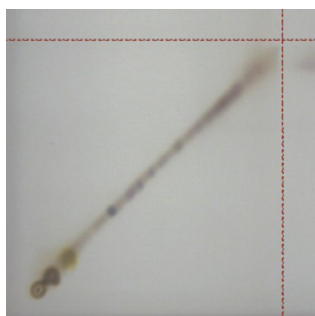
Criteria for selecting a suitable method are determined by the analytical goal but also include safety factors, time requirements, and simplicity.

Optimization of method

During optimization all TLC methods are converted into HPTLC methods and a standardized methodology is applied. At this point botanical reference materials (BRM) of known adulterants are included to ensure sufficient specificity of the method.

Stability

The stability of the analyte on the plate, in solution, and during chromatography as well as the stability of the visualized chromatogram are to be investigated. Stability of the sample during chromatography is investigated by two-dimensional (2D) development. Therefore one portion of the BRM is prepared and applied as spot at the lower right corner of a plate. The plate is developed, dried, turned 90° to the right, and developed a second time. If the sample is stable during chromatography, all components can be detected on the line connecting the application position and the intersection of the two solvent fronts. Spots located off this line indicate the formation of artifacts. Methods that produce artifacts have to be improved. If visualization of the fingerprint requires a derivatization step, the stability of result must be evaluated over time (e.g. up to one hour).



Investigation of stability during chromatography for Eleuthero (stable, left) and Angelica (not stable, right)

Validation protocol

The validation protocol is a key instrument for structuring, managing and documenting the validation process. The following elements must be included:

1. Goal of the method to be validated
2. General acceptance criteria
3. Personnel
4. Detailed description of the method
5. Validation
 - 5.1 Material
 - 5.2 Stability
 - 5.3 Specificity
 - 5.4 Precision
 - 5.4.1 Repeatability
 - 5.4.2 Intermediate precision
 - 5.4.3 Reproducibility (optional)
 - 5.5 Robustness
6. Results, releases, signatures

Specificity

Authenticated samples of the target species, if possible from different origin, and samples of known adulterants are chromatographed on the same plate. Based on electronic images the sequence (number, color, intensity, and position) of the zones in each fingerprint is evaluated by visual comparison. A method is specific, if an authentic sample gives a fingerprint similar to that of the BRM and any adulterated sample fails the similarity test.

Precision

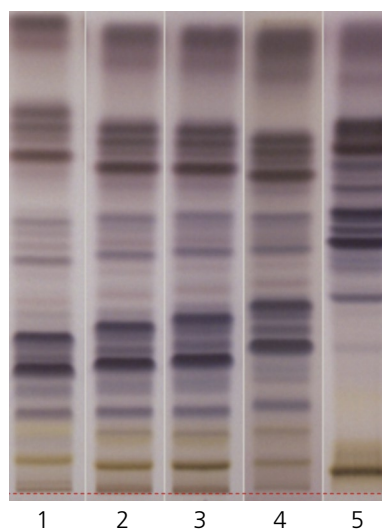
The precision of a qualitative analysis (generation of HPTLC fingerprint) can be expressed as precision of the positions of separated zones (hR_F -values). We propose to look at three levels: repeatability, intermediate precision, and reproducibility. Precision is acceptable if the variability of the hR_F -values of three markers does not

exceed ΔhR_F 1 across each plate, ΔhR_F 2 for repeatability, and ΔhR_F 5 for intermediate precision and reproducibility [1].

Note: It is recommended to use the Automatic Developing Chamber 2 that controls the plate activity.

Robustness

Effects of many experimental parameters can be evaluated already during method development. As the HPTLC result is generally affected by the relative humidity in the laboratory it is important to evaluate the range over which the method performs as expected.



Comparison of the fingerprints of *Hoodia gordonii* obtained at different relative humidity (1: 3% RH, 2: 33% RH, 3: 47% RH, 4: 54% RH, 5: 75% RH)

Further information is available on request from the author.

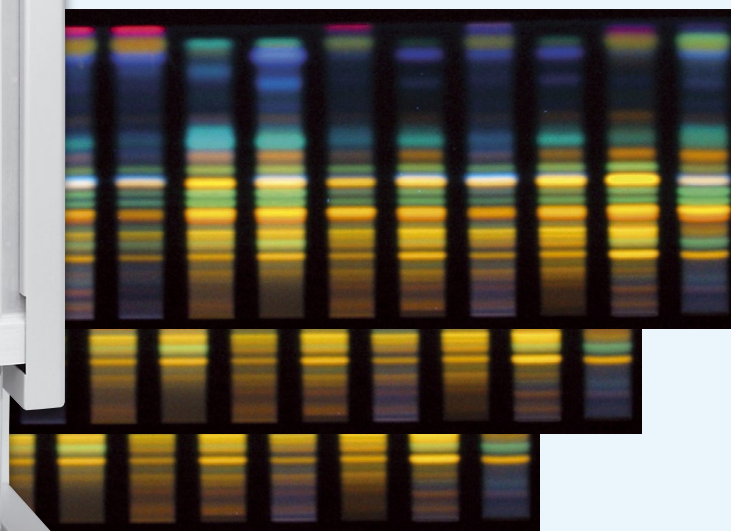
- [1] E. Reich, A. Schibli, A. DeBatt, J AOAC INT 91 (2008) 13
- [2] E. Reich, A. Schibli, High-performance thin-layer chromatography for the analysis of medicinal plants, Thieme Medical Publishers Inc., New York, 2007.

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Reproducibility, comfort
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plates



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Chromatogram development is the most critical step of Planar Chromatography. In the Automatic Developing Chamber ADC 2 this step is fully automatic and reproducible, independent of environmental effects.

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- Operation in stand-alone mode or under winCATS
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