CBS 92 • MARCH 2004

CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY

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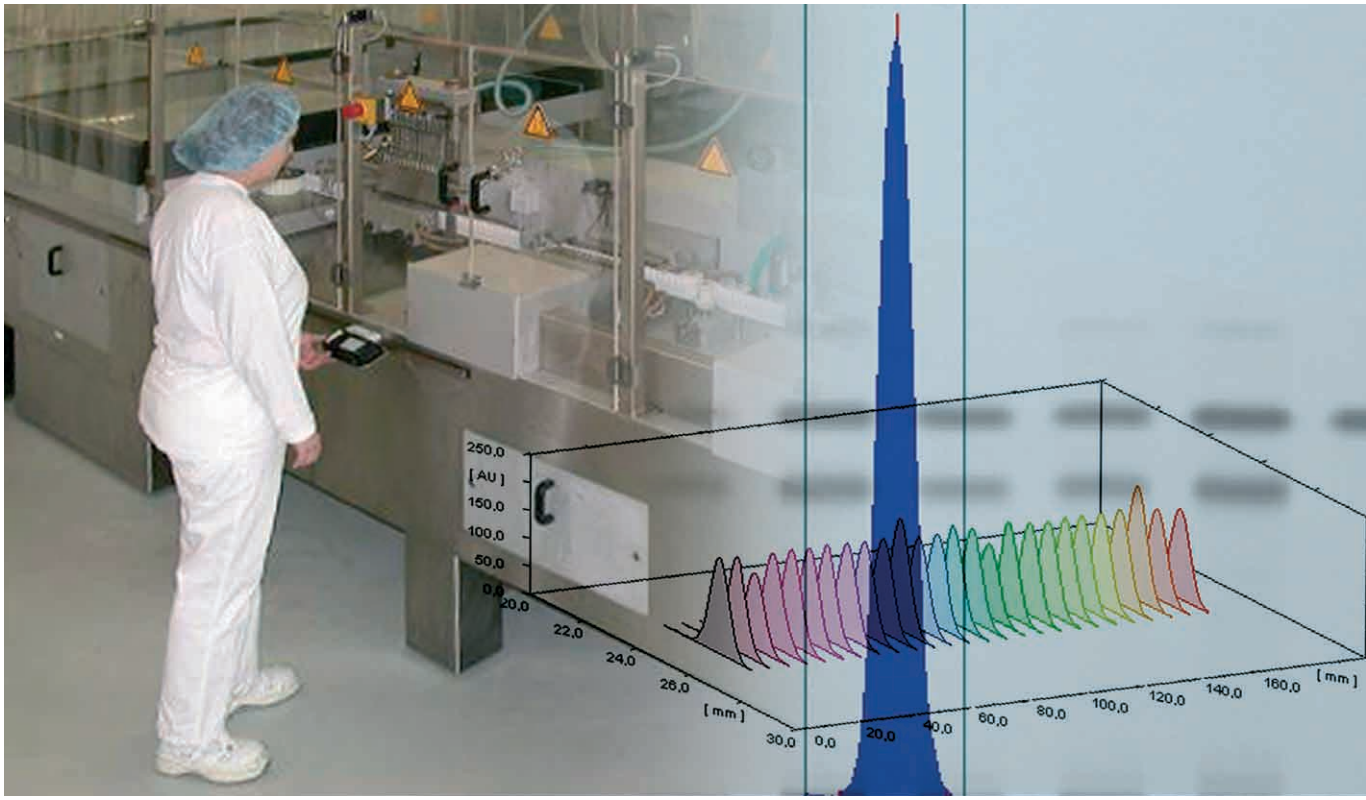

Success story: Planar Chromatography for Content Uniformity Test

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92

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No. 92, March 2004

CAMAG Bibliography Service
Planar Chromatography
Edited by Gerda Morlock
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IN THIS ISSUE

Procedures, applications, events

CAMAG products featured in this issue

Column: Know CAMAG?

CAMAG

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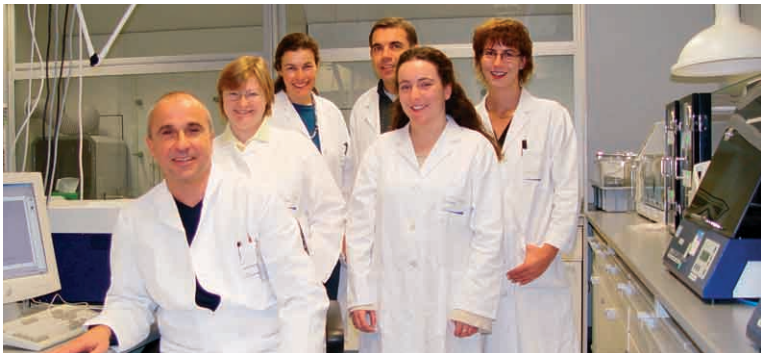
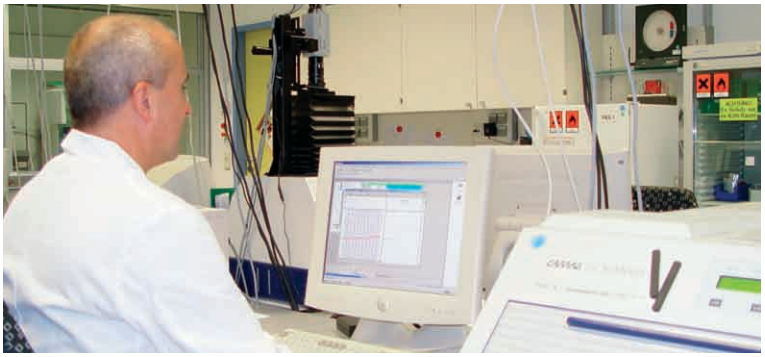
Success story planar chromatography



1

▲ *Istvan Mesaros, Silvia Siggert, Barbara Wochner, Harald Jehle, Daniela Kleiber, Nadine Sinner (from left to right)*



2

▲ *Istvan Mesaros at the HPTLC work station*

ALTANA Pharma is an international organization of the pharmaceutical research industry located in Konstanz, Germany. More than 7.500 employees of 30 daughter and partner companies are working in the business branches therapeutics, diagnostics, and self-medication.

Since the 70s planar chromatography is used by the company also for quantitative determinations. The following application, a contribution by the team of Mr. Jehle*, department of quality control of non-sterile products, illustrates why planar chromatography is of great importance for an innovative and cost oriented business. Planar chromatography is especially suitable because in comparison to other chromatographic techniques analysis time for a content uniformity test is unbeatably short. For example compared to HPLC planar chromatographic determination is about 70% faster. In addition matrix effects can be eliminated due to selective detection.

CUT of cinchocaine hydrochloride

Test for content uniformity of single dose preparations (CUT) according to the European Pharmacopoeia on the example of cinchocaine hydrochloride in suppositories

Introduction

Cinchocaine containing suppositories are used for therapy of hemorrhoids (proctologic), particularly when combined with inflammation and bleeding, of anal fissures, rhagades, eczemas, or pruritus and for treatments of wounds following proctological operations. According to the European Pharmacopoeia content uniformity must be proven for contents of active ingredients of less than 2% related to the total mass. For 10 randomly selected samples of cinchocaine containing suppositories the content of active ingredient is individually determined as follows.

Sample preparation

10 suppositories are individually placed in 250 mL volumetric flasks together with 60 mL methanol and 30 mL water and melted at 50° C in a shaken water bath for 20–30 min. Then the suspension is vigorously shaken by hand and while continuously shaken with a shaker cooled down to room temperature so that the fat is almost quantitatively precipitated as fine flakes. The volumetric flasks are filled to the 250 mL mark with ethanol. 9 mL of this solution are centrifuged for 15 min at 0° C and 2000 rpm. Any remaining traces of fat are thus precipitated. The sample solutions are filtered through a disposable filter (0.45 µm) into individual vials.

Standard solution

50 mg cinchocaine hydrochloride are weighed into a 100 mL volumetric flask and filled to the mark with methanol. 0.4, 0.5 and 0.6 mL of this solution are diluted to 25 mL with methanol.

3

CAMAG TLC Scanner 3

Classical densitometry with the CAMAG Scanner 3 offers highest accuracy for quantitative evaluation. In these days of rapidly spreading image processing technology the classical densitometer is indispensable when spectral selectivity and sensitivity is essential.

The complete spectral range from 190 to 800 nm can be utilized for evaluation. Within the complete range also absorption spectra or (fluorescence) excitation spectra can be recorded for identification of substances.

CAMAG TLC Scanner 3 with the software "winCATS Planar Chromatography Manager" is the most advanced work station for densitometric evaluation of planar chromatograms. For additional requirements various options are available such as the option "21 CFR Part 11 compliance ready" (see p. 16) available for this year.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm

Sample application

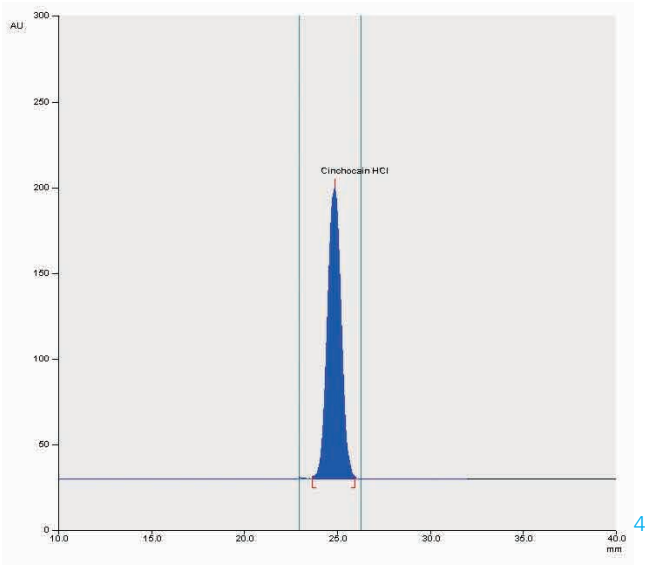
With Automatic TLC Sampler 4, 26 tracks using the data pair technique, band length 5 mm, application volume 1.6 µL, distance from lower edge 10 mm, distance from the side 20 mm, track distance 6.4 mm. Following application the plate is dried for 30 s at 110° C using a plate heater.

Chromatography

In a CAMAG flat bottom chamber with 1-butanol – toluene – ethanol – water – acetic acid 100% 10:8:7:4:1, developing distance 30 mm from lower edge of plate, development time 21 min. Following chromatography the plate is dried for 10 min at 110° C using a plate heater.

Densitometry

TLC Scanner 3 with winCATS software, fluorescence measurement at 313/>400 nm, evaluation of peak areas with linear regression.

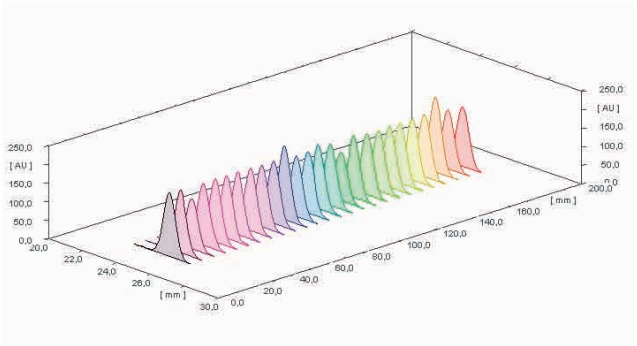


4

▲ *Display of a cinchocaine peak*

Results and discussion

The method is validated. Precision is determined to be 2% and recovery 101%. The linear calibration function has a correlation coefficient of 0.993 and a relative standard deviation of 1.91%.



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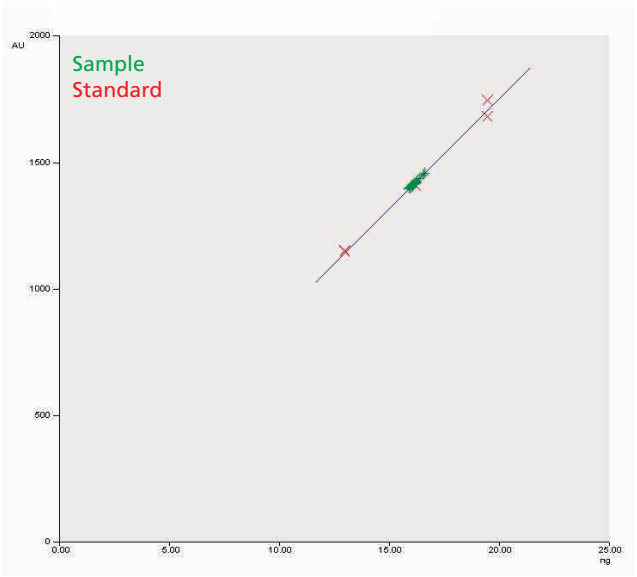
▲ *3D-display of 26 tracks*



6

▲ *Linear calibration function of cinchocaine HCl*

Further information is available on request from the author.

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Does the use of spherical adsorbents for HPTLC pay off?


7

Dr. H. Hauck, M. Schulz, C. Lorenz


8

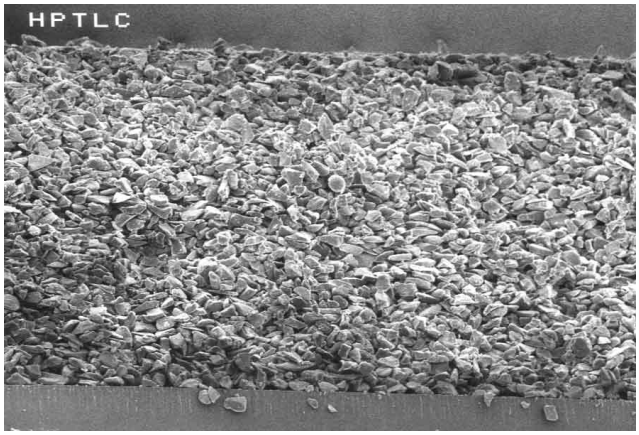
Dr. A.Koch

Dr. Heinz Hauck* is manager of the laboratory which, as a part of the department A&R/R&D of Merck KGaA Darmstadt, is responsible for research and development in the area of planar chromatography. This article is a result of the collaboration with Dipl.-Ing. Michael Schulz, Carsten Lorenz, trainee at the department, and Dr. Angelika Koch*, Frohme Apotheke, Hamburg.

Scope

In 1975 the introduction of precoated HPTLC layers with a narrow particle size distribution and average particle size significantly smaller than that of the adsorbents traditionally used for thin-layer chromatography resulted in a drastical improvement of separation power and optical properties. Further significant improvements have now been achieved by utilizing spherical adsorbents.

These spherical precoated HPTLC layers with the registered trademark LiChrospher® are characterized by an even narrower particle size distribution within an optimized average particle size. The electron-microscopic image clearly shows the difference between a normal HPTLC plate and a LiChrospher layer. Both adsorbents represent silica gel with an average pore size of 6 nm (silica gel 60).



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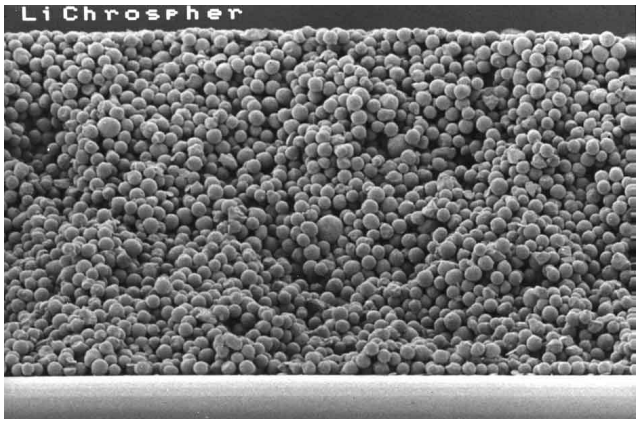


10

▲ *Comparison of an HPTLC plate Si 60 (top) and an HPTLC plate LiChrospher® Si 60 (bottom)*

Further applications and details for separation on spherical plates are available from the authors on request.

*Dr. Heinz E. Hauck, Merck KGaA, A&R/R&D Synthesis and Derivatization, Frankfurter Str. 250, D-64293 Darmstadt, Tel. +49 (0)6151-722830, Heinz-Emil.Hauck@merck.de und Dr. Angelika Koch, Frohme-Apotheke, Frohme Str. 14, D-22457 Hamburg, Germany.

Comparison of development time

In the table below development times are shown for both kind of HPTLC plates over a distance optimized for the respective developing solvents. It can be seen that the developing time for HPTLC LiChrospher plates on average is about 25% shorter than that for HPTLC plates with irregularly broken silica gel.

Developing solvent	Developing distance [cm]	Developing time [s]	
		HPTLC silica gel 60	HPTLC LiChrospher® Si 60
Toluene	4	345	240
Ethyl acetate – toluene 19:1	5	470	360
Methylethyl ketone – 1-propanol – water – acetic acid 8:8:4:1	5	186	120
n-Hexane – toluene – acetone 7:2:1	7	1140	780
Petroleum ether (40–60°) – acetone 7:3	7	1200	1020

Comparison of retention und selectivity

For comparison of the retention behavior and selectivity of both HPTLC plates the table contains hR_F-values and the corresponding selectivity values for the pesticides hexazinon, aldicarb and trifluralin on normal phases with petroleum ether (40–60°) – acetone 7:3 as mobile phase. On reversed phases these values were determined for the pharmaceuticals phenacetin, caffeine and acetylsalicylic acid with the developing solvent acetonitrile – water 4:6. The results show only small differences in retention behavior and almost identical selectivity so that the possibility of transferring separations to LiChrospher layers is quite good in this case.

Value	HPTLC silica gel 60	HPTLC LiChrospher® Si 60
$hR_{F\ Hexazinon}$	19,7	24,6
$hR_{F\ Aldicarb}$	52,3	57,0
$hR_{F\ Trifluralin}$	88,2	93,0
$Selectivity_{\ Aldicarb/Hexazinon}$	2,6	2,3
$Selectivity_{\ Trifluralin/Aldicarb}$	1,7	1,6

Value	HPTLC RP-18 W	HPTLC LiChrospher® RP-18 W
$hR_{F\ Phenacetin}$	29,8	34,5
$hR_{F\ Caffeine}$	45,8	48,3
$hR_{F\ Acetylsalicylic\ acid}$	57,2	64,3
$Selectivity_{\ Caffeine/phenacetin}$	1,5	1,4
$Selectivity_{\ Acetylsalicylic\ acid/caffeine}$	1,3	1,3

Comparison of separation power

For characterization and comparison of the respective separation power of HPTLC plates with spherical and irregularly broken adsorbents separation numbers were determined for the following systems:

a) HPTLC LiChrospher® Si 60 and HPTLC silica gel 60
Separation of the steroids hydrocortisone, Reichstein's substance S, methyl testosterone with ethyl acetate – toluene 19:1

b) HPTLC LiChrospher® Si 60 and HPTLC silica gel 60
Separation of the pesticides hexazinon, aldicarb, trifluralin with petroleum ether (40-60°) – acetone 7:3

c) HPTLC LiChrospher® RP-18 W and HPTLC RP-18 W
Separation of the pharmaceuticals phenacetin, caffeine, acetylsalicylic acid with acetonitrile – water 4:6

The separation numbers listed in the following table clearly prove better separation power of the spherical HPTLC adsorbents compared to the irregularly broken material not only for unmodified silica gels but also for the RP 18 modifications.

Plate type	Separation number $_{Steroids}$	Separation number $_{pesticides}$	Separation number $_{pharmaceuticals}$
HPTLC silica gel 60	12,5	12,5	–
HPTLC LiChrospher® Si 60	13,5	15,8	–
HPTLC RP-18 W	–	–	6,7
HPTLC LiChrospher® RP-18 W	–	–	7,6

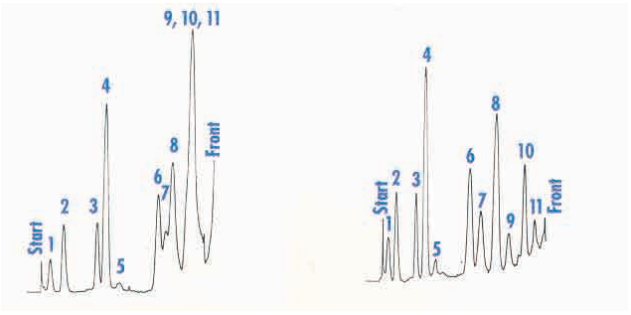
Comparison of sensitivity of detection

The increased separation power, that means the formation of more compact zones on HPTLC plates LiChrospher®, also causes an increase in sensitivity of detection in comparison to HPTLC layers with broken silica gel. The table illustrates this fact using as example the limits of detection for several UV-absorbing substances for visual evaluation under a UVlamp at 254 nm and spectrophotometric determination with a scanner.

Substance	Limit of detection at UV 254 nm [ng/zone]			
	Visual		Scanner	
	silica gel 60 F_{254}	LiChrospher® Si 60 F_{254s}	silica gel 60 F_{254}	LiChrospher® Si 60F_{254s}
2-Aminophenol	50	25	25	5
3-Aminophenol	10	5	10	5
4-Aminophenol	>100	50	50	25
Ascorbic acid	100	100	100	25
Atrazin	50	25	10	5
Cortisone	50	25	25	10
Prometryne	25	10	10	5
Theophylline	50	25	25	10

Applications

The separation of pharmaceuticals chromatographed with ethyl acetate – methanol – 25% ammonia 17: 2:1 is shown in fig. 11. The developing time for 8 cm developing distance is 18 min on HPTLC plates LiChrospher® Si 60 in comparison to 26 min on HPTLC plates silica gel 60.



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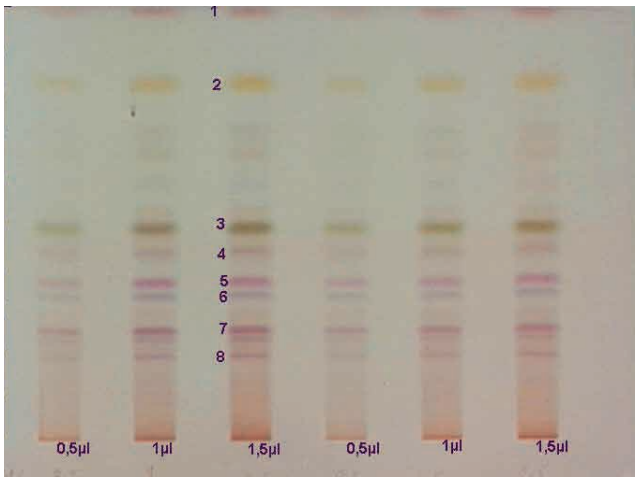
▲ *Separation of pharmaceuticals on HPTLC plates silica gel 60 (left) and LiChrospher® Si 60 (right), measurement of absorption at UV 220 nm: nitrofurantoin (1), theophylline (2), atenolol (3), hydrochlorthiazide (4), theobromine (5), metoprololtartrate (6), propafenone hydrochloride (7), molsidomine (8), phenazopyridine hydrochloride (9), spironolactone (10), impurity (11)*

The separation of a *Boswellia carteri* extract on HPTLC plates LiChrospher® Si 60 F_{254s} with toluene – ethyl acetate – formic acid (water-free) – n-heptane 80:20:3:10 is illustrated below.



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▲ *Separation of a* Boswellia carteri *extract on HPTLC plates LiChrospher® Si 60, post chromatographic derivatization with anisaldehyde sulfuric acid reagent: verticillatriene (1, purple), incensol acetate (2, ochre), incensol (3, greenish ochre), 3-acetyl-8,24-diene-tirucallic acid (4), 3-acetyl-ß-boswellic acid (5, purple), 3-oxo-8,24-diene-tirucalic acid (6, blue), ß-boswelic acid (7, purple), 11-hydroxy boswellic acid (8)*

Results and Discussion

When compared to corresponding precoated layers of broken materials of similar retention and selectivity behavior, the use of spherical adsorbents in High Performance Thin-layer Chromatography results in a significant improvement with respect to:

- Analysis time (about 25% shorter development time)
- Separation power
- Sensitivity of detection (about 50% better).

These advantages can be related to improved and more homogenous packing of the layers with spherical adsorbents.

The only question remaining is, how much do these advantages cost us? In 90% of all cases nothing, on the contrary – LiChrospher plates Si 60 F_{254s} are currently about 15 % cheaper than the corresponding HPTLC plates with irregularly broken silica gel. However, some special LiChrospher plates such as Si 60 WRF_{254s} or RP-18 WF_{254s} are 25–30% more expensive than the corresponding plates with irregularly broken adsorbents.

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Come visit CAMAG´s new home page!

Clear, structured and transparent – there is no better way to describe the new homepage. It invites you to browse and inform yourself about the latest developments in planar chromatography.

6 sections quickly provide the information you are searching for:

- Under "Products" you find information, explanation and hints regarding the individual instruments and an overview of the entire CAMAG product range. Brochures for all instruments can be downloaded as .pdf files.
- "Support" provides a general description of planar chromatography as well as the valuable data bank CCBS, including more than 7500 abstracts for free download.
- Check out "News and Events" for dates of courses, exhibitions, conferences and other events.
- The section "Services" lists contact addresses for application support and informs about instrument qualifications (IQ, OQ, PQ).
- "Literature" includes further links.
- The last section is devoted to CAMAG and its worldwide distributor network.

Filled with useful information, clearly structured and logically grouped – the CAMAG website. Take some time and explore the world of modern planar chromatography.

CBS

CAMAG BIBLIOGRAPHY SERVICE PLANAR CHROMATOGRAPHY

Liebe Freunde

Vielleicht kennen Sie mich noch aus der Zeit als ich das CAMAG Labor leitete. Seit der letzten Ausgabe habe ich die Redaktion von Herrn Reich übernommen. In Form des CBS möchte ich den Anwender mit einer Fachdatenbank unterstützen, hervorragende Anwendungsbeispiele zur Anregung aufzeigen und über den aktuellen Stand der Geräte-Technik informieren.

Die Literaturabstracts werden von einem qualifizierten, internationalen Referentenkreis gesammelt. Die Datenbank hat so manchem Anwender, der vor einer Methodenentwicklung stand, die ersten Schritte erleichtert. Seit CBS 51 (1983) sind ca. 7500 Abstracts in der planar-chromatographischen Datenbank integriert. Diese Dienstleistung stellt CAMAG als CD-Rom und unentgeltlich als Download (unter www.camag.com) zur Verfügung.

Durch unsere vielfältigen Kundenkontakte fallen uns immer wieder hervorstechende planar-chromatographische Arbeiten auf. Diese möchten wir einem breiten Fachpublikum vorstellen. Immer wieder staunt man über die Leistungsstärke dieser einfachen Trennmethode und vor allem darüber, wie kreativ sie eingesetzt werden kann. Erinnern wir uns an den letzten CBS, an die spektakulären Lipid-Trennungen oder an Ajmalicin, das Prof. Creche et al. durch UV-Bestrahlung zum „Leuchten“ gebracht hat. Er hat sich das in allen Teilschritten vollautomatisierte und GLP konforme off-line-Prinzip zunutze gemacht.

In diesem CBS möchte ich vor allem auf das „Jahr der Chemie“ hinweisen. Die Planar-Chromatographie eignet sich besonders gut, um das Prinzip der Chromatographie an sich zu verdeutlichen.

Herzlichst Ihre

Gerda Morlock

Gerda Morlock

Dear friends

Possibly you remember me from the days when I was heading the CAMAG laboratory. Beginning with the last issue I have taken over the editorship from Eike Reich. By means of the CBS I would like to support the user with a professional database, outstanding examples of applications and information about the latest development of the technique.

The literature abstracts are collected by a qualified group of international referees. The database has inspired many users, who were faced with method development. Beginning with CBS 51 (1983) about 7500 abstracts have been incorporated into the database. CAMAG offers this service on a CD-ROM and, free of charge, for download from our website www.camag.com.

Through many of our contacts with clients we continue to learn about outstanding work in TLC. This we would like to share with a broad professional audience. One is always surprised by the power of this simple separation technique and particularly by the creativity of its users. Let’s recall the last CBS and spectacular lipid separation, or ajmalicine, which Prof. Creche et al. excited by UV light to “glow”. He utilized the off-line principle, which is however fully automated and GLP compliant for each step.

In this CBS I would particularly like to emphasize the “Year of Chemistry”. Planar chromatography can very effectively demonstrate the principle of chromatography.

Sincerely yours,

Gerda Morlock

Gerda Morlock

CAMAG

MARCH 2004 92

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**

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

1. Reviews and Books

92 001 M. BATHORI*, H. KALASZ, G. JANICSAK, Z. PONGRACZ, J. VAMOS, (*Dept. of Pharmacogn., Univ. of Szeged, Eötvös utca 6, H-6720 Szeged, Hungary): Thin-layer chromatography of phytoecdysteroids. J. Liq. Chrom. & Rel. Technol. **26**, 2629-2649 (2003). Review of planar chromatography of ecdysteroids. Separation of various ecdysteroids is detailed using both straight-phase and reversed-phase thin-layer chromatography. The generally used special techniques, such as two-dimensional TLC, forced-flow TLC, displacement mode of development, etc., are also specified. The particular behavior of certain ecdysteroids is discussed. Materials and methods frequently used for ecdysteroid separation are described like e.g. stationary phases, mobile phases, detection methods, screening and identification of plant ecdysteroids, analysis of 20-hydroxyecdysone through the vegetation of plants, lipophilicity of ecdysteroids, analysis of ecdysteroids on RP-TLC plates, FF-TLC, AMD, 2D-TLC, displacement TLC, and hyphenated techniques.

Biochemistry 01

2. Fundamentals, Theory and General

92 005 N.U. PERISIC-JANJIC*, B. LUCIC, D. AGBABA, (*Inst. of Chem., Fac. of Sci., Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia and Montenegro): Study of lipophilicity and retention behavior of some beta-adrenoceptor antagonists. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 369-375 (2003). TLC of betaxolol HCl, propanolol HCl, celiprolol HCl, oxprenolol, karvedilol, metoprolol tartrate, and atenolol on RP-18 with water - acetonitrile and water - dioxane in different concentrations. Visualization under UV 254 nm.

Pharmaceutical research, qualitative identification 02

92 002 M. JANICKA*, J.K. ROZYLO, (*Fac. of Chem., Maria Curie-Sklodowska Univ., Maria Sklodowska Sq. 3, 20-031 Lublin, Poland): OPLC and TLC in the prediction of retention factors of solutes in pure water. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 13-23 (2003). TLC of newly synthesized benzanilides, benzamide and a group of pesticides on RP-18 with aqueous solutions of acetonitrile or methanol with different organic modifier concentrations. Visualization under UV 254 or 366 nm.

02

92 003 B. OSZIK-MENDYK, (Fac. of Chem., Dept. of Planar Chromatography, Maria Curie-Sklodowska Univ., Maria Curie-Sklodowska Sq. 3, 20-031 Lublin. Poland): LC in studies on molecular interactions in mobile phase. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 347-355 (2003). Discussion of a model of chromatographic process which takes into account molecular interactions in mobile phase. TLC of i.e. 2-, 3-, 4-nitroaniline, 4-nitro-2-, 2-nitro-4-, 5-nitro-2-toluidine, 2-, 3-nitrotoluene, 2-, 4-chlorophenol, 2-, 4-nitrophenol, 2,3-, 2,4-, 2,5-, 2,6-dichlorophenol, and naphthalene on silica gel with benzene - acetone, benzene - methanol, carbon tetrachloride - ethyl acetate, and toluene - isopropanol.

Theoretical considerations 02

92 053 N.U. PERISIC-JANJIC et al., see section 24

92 004 N.U. PERISIC-JANJIC*, B.Z. JOVANOVIC, O.S. RAJKOVIC, D.G. ANTONOVIC, (*Inst. of Chem., Fac. of Sci., Trg Dositeja Obradovica 3, 21 000 Novi Sad, Serbia and Montenegro): Correlation of retention behavior of some newly synthesized s-triazine derivatives in various RP-TLC systems. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 363-368 (2003). HPTLC of triazine derivatives (2,4-bis(cyclopropylamino)-6-chloro-s-triazine, 2,4-bis(cyclo-dodecyl-amino)-6-chloro-s-triazine) on RP-18 and on silica gel, impregnated with paraffin oil, with water - acetone. Visualization under UV 254 nm.

Qualitative identification, s-triazine derivatives 02

92 006 M. SAJEWICZ, A. PIENIAK, R. PIETKA, K. KACZMARSKI, T. KOWALSKA, (Inst. of Chem., Silesian Univ., 9, Szkolna Street, 40-006 Katowice, Poland): A densitometric comparison of solute retention in the classical and sandwich-type chromatographic chambers. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 399-405 (2003). TLC of 4-phenyl-1-butanol, 5-phenyl-1-pentanol, 3-phenylpropionic acid and 3-phenylbutyric acid on cellulose with i.e. decalin in the classical chamber of Stahl-type and the sandwich-type. Densitometry at 260 nm in reflectance mode. It was clearly demonstrated that, under the conditions used, the sandwich-type chamber can by no means be considered as superior to the Stahl-type chamber.

Densitometry, comparison of chromatographic conditions 02

92 007 M. WAKSMUNDUKA-HAJNOS, A. PETRUCZYNIK, A. HAWRYL, (*Dept. of Inorg. and Anal. Chem., Med. Univ., Staszica 6, 20-081 Lublin, Poland): Polar bonded stationary phases, their chromatographic properties and use in TLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 75-88 (2003). Characterization of CN-, diol-, amino-, and RP-modified silica gel by e.g. LSER parameters, solid surface free energy components, RM vs. RM correlations, quantitative structure - retention relationships (QSRR), and retention - modifier concentration relationships.

Biology, characterization of sorbents 02

3. General Techniques

92 008 V. COMAN, S. KREIBIK, ('Raluca Ripan' Inst. for Res. in Chem., 30 Fântânele Street, P.O. Box 702, RO-400294 Cluj-Napoca, Romania): Planar dielectrochromatography - a perspective technique. J. Planar Chromatogr. **16**, 338-346 (2003). In normal TLC migration of the mobile phase through the layer is controlled by capillary forces. The velocity and migration distance of the mobile-phase front in porous media can be increased by application of an external electric field. This effect is observed i.a. on different TLC plates developed with non-polar and polar solvents. As a result the separation of some compounds was improved. This method is a hybrid of electric forced flow and classical TLC; it was named 'planar dielectrochromatography' (PDEC). TLC of lipophilic dyes on aluminium oxide and silica gel with benzene. Also TLC of a hydrophilic test dye mixture on cellulose with propanol - water - ethyl acetate 6:3:1.

Planar dielectrochromatography 03

92 009 V. COMAN, S. KREIBIK, ("Raluca Ripan" Inst. for Res. in Chem., 30 Fântânele Street, P.O. Box 702, 3400 Cluj-Napoca, Romania): Planar electrochromatography - a perspective technique. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 89-109 (2003). The velocity and migration distance of the mobile-phase front in porous media, in normal TLC practice controlled by capillary forces, can be increased by application of an external electric field. The effect is observed on different TLC plates developed with non-polar and polar solvents. As a result the separation resolution of some compounds was improved. The

method, a hybrid of electric forced flow and classical TLC, was named "planar dielectrochromatography" (PDEC).

Electrochromatography, electric forced flow 03

92 010 K. DEAK, SZ. NYIREDY*, (*Res. Inst. for Med. Plants, Lupaszigeti út 4, 2011 Budakalász, Hungary): Comparison of on-line injection and off-line sample application as well as on-line and off-line detection using continuous development HPTLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 211-221 (2003). Description of the applications of continuous development HPTLC techniques combining on-line and off-line chromatographic principle steps (sample application, separation and detection). Fully on-line combination of off-line sample application and on-line detection as well as fully off-line methods are compared by separating Test Dye Mixture III and furocoumarin isomers. HPTLC of Test Dye Mixture III on silica gel with toluene and different compositions of toluene and chloroform mixtures 9: 1, 15:3, 4:1 and HPTLC of furocoumarin isomers on silica gel with ether - dichloromethane - tetrahydrofuran - hexane 10.71:9.68:7.5:72.11, diluted with 50%, 75%, and 100% hexane. All experiments were performed in a further developed prototype apparatus. Detection by densitometry in a range of 200 -1000 nm.

03

92 011 T.H. DZIDO*, R. MAJEWSKI, (*Dept. of Inorg. and Anal. Chem., Med. Univ., Staszica 6, 20-081 Lublin, Poland): Planar electrochromatography in horizontal chamber with cooling of the chromatographic plate. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 129-138 (2003). Conventional HPTLC of aromatic hydrocarbons with polar groups (2-naphthol, 1-nitro-naphthalene, 1,7-dihydroxynaphthalene, 2-ethylanthraquinone, 4-nitroaniline, anthraquinone) and dyes (1-aminoanthraquinone, fat brown, 4-diethylaminoazobenzene, 4-hydroxybenzene-azonaphthol-2, 4-(4-(N,N-ethylethanol)benzeneazo)-N-methylphthalimide, 4-nitroaniline) on RP-18 with e.g. 90% methanol in pH 10 buffer (diluted 1:7 with water) or acetonitrile - buffer in a presaturated horizontal chamber. Planar electrochromatography was performed under similar conditions with the exception of e.g. using plates with 0.5 cm margins of paraffin oil along their longer parallel edges and pre-wetting after spotting of the sample with mobile phase from both sides leaving within approximately 1 mm dry zone of start spot position. Then the chromatographic plate was pressed face-to-face with a counter-plate using clips. 200-250 V /cm field was applied to the plate. Higher efficiency and shorter development time of electrochromatography systems relative to conventional TLC separations were demonstrated.

Quantitative analysis, densitometry, qualitative identification, aromatic hydrocarbons 03

92 012 M. FILIP, V. COMAN, R. GRECU, Z. MOLDOVAN, ("Raluca Ripan" Inst. for Res. in Chem., 30 Fântânele Street, P. O. Boc 702, 3400 Cluj-Napoca, Romania): Characterisation of some chemically modified acidic alumina samples for TLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 231-242 (2003). Preparation and characterization of octadecyl-, 3-mercaptopropyl-, and N-(2-aminoethyl)-3-aminopropyl modified alumina by elemental analysis, specific surface area, FTIR spectroscopy, mass spectrometry and thermal analyses. The TLC behavior of unmodified and modified acidic alumina was tested for the separation and identification of some dyes and benzo[a]pyrene derivatives.

03

92 013 G. GRYGIERCZYK*, W. KLIMCZOK, D. LOMNKIEWICZ, (*Inst. of Chem., 9 Szkolna Street, 40-006 Katowice, Poland): Study on the influence of physico-chemical modification of chemically bonded stationary phases on the retention mechanism of selected organic compounds. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 251-255 (2003). TLC

of lauric, myristic, palmitic, stearic, and arachidic acid and lauryl, myristyl, palmityl, stearyl, and arachyl alcohols on silica gel, RP-2, RP-8, and RP-18, impregnated with 1, 5, and 10% solutions of squalane and octadecane in hexane, with methanol - water 39:1. Detection by exposure to iodine vapor.

Retention mechanism of fatty alcohols, impregnated phases 03

92 014 J.M. KOERS, M.A. CARMICHAEL, A.L. NOVOTNY, J.J. KOSIBA, D. NUROK, G.L. HAWKINS, R.W. REPLOGLE, R.E. SANTINI, (Dept. of Chem., Indiana Univ. Purdue Univ. Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202, USA): Performance of planar electrochromatography at elevated pressure. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 123-128 (2003). TLC by Pressurized Planar Electrochromatography (PPEC) of 4-cholesten-3-one, 17α-acetoxyprogesterone, 2'acetonaphthone, benzanilide, and o-nitroaniline on RP-18 with 55% aqueous acetonitrile containing 50 mM acetate buffer at a nominal pH of 4.5. The applied potential was 6 kV, and the pressure was elevated. Details are omitted based on the current patent application and intellectual property considerations. Improved new technique.

Pressurized Planar Electrochromatography (PPEC) 03

92 015 M. LAN, D. WANG*, W. WEI, W. LU (*Dept. of Anal. Chem., Shenyang Pharm. Univ., Shenyang 110016, P. R. China): Multidimensional relay development in TLC. J. Planar Chromatogr. **16**, 461-464 (2003). TLC of 13 dyes (light yellow G, acid red B, disperse deep blue H-GL, disperse scarlet BWFL, disperse orange 2BFL, disperse balas 2GFL, disperse blue BGL, disperse red 3B, disperse yellow 54, disperse turquoise GL, 10GN disperse fluorescent yellow, S-BGL disperse blue, and 2B disperse black) on silica gel (5 x 40 cm; layer thickness approx. 0.5 mm) with a new mode of 'multidimensional relay' development. Multidimensional relay development with the half-way development device transfers unseparated spots to a new TLC plate on the basis of the first separation, then re-develops this new plate.

Multidimensional relay development 03

92 016 M. LAN, D. WANG*, J. HAN, (*Dept. of Anal. Chem., Shenyang Pharm. Univ., Shenyang, 110016 (or 110015), P. R. China): A new distributor for half-way development. J. Planar Chromatogr. **16**, 402-404 (2003). Description of a new distributor which can be used more perfectly for 'half-way development' consisting of two microscope slides which are specially treated and glued together like a funnel. Mixtures of dyes have been used to verify this supposition, with satisfactory results. TLC of 5 dyes on silica gel (400 mm x 55 mm plates) with cyclohexane - ethyl acetate 3: 1 and cyclohexane - ether 3:1.

03

92 017 M. MANACH, S. LAROCHE, C. TRUCHY, D. PAPILLARD, E. MINCSOVICS, (OPLC-NIT Ltd., Andor u. 60, 1119 Budapest, Hungary): Parallel purification of synthetic molecules and plant extracts using optimum performance laminar chromatography (OPLC). Proc. Intern. Symp. on Planar Separations Plan. Chrom. 153-161 (2003). Optimum Performance Laminar Chromatography (OPLC) is a liquid chromatography technique using planar sorbent beds of low thickness (200 to 500 μm) in which the eluent is injected under pressure allowing parallel separation of samples. OPLC separation and isolation of a mixture of synthesized molecules and plant extract on planar sorbent beds (HTSorb) sealed on 4 edges on silica with e.g. 1)toluene or dichloromethane - ethyl acetate and on 2) RP-18 with a gradient water - acetonitrile 13:7 to 1:1 in 18 min or hexane - ethyl acetate from 99:1 to 1:99. The HTSorb were equilibrated before their use with the starting solution. Detection with optic fibers and Diode Array Detector (200-600 nm). New technique for chromatogram development.

Optimum Performance Laminar Chromatography, HTSorb 03

92 018 E. MINCSOVICS, M. MANACH, D. PAPILLARD,. (Bionisis-OPLC, 18-20 Ave Edouard Herriot, F-92 350 Le Plessis Robinson, France): Flowing eluent wall as a tool of parallel on-line OPLC separations on non-segmented sorbent bed. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 163-178 (2003). Application of a new concept, the process of flowing eluent wall (FEW), for single and multi-channel on-line overpressured layer chromatography (OPLC) separation with non-segmented sorbent bed for operating segmentation. OPLC of dyes, chamomile oil, ascorbigen, and homogenized cabbage on silica gel with different mixtures of hexane - ethyl acetate, chloroform - methanol - acetic acid 90: 10:1. Derivatization with freshly prepared vanillin-sulfuric acid reagent or 10% molybdatophosphoric acid in n-propanol (120°C for 5 min). Detection under UV 254 and 285 nm.

Multi-channel on-line overpressured layer chromatography 03

92 019 E. MINCSOVICS*, M. MANACH, L. KECSKES, B. TAPA, D. PAPILLARD, E. TYIHAK, (*OPLC-NIT, Ltd. , Andor u. 60, H-1119 Budapest, Hungary): Single- and multi-channel OPLC separation on non-segmented sorbent bed using flowing eluent wall for operating segmentation. J. Liq. Chrom. & Rel. Technol. **26**, 2611-2627 (2003). A new OPLC separation procedure has been developed for single- and multi-channel separation using a non-segmented sorbent bed and flowing eluent wall (FEW) for operating segmentation. The FEW detaches the sorbent bed into active and non-active parts regarding separation during the process. Only mobile phase is introduced into the non-active part, while, for the active part, eluent and also the sample can be admitted, thus the non-homogeneous part of the sorbent is excluded from the separation process. OPLC of ascorbigen, cabbage extract and a dye mixture on silica gel with e.g. chloroform - methanol - acetic acid 90:10:1 resp. toluene. Detection under UV 285 nm.

Forced flow 03

92 020 S. NYIREDY, (Res. Inst. for Med. Plants, Lupaszigeti út 4, 2011 Budakalász, Hungary): Advancement in forced-flow planar separations. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 41-60 (2003). Summary of the progress in forced-flow planar separations (FFPS) and demonstration of the importance of the different forced-flow planar chromatographic (FFPC) techniques like rotation planar chromatography (RPC), overpressured layer chromatography (OPLC), and electro-planar chromatography (EPC) as well as rotation planar extraction (RPE). Special attention was paid to a novel analytical forced-flow high-performance (HP) thin-layer chromatographic (TLC) method, in which continuous development and evaporation of the mobile phase from the end of the chromatographic plate ensure forced-flow development.

Comparison of methods, 03

92 021 C. SCHÄFER*, H.-E. HAUCK, M. SCHULZ, (*Merck KGaA, Analytics and Reagents, Frankfurter Str. 250, 64293 Darmstadt, Germany): New stationary phases for planar chromatography: Ultra-thin monolithic silica and ultra-bright TLC layers. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 71-74 (2003). Ultra-thin layers exhibit a thickness of 10 μm and consist of monolithic silica eliminating the need for a binder to fix the layer on the glass surface of a plate resulting in the combination of short migration distances, fast development times, and extremely low eluent consumption. LuxPlate, a new TLC plate, is characterized by a higher amount of fluorescent indicator and are twice as bright under UV light when compared to conventional TLC plates.

Quantitative analysis, qualitative identification, ultra-thin layers 03

92 024 B. SPANGENBERG et al., see section 5

92 025 B. SPANGENBERG et al., see section 5 03

92 022 K. TYRPIEN*, R.R. SCHEFER, S. BACHMANN, K. ALBERT, (*Med. Univ. of Silesia, Jordana 19, 41808 Zabrze, Poland): Development and application of new C30-modified TLC plates. J. Planar Chromatogr. **16**, 256-262 (2003). TLC of a-, ß-, g-, d-tocopherol and α-tocopherol acetate on silica gel and RP-18 and 30 modified silica gel. The polymeric C30 bonded phase was prepared by chemical modification of LiChrospher WP 120 silica (3 μm particle size and 120 Å average pore diameter, research sample, Merck) with triacontyltrichlorosilane by a procedure described by M. Pursch et al., Anal.Chem. 68, 386-393 (1996) and L.C. Sander et al., Anal. Chem. 66, 1667-1674 (1994). Chromatography was performed in a horizontal chamber with methanol as mobile phase. Quantitation by densitometry at 280 nm. Results showed that the new TLC phase can be used for rapid separation of a tocopherol mixture; these new plates could therefore be of great interest for rapid, high-throughput screening.

New sationary phase, C30, tocopherol 03

4. Special Techniques

92 023 M. PROSEK, A. GOLC-WONDRA, I. VOVK, S. ANDRENSEK, (Nat. Inst. of Chem., Hajdrihova 19, 1001 Ljubljana, Slovenia): On-line TLC-MS. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 149-152 (2003). Description of an on-line system which provides computer controlled elution of spots from the TLC plate and injection of the eluted substances into MS. All flexibility of TLC is retained, small fraction of MS working time is needed, and any spot on an unlimited number of plates can be selected.

On-line TLC-MS 04

5. Hydrocarbons and Halogen Derivatives

92 024 B. SPANGENBERG*, K. LORENZ, S. NASTERLACK, (*Univ. of Appl. Sci. Offenburg, Badstrasse 24, DE-77652 Offenburg, Germany): Fluorescence enhancement of pyrene measured by thin-layer chromatography with diode-array detection. J. Planar Chromatogr. **16**, 331-337 (2003). Literature recommends dipping TLC plates in viscous liquids to enhance fluorescence. Measurement of the fluorescence and absorbance spectra of pyrene spots reveals the mechanism of enhancement of plate dipping in viscous liquids - blocked contact of the fluorescent molecules with the stationary phase or other sample molecules is responsible for the enhanced fluorescence at lower concentrations. TLC and HPTLC of pyrene on silica gel and on RP-18 with methanol - acetone 8:3. The dipping solution was prepared by dissolving 3.8 g pentane sulfonic acid in 20 mL water. Densitometry in the range of 198 to 610 nm.

Densitometry, fluorescence enhancement 05

92 025 B. SPANGENBERG*, K. LORENZ, S. NASTERLACK, (*Univ. of Appl. Sci. Offenburg, Badstrasse 24, DE-77652 Offenburg, Germany): Fluorescence lifetime measurements of pyrene on HPTLC plates. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 3-11 (2003). HPTLC of pyrene on silica gel with hexane and on RP-18 with methanol - acetone 8:3 in a horizontal developing chamber. Densitometry at 198 to 610 nm after dipping in a solution of 3.8 g pentane sulfonic acid in 20 mL water.

Densitometry, pyrene 05

6. Alcohols

92 006 M. SAJEWICZ et al., see section 2

7. Phenols

92 026 T. CSERHATI, E. FORGACS*, (*Inst. of Chem., Chem. Res. Centre, Hungarian Acad. of Sci., P.O. Box 17, 1525, Budapest, Hungary): Effect of pH and salts on the binding of ring-substituted phenol derivatives to the corn protein zein, studied by thin-layer chromatography. J. Liq. Chrom. & Rel. Technol. **26**, 2303-2313 (2003). Study of the interaction of seven ring-substituted phenol derivatives with the corn protein zein by RP-TLC carried out on zein-impregnated cellulose layers, and determination and elucidation of the effect of pH and salts on the strength and selectivity of the interaction by using spectral mapping techniques (SPM) and stepwise regression analysis. (SRA). TLC of 4-nitro-, 4-amino-, 2-amino-, 3-amino-, 3-hydroxy-, 4-cyano-, and 2,6-dimethoxyphenol on zein-impregnated cellulose, prepared by dissolving 1.0 g zein in the mixture of 160 mL of n-propanol and 40 mL of water at 70°C. After the dissolution of the protein, 20 g of microcrystalline cellulose was added and the mixture was stirred for 3 h at the same temperature. Solvents have been removed at 70°C in vacuum. Plates of 20 x 20 cm containing 5 g of stationary phase have been prepared. TLC with distilled water and i.a. 0.16 M aqueous solutions of acetic acid, sodium acetate, sodium chloride, calcium chloride, and magnesium chloride. Detection by exposure to iodine vapor.

Pharmaceutical research, qualitative identification, 4-nitro-, 4-amino-, 2-amino-, 3-amino-, 3-hydroxy-, 4-cyano-, and 2,6-dimethoxyphenol 07

92 027 H. MAJSTOROVIC, D. RATKOV-ZEBELJAN, Z.L. TESIC, D.M. MILOJKOVIC-OPSENICA*, (*Fac. of Chem., Univ. of Belgrade, P.O. Box 158, 11 001 Belgrade, Serbia and Montenegro): Interpretation of the mechanisms of chromatographic separation on CN-silica. Part II: TLC of some phenols. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 297-303 (2003). Study of the chromatographic behavior of 9 phenols (phloroglucinol, 2-aminophenol, 4-hydroxybenzaldehyde, 4-methoxyphenol, salicylic acid, phenol, 4-tert-butylphenol, 2,4-dichlorophenol, 2,6-dimethylphenol) on CN-modified silica gel, silica gel and polyacrylonitrile sorbent with 14 mobile phases. Detection by spraying with 0.05% ethanolic fluorescein solution and under UV 356 nm.

Qualitative identification, phenols 07

8. Substances Containing Heterocyclic Oxygen

92 082 Z. JANECZKO et al., see section 32

92 028 I. MALINOWSKA, M. KRAUZE-BARANOWSKA*, (*Dept. of Pharmacogn., Med. Univ. of Gdansk, Gen. J. Hallera str. 107, 80-416-Gdansk, Poland): Separation of some flavonoids by use of the Prisma model and forced-flow planar techniques. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 305-307 (2003). TLC of flavonoids (7-O-glucoside luteolin, 7-O-glucoside apigenine, 5'-O-glucoside tricetin, 3-O-rhamnoside quercetin, 3-O-rhamnoside kaempferol, luteolin, quercetin, kaempferol, isoginkgetin, ginkgetin) on silica gel in a sandwich chamber, in a Personal OPLC chamber, and by planar electrochromatography method with 11 monocomponent, 8 binary phases in different concentration, and ternary

and quaternary mobile phases. The best separation of flavonoids, biflavones, aglycones, glycosides was obtained with ethanol - ethyl acetate -dioxane - hexane (as solvent strength modifier).

Herbal, flavonoids 08

10. Carbohydrates

92 029 B. SPANGENBERG*, J. SROKA, I. ARRANZ, E. ANKLAM, (*Univ. of Applied Sci. Offenburg, Badstrasse 24, D-77652 Offenburg, Germany): A simple and reliable HPTLC method for the quantification of the intense sweetener sucralose®. J. Liq. Chrom. & Rel. Technol. **26**, 2729-2739 (2003). HPTLC of sucralose® (4-chloro-4-deoxy-α-*D*-galactopyranosyl-1,6-dichloro-1,6-dideoxy-ß-*D*-fructofuranoside) on amino-bonded silica gel with acetonitrile - water 4:1. Subsequently, the wet plate was heated for 20 min at 190°C in an oven resulting in the formation of a brilliant fluorescent spot. Inspection under UV 365 nm directly or after dipping for 5 s in a cetyl-trimethylammonium bromide solution (5 g in 100 mL methanol), which resulted in fluorescent enhancement by a factor of two. Quantitation by measurement either in the absorption or fluorescence mode. Excellent repeatability (RSD = 3.4%) and recovery (95%).

Food analysis, quantitative analysis, densitometry, sucralose® 10

92 030 I. VOVK*, B. SIMONOVSKA, L. KOMPAN, M. PROSEK, (*Nat. Inst. of Chem., Lab. for Food Chem., Hajdrihova 19, SI-1000 Ljubljana, Slovenia): TLC determination of mannitol and lactulose on amino HPTLC plates. J. Planar Chromatogr. **16**, 374-376 (2003). HPTLC of mannose and lactulose from urine on amino-modified silica gel with acetonitrile - water 7:3. After drying the plates were heated at 170°C for 20 min. Determination of lactulose by densitometry at 366 nm. Visualization after dipping into paraffin - hexane 1:2, and drying for 5 min at 100°C. The plate was immersed in three detection reagents: 1 L 0.1 mol/L $AgNO_3$ and 200 mL acetone for 2 s, drying in air; 4 g NaOH in 4 mL water and 200 mL methanol for 2 s, drying for 2 min at 100°C; 20 g sodium thiosulfate dissolved in 100 mL water and 100 mL ethanol for 6 s, drying for 2 min at 100°C. Determination of mannitol by densitometry in absorbance mode at 660 nm.

Clinical chemistry research, quantitative analysis, densitometry, mannose, lactulose 10

11. Organic Acids and Lipids

92 031 T. HAYAKAWA, M. HIRAI* (*Dept. of Phys., Gunma Univ., 4-2 Aramaki, Maebashi 371-8510, Japan): An assay of ganglioside using fluorescence image analysis on a thin-layer chromatography plate. Anal. Chem **75**, 6728-6731 (2003). Fluorometric method for the determination of quantities of gangliosides ranging from pico- to nanomoles. HPTLC of sugars (D-(+)-glucose, D(+)-galactose, D(+)-frucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-neuramic acid) and gangliosides (GM1, asialoGM1, GD1a, GT1b) on silica gel, prewashed with methanol - chloroform 1:1, with chloroform - methanol - 0.2% $CaCl_2$ (aq) 60:35:8 as solvent system in a well-saturated TLC chamber. Detection by spraying with 18% hydrochloric acid thoroughly drying at 40°C in an drying oven and heating for 12 min at various temperatures (from 50 to 180°C). The fluorescence (UV 365 nm) of each sample was greatly dependent on the heating temperature. Calibration curves for gangliosides were obtained by HPTLC and an image-analyzing system equipped with a CCD camera and they showed a high linearity in a wide range from 47 pmol to 4.5 nmol. New and simple procedure.

Quantitative analysis, biochemistry 11

92 032 H.P. KORNENA, H.A. BUTINA, E.O. GERASIMENKO, H.V. GRUSHENKO, V.V. NOSACHOVA, A.Y. BUDUNOVA, B.M. SOGOLOVSKY*, (Kuban State Techn. Univ., Moskovskaya 2, 350072, Krasnodar, Russia): Use of TLC modern methods for the research of phospholipid products. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 267-273 (2003). TLC of phospholipids (i.a. phosphatidylinositols, -cholines, -serines, -ethanolamines, -glycerines, neutral lipids) on silica gel with chloroform - methanol - water 65:25:4 and chloroform - methanol - acetic acid 65:25:8. After drying 5% phosphomolybdic acid was used for spraying or immersion; also iodine vapor, Dragendorff, butanolic ninhydrin solution and ammoniacal silver nitrate solution. Quantitation by densitometry. Effective quantitative analysis.

Clinical chemistry research, quantitative analysis, densitometry, phospholipids 11

92 033 M. KOZYRA*, K. GLOWNIAK, A. ZADUBIEC, (*Dept. of Pharmacogn., Med. Univ., 12 Peowiaków St., 20-007 Lublin, Poland): Phenolic acids in the herb, fruits and roots of Peucedanum verticillare L. Koch ex DC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 274-283 (2003). TLC of phenolic acids (e.g. p-coumaric, chlorogenic, hydroxybenzoic, isovanillic, caffeic, rosmarinic, syringic, vanillic, protocatechuic, ferulic, γ- and ß-resorcylic and gentisic acid) on cellulose with toluene - sodium ethyl - formic acid 5:4:1, sodium formate - formic acid - water 10:1:200, and 15% aqueous acetic acid. After preconditioning with benzene – methanol – acetic acid 94:1:5 for 2D-TLC with benzene - methanol - acetic acid - acetonitrile 16:2:1:1 in the first direction and sodium formate - formic acid - water 10:1:200 in the second direction. Examination under UV 254 and 366 nm. Visualization with 3% methanolic solution of iron(III) chloride, diazotized sulfanilic acid in 20% sodium carbonate solution 1:1.

Herbal, phenolic acids 11

92 034 J. NOWAKOWSKA*, M. MARSZALL, (*Med. Univ. of Gdansk, Fac. of Pharm., Dept. of Phys. Chem., Al. Gen. J. Hallera 107, 80-416 Gdansk, Poland): Retention properties of rosmarinic and valerenic acids on an amino-modified hydrophilic layer. J. Planar Chromatogr. **16**, 369-373 (2003). TLC of rosmarinic acid and valerenic acid on amino-modified silica gel with 8 binary phases (methanol - water, ethanol - water, propanol - water, acetonitrile - water, THF - water, acetonitrile - buffer, and methanol - buffer), in which the concentration of organic modifier varied from 0 to 100%, with chamber saturation at 20°C. Visualization by spraying with sulfuric acid conc. - methanol 1:10 and heating for 10 min at 120°C. Visualization under UV.

Pharmaceutical research, qualitative identification, rosmarinic acid, valerenic acid 11

92 035 A. PYKA*, K. BOBER, (*Silesian Acad. of Med., Fac. of Pharm., Dept. of Anal. Chem., 4 Jagiellonska Street, PL 42-200 Sosnowiec, Poland): Investigation of a homologous series of fatty acids by TLC. Part III. Application of terms describing the separation of homologous series of saturated fatty acids in TLC. J. Planar Chromatogr. **16**, 303-307 (2003). HPTLC of 19 fatty acids (from pentanoic to tricosanoic acid) on RP-18, with and without concentrating zones. The best chromatographic conditions for separation were on RP-18 without concentrating zone with methanol - water 9:1, 19:1 and ethanol - water 9:1, and on RP-18 with concentrating zone with methanol - water 9:1 and 19:1, 100% ethanol and ethanol - water 9:1 and 19:1. Visualization by exposure to iodine vapor. Optimization of separation conditions.

Qualitative identification, fatty acids 11

92 036 A. PYKA*, K. BOBER, (*Dept. of Anal. Chem., Fac. of Pharm., Silesian Acad. of Med., 4 Jagiellonska Str., PL-41-200 Sosnowiec, Poland): Investigation of an homologous series of fatty acids by TLC. II. Comparison of separation of fatty acids on RP-18 plates with different mobile phases. J. Liq. Chrom. & Rel. Technol. **26**, 2663-2671 (2003). TLC of fatty acids (octa-, nona-, deca-, undeca-, dodeca-, trideca-, tetradeca-, pentadeca-, hexadeca-, heptadeca-, and octadecanoic acid) on RP-18 (with and without concentrating zones). Visualization by exposure to iodine vapor. The best separation of the acids investigated was obtained on RP-18 with a concentrating zone, and methanol - water 19:1.

Systematic investigation 11

92 006 M. SAJEWICZ et al., see section 2

92 037 J.L. SCHNECK, B. FRIED*, J. SHERMA, (*Dept. of Biol., Lafayette Coll., Easton, PA 18042, USA): High-performance thin-layer chromatographic analysis of lipids in juvenile Helisoma trivolvis (Colorado strain) maintained on a hen's egg yolk diet. J. Planar Chromatogr. **16**, 405-407 (2003). HPTLC of neutral lipids (e.g. cholesteryl oleate, methyl oleate, triolein, oleic acid, and cholesterol) on silica gel (with prescored lanes), after prewashing with dichloromethane - methanol 1:1, for the methyl ester, triacylglycerol, free fatty acids, and free sterol content with petroleum ether - ether - acetic acid 80:20:1 and for cholesteryl esters with hexane - petroleum ether - ether - acetic acid 50:25:5:1. Development in a presaturated chamber at 22°C and a humidity of 50%. Visualization by spraying with a solution of 5 g phosphomolybdic acid in 100 mL absolute ethanol and heating for 10 min at 115°C. TLC of polar lipids (cholesterol, phosphatidylethanolamine, phosphatidylcholine, and lysophosphatidylcholine) on silica gel with chloroform - methanol - water 65:25:4. Visualization by spraying with a 10% cupric sulfate solution (prepared by dissolving 100 g $CuSO_4$ and 100 mL phosphoric acid in water and making up to 1 L), followed by heating for 10 min at 140°C. Quantitative densitometry at 610 nm for neutral lipids and at 370 nm for polar lipids.

Biochemistry 11

13. Steroids

92 001 M. BATHORI et al., see section 1

92 038 A. BETERINGHE, I. BACIU, M.T. CAPROIU, T. CONSTANTINESCU, A.T. BALABAN*, (*Texas A&M Univ. at Galveston, Galveston, TX 77553-1675, USA): O-Methyloximes of testosterone and of 17α-methyltestosterone: TLC and QSPR study of RF values. J. Planar Chromatogr. **16**, 268-270 (2003). TLC of the Z and E diastereomers of the O-methyloximes of testosterone and 17 α-methyltestosterone on silica gel with ether - petroleum ether (30-60°C) 1:1. The RF values of these compounds could be rationalized by a quantitative structure-property relationship (QSPR) using one topological/topographical index.

Qualitative identification, biomedical application, O-methyloximes of testosterone, 17α-methyltestosterone 13

92 039 A. PYKA*, M. DOLOWY, (*Dept. of Anal. Chem., Fac. of Pharm., Silesian Acad. of Med., 4 Jagiellonska Street, PL-41-200 Sosnowiec, Poland): Lipophilicity of selected bile acids as determined by TLC. J. Liq. Chrom. & Rel. Technol. **26**, 2741-2750 (2003). HPTLC of bile acids (cholic, chenodeoxycholic, deoxycholic, lithocholic, glycocholic, glycodeoxycholic, and glycolithocholic acid) on RP-18 with mixtures of methanol - water. The methanol content was varied by 5% volumes from 60% to 100%. Detection after drying at room temperature by spraying with a 10% solution of sulfuric acid in water and heating at 120°C for 20 min.

The retention parameters RMW may be used as a measure of lipophilicity of the investigated bile acids.

Qualitative identification, bile acids 13

14. Steroid Glycosides, Saponins and Terpenoid Glycosides

92 040 X.-L. CAO, Y. TIAN, T.-Y. ZHANG*, Q.-H. LIU, L.-J. JIA, Y. ITO, (*Beijing Inst. of New Techn. Appl., Xizhimen South Street, No. 16, Beijing 100035, P. R. China): Separation of dammarane-saponins from Notoginseng, root of Panax notoginseng (Burkh.) F.H. Chen, by HSCCC coupled with evaporative light scattering detector. J. Liq. Chrom. & Rel. Technol. **26**, 1579-1591 (2003). TLC of ginsenoside-Rg1, ginsenoside-Re, notoginsenoside-R1 on silica gel with chloroform - methanol - 2-butanol - water 5:6:1:4 and ethyl acetate - 1-butanol - water 1:1:2. Detection under UV.

Pharmaceutical research, qualitative identification, ginsenosides 14

92 041 Y. IKEDA*, Y. FUJII, (*Fac. of Pharm. Sci., Hokuriku Univ., Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan) Quantitative determination of lanatosides in the hybrid Digitalis ambigua x Digitalis lanata leaves by HPLC. J. Liq. Chrom. & Rel. Technol. 26, 2013-2021 (2003). (1994). Quick and simple TLC procedure of lanatoside A, B, C, desacetyllanatoside A, 14a,15a-epoxy-”ß”-anhydrodesacetyl-lanatoside on silica gel with chloroform - methanol - water 32:8:1 and on RP-18 with acetonitrile - 0.5 M sodium chloride 10:13. Detecion by spraying with concentrated sulfuric acid, and heating at 120°C for 10 min.

Pharmaceutical research, qualitative identification, lanatoside A, B, C, desacetyllanatoside A, 14α,15α-epoxy-”ß”-anhydrodesacetyl-lanatoside 14

92 042 A. LUDWICZUK*, T. WOLSKI, S. NYIREDY, (*Dept. of Pharmacogn., Med. Univ., Peowiakow 12, 20-007 Lublin, Poland): Circular and linear OPLC of ginsenosides in Panax quinquefolium L. cultivated in Poland. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 291-296 (2003). TLC, HPTLC and OPLC of ginsenosides (e.g. Rb 1, Rc, Re, Rd, Rg1, and Rg 2) on normal and HPTLC silica gel with chloroform - methanol - ethyl acetate - water 15:22:40:9 (I) and chloroform - methanol - ethyl acetate - water - hexane 10:11:30:4:2 (II). Mobile phase II gave better results for circular and linear forced-flow OPLC. Visualization by spraying with Godin’s reagent (5% sulfuric acid in ethanol and 1% vanillin in ethanol) and heating at 105°C for 10 min. Quantitation by densitometry at 540 nm.

Herbal, qualitative identification, ginsenosides 14

15. Terpenes and other Volatile Plant Ingredients

92 043 J.K. LALLA*, P.D. HAMRAPURKAR, P.S. PATIL, (*Dept. of Pharm. Anal., Principal K. M. Kundnani Coll. of Pharm., Plot Nr. 47, Dr. R. G. Thadani Marg, Worli, Mumbai 400 018, India): Azadirachtin as a biomarker compound in HPTLC assay of seed and seed oil of Azadirachta indica A. Juss. J. Planar Chromatogr. **16**, 311-314 (2003). HPTLC of azadirachtin on silica gel with toluene - ethyl acetate - formic acid 10:8:1 with chamber saturation for 20 min. at 25°C. After drying of the plate visualization by spraying with vanillin-sulfuric acid reagent (3% vanillin in 1% ethanolic sulfuric acid) and heating at 100°C for 2 min. Quantitation by densitometry at 677 nm. Development of a simple, precise, and specific method.

Quality control, quantitative analysis, densitometry, azadirachtin 15

17. Amines, Amides and Related Compounds

92 002 M. JANICKA et al., see section 2

92 044 L. SIMON-SARKADI*, G. KOCSY, K. LESKO, A. VARHEGYI, Z. VEGH, (*Budapest Univ. of Techn. and Economics, Dept. of Biochem. and Food Techn., P.O. Box 91, 1521 Budapest, Hungary): Investigation of the effect of drought stress on polyamine accumulation in soybean by OPLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 415-421 (2003). OPLC of polyamines (spermine, spermidine, putrescine, tyramine) after pre-chromatographic derivatization with dansyl chloride by stepwise gradient elution on HPTLC silica gel with mobile phase A (first step), hexane - butanol - triethylamine 900:100:91, eluent B (second step), hexane - butanol 4 1. Densitometry at 313/400 nm in fluorescent mode. Variation of detection procedure.

Food analysis, qualitative identification, biochemistry, polyamines 17

18. Amino Acids and Peptides, Chemical Structure of Proteins

92 045 E.L. PONDER, B. FRIED*, J. SHERMA, (*Dept. of Biol., Lafayette Coll., Easton, PA 18042, USA): Thin layer chromatographic analysis of free pool amino acids in cercariae, rediae, encysted metacercariae, and excysted metacercariae of Echinostoma caproni. J. Liq. Chrom. & Rel. Technol. **26**, 2697-2702 (2003). HPTLC of 19 amino acids (alanine, arginine, asparagine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) on silica gel, cellulose, strong acid cation-exchange sheets, and on RP-18, pre-cleaned by development with dichloromethane - methanol 1:1. Propanol - 0.5 M NaCl 2:3 was used as eluent for the RP phase, pH 3.3 citrate buffer for the ion exchange layer, and n-butanol - acetic acid - water 3:1:1 for the other two layers. Detection by spraying heavily with ninhydrin reagent (0.3 g ninhydrin and 3 mL acetic acid in 100 mL butanol), air drying for 30 min, and heating for 10 min at 110°C on a plate heater. Quantitation by densitometry at 495 nm for histidine and 610 nm for all other amino acids.

Agricultural, densitometry, qualitative identification, biology 18

19. Proteins

92 046 G. IONITA*, C. POSTOLACHE, C. TILIMPEA, D. DINU, V.E. SAHINI, (*Inst. of Phys. Chem., Univ. of Bucharest, Spl. Independentei 202, Bucharest, 77208, Romania): Planar chromatographic and electrophoretic study of thermally induced conformational modifications of protein structure. J. Planar Chromatogr. **16**, 308-310 (2003). TLC of bovine serum albumin on silica gel impregnated by overnight predevelopment with chloroform - paraffin oil 19:1 with buffer solutions of pH 1, 4, 7, 10, or 12. After drying of the chromatogram visualization by treatment with a 2% solution of ninhydrin in acetone.

Qualitative identification, bovine serum albumin 19

22. Alkaloids

92 047 M. LUCZKIEWICZ*, P. MIGAS, A. KOKOTKIEWICZ, M. WALIJEWSKA, W. CISOWSKI, (*Dept. of Pharmacogn., Med. Univ. of Gdansk, al. Gen. J. Hallera 107, 80-416 Gdansk, Poland): Two-dimensional TLC with sorbent gradient for the analysis of quinolizidine alkaloids in the herb and in vitro cultures of several Genista species. Proc. Intern. Symp. on

Planar Separations Plan. Chrom. 285-289 (2003). One-dimensional TLC of 8 quinolizidine alkaloids (sparteine, α-isosparteine, retamine, hydroxylupanine, cytisine, methylcytisine, sophocarpine, lupanine) on silica gel with hexane - diethyl amine 100:0 to 1:1, on silica gel and DIOL with chloroform - methanol - NH_3 100:0:1, 95:5:1 to 85:15:1, and on DIOL and RP-18 with acetonitrile - water - hydrochloric acid 30:100:5, 30:100:10, 30:100:15 and 30:100:20.. 2D-TLC on DIOL with chloroform - methanol - NH_3 85:15:1 (I) in the first direction and after drying at room temperature with acetonitrile - water - hydrochloric acid 30:100:7 (II) in the second direction. In adsorbent TLC - diol plates were used in the first direction with mobile phase I. Then the alkaloids were transferred by a specially designed device on the second RP-18 plate with the mobile phase II. The same phase was then used for the second development in the perpendicular direction. Visualization by spraying with Dragendorff reagent and documentation by photography.

Herbal, qualitative identification, quinolizidine alkaloids, plate transfer 22

92 048 A. PETRUCZYNIK, M. GADZIKOWSKA, M. WAKSMUNDZKA-HAJNOS, M. HAWRYL, (Dept. of Inorg. and Anal. Chem., Med. Univ., Staszica 6, 20-081 Lublin, Poland): Quantitative and qualitative analysis of the tropane alkaloids from Datura innoxia by TLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 377-383 (2003). TLC of tropane alkaloids (i.e. atropine, homatropine, L-hyoscamine, scopolamine, scopolamine N-oxide, tropine, tropic acid) on silica gel with methanol - acetone - NH_3 10:8:1 (I) and methanol - acetone - diethylamine 25:24:1 (II). Also TLC on RP-18 with 25% methanol - water (buffered to pH 3.4) containing 0.01 mL HDEHP (III). 2D-TLC on silica gel with mobile phase II in the first direction and on RP-18 with mobile phase III in the second direction. Development in horizontal chambers after preconditioning. Quantitation by densitometry after spraying with Dragendorff reagent at 520 nm.

Herbal, quantitative analysis, densitometry, tropane alkaloids 22

92 049 A. PETRUCZYNIK, M. WAKSMUNDZKA-HAJNOS, M.L. HAJNOS, L. LOSZAJ, (Dept. of Inorg. and Anal. Chem., Med. Univ., Staszica 6, 20-081 Lublin, Poland): Effect of chromatographic conditions on the separation of some alkaloids in RP-HPTLC. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 385-392 (2003). HPTLC of alkaloids (yohimbine, boldine, novocain, quinine, colchicin, brucine, strychnine, theobromine, narcotine, glaucine, caffeine, protopine) on RP-18 in horizontal chambers after pre-conditioning for 20 min with e.g. binary mixtures of organic modifier, methanol, acetonitrile, or tetrahydrofuran, isopropanol, dioxane with buffered water. Visualization under UV 254 nm. Densitometry at 254 nm. The incorporation of amine to the aqueous mobile phase gives the best results for the separation of alkaloids investigated; the peaks are narrow, in most cases symmetric, and the selectivity of separation is improved.

Quantitative analysis, densitometry, alkaloids 22

92 050 B. SZABO*, A. LAKATOS, T. KÖSZEGI, L. BOTZ, (*Dept. of Botany, Fac. of Sci., Pécs Univ., Ifjúság u. 6, H-7624 Pécs, Hungary): HPTLC and HPLC determination of alkaloids in poppies subjected to stress. J. Planar Chromatogr. **16**, 293-297 (2003). TLC and HPTLC of narceine, morphine, codeine, thebaine, papaverine and narcotine on silica gel with concentration zone with toluene - acetone - ethyl acetate - NH_3 (25%) 20:20:3:1 and toluene - acetone - ethanol - NH_3 20:20:3:1, respectively. Detection by spraying with Dragendorff's reagent with sodium nitrite or by treatment with formaldehyde-sulfuric acid reagent (Marquis's reagent) and densitometry at 600 nm. Evaluation by densitometry at 520 nm. Simple and quick procedure.

Toxicology, quantitative analysis, densitometry, biology, alkaloids 22

23. Other Substances Containing Heterocyclic Nitrogen

92 051 J. HABDAS, G. MATYSIK*, (*Dept. of Inorg. and Anal. Chem., Med. Acad., Lublin, Poland): Determination of the yields of meso-tetraphenylporphyrins by densitometry. J. Planar Chromatogr. **16**, 289-292 (2003). HPTLC of 6 porphyrins (5,10,15,20-tetratolylporphyrin, 5-(4-methoxyphenyl)-10,15,20-tritolylporphyrin, 5,10-di(4-methoxyphenyl)-15,20-ditolylporphyrin, 5,15-di(4-methoxyphenyl)-10,20-ditolylporphyrin, 5,10,15-tri(4-methoxyphenyl)-20-tritolylporphyrin, 5,10,15,20-tetra(4-methoxyphenyl)-porphyrin) on diol modified silica gel with dichloromethane- ethyl acetate 97:3. Quantitation by densitometry at 420 nm. Convenient and accurate procedure.

Qualitative identification, biomedical, porphyrins 23

24. Organic Sulfur Compounds

92 052 G. GRYGIERCZYK, J. WASILEWSKI, D. LOMANKIEWICZ, W. KLIMCZOK, T. KOWALSKA*, (*Inst. of Chem., Silesian Univ., 9 Szkolna Str., 40-006 Katowice, Poland): Use of complexation TLC to investigate monosulfides. II. Silica impregnated with the Cd(II), Sr(II), Eu(III), and V(IV) cations as stationary phase. J. Liq. Chrom. & Rel. Technol. **26**, 2651-2661 (2003). TLC of 10 monosulfides (1-(methylthio)octadecane, didecylsulfide, dioctadecylsulfide, octadecylthiobenzene, 2-(methylthio)naphthalene, 2-(octadecylthio)naphthalene, 2-(benzylthio)naphthalene, 2-(dodecylthiomethyl)naphthalene, 9-(dodecylthiomethyl)anthr acene, 1-(triphenylmethylthio)dodecane) on silica gel and on silica gel impregnated with 5% aqueous solutions of salts of Cd(II), Sr(II), or V(IV), or with a 1% aqueous solution of europium oxide containing the Eu(III) cation. Carbon tetrachloride, hexane - chloroform 9:1, hexane - carbon tetrachloride 9:1, hexane - toluene 19:1, and carbon tetrachloride - hexane - chloroform 7:2:1 were used as mobile phases. After development and drying, visualization under UV 254 nm.

Complexation TLC 24

92 053 N.U. PERISIC-JANJIC*, T.L. DJAKOVIC-SEKULIC, K. POPOV-PERGAL, (*Inst. of Chem., Fac. of Sci., Trg D. Obradovica 3, 21 000, 21 000 Novi Sad, Serbia and Montenegro): Correlation between reversed-phase chromatographic retention data and structure of several thiazole derivatives. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 357-362 (2003). Investigation of the retention behavior of thiazoles as function of organic modifier content of the eluents using two types of stationary phases. TLC of 12 3-benzyloxycarbonyl-5-substituted 2,4-dioxotetrahydro-1,3-thiazoles on silica gel, impregnated with paraffin oil, and rice starch with NH_3 conc. - methanol, conc. NH_3 conc. - dioxane, and NH_3 conc. - acetone with different modifier content (from 0.01 to 0.34) for methanol, dioxane, and acetone. Visualization under UV 254 nm.

Influence of organic modifier content 24

27. Vitamins and Various Growth Regulators

92 054 S. TAKENAKA, T. ENOMOTO, S. TSUYAMA, F. WATANABE*, (*Dept. of Health Sci., Kochi Women's Univ., Kochi 780-8515, Japan): TLC analysis of corrinoid compounds in fish sauce. J. Liq. Chrom. & Rel. Technol. **26**, 2703-2707 (2003). TLC of vitamin B12 and corrinoid compounds (5-hydroxybenzimidazolyl cyanocobamide, benzimidazolyl cyanocobamide, 7-adenylcyanocobamide) on silica gel with 1-butanol - 2-propanol - water 10:7:10 in the dark at 24°C. The TLC sheet was dried and cut into small pieces; B12 was extracted from the pieces in 80% methanol containing 20 mg/L KCN several times, evaporated to dryness

Parameters of Planar Chromatography

The articles in this series are dedicated to the important steps of planar chromatography and their parameters which influence the chromatographic result. Hints for optimization are given to help the reader to use planar chromatography most efficiently.

Collecting these pages is recommended.

Documentation of TLC Plates

It is an inherent advantage of planar chromatography that the chromatographic result can be visualized and documented as an image. This allows a convenient qualitative evaluation of multiple samples on the same plate. For example, the plate in Fig. 1 shows fingerprints of several ginseng species in comparison to a set of reference substances (on left tracks).

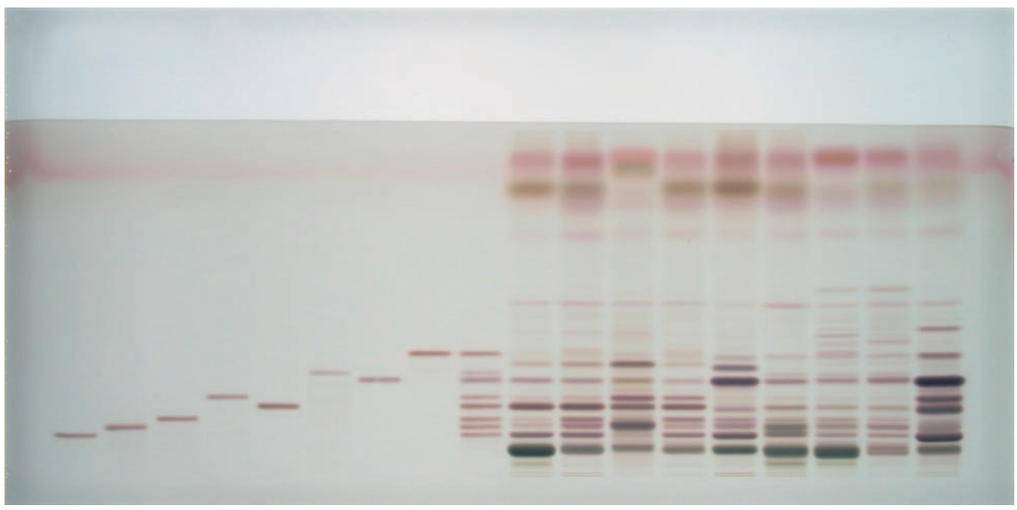


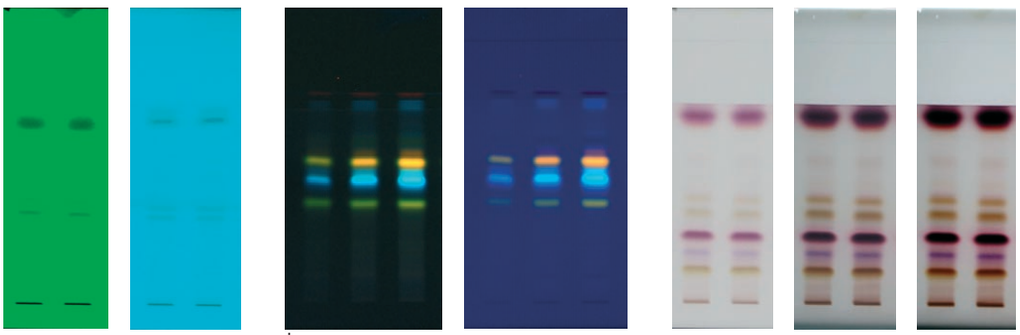
Fig. 1: HPTLC of Ginseng samples
Lane 1 Ginsenoside Rb1
Lane 2 Ginsenoside Rb2
Lane 3 Ginsenoside Rc
Lane 4 Ginsenoside Rd
Lane 5 Ginsenoside Re
Lane 6 Ginsenoside Rf
Lane 7 Ginsenoside Rg1
Lane 8 Ginsenoside Rg2
Lane 9 Mixture of all standards
Lane 10 Panax quinquefolius 3 yr old root
Lane 11 P. quinquefolius 2 yr old root
Lane 12 P. quinquefolius Leaf of 4 yr old root
Lane 13 P. quinquefolius root tails
Lane 14 P. quinquefolius Wild 26 yr old root body,
Lane 15 P. ginseng wood-grown root
Lane 16 P. ginseng Kirin (red)
Lane 17 P. ginseng White root 6 yr old
Lane 18 P. pseudoginseng, Black (Notoginseng)

For proper identity each sample must comply with a specification considering number, color, intensity, position and sequence of zones. Similarities and differences of the individual samples are easily detected. Samples representing different species, plants, plant parts, or treatment are clearly distinguishable. Whereas scanning densitometry has its strength in spectral selectivity and the possibility of comparing e. g. all samples on a plate at one wavelength or one sample at multiple wavelengths (MWL scan), the visual inspection of the image allows side-by-side comparison of all samples at all wavelengths of the visible range (colors). In the early days

of TLC the visual impression of a plate had to be verbally described. Often a schematic image was drawn. Later, plates were photographed, which allowed producing permanent records of the result. Today capturing electronic images with video or digital cameras is the most efficient way of documenting TLC plates in a reproducible manner. Flatbed scanners are also used for documentation of colored zones under white light. Electronic images can be annotated, saved, transmitted, and printed with full compliance to GxP regulations, if suitable software is used.

Requirements for proper documentation

1. The principal requirement for documentation is the "visibility" of the chromatogram with or without derivatization. In modern documentation systems one of three light sources can be selected. Substances absorbing UV light of about 254 nm are visualized as dark zones on plates containing a fluorescence indicator, which is excited to emit green or blue light by a short wave (254 nm) UV lamp as seen in Fig. 2a. A long wave (366 nm) UV lamp is used to excite substances, which are able to fluoresce, as Fig. 2b shows. A cutoff filter must be used to prevent the illuminating UV light from reaching the camera, while the produced/emitted (visible) fluorescent light of different wavelengths passes. White light is used to visualize colored substances. Three illumination modes are used (Fig. 2c): reflectance, transmission, and reflectance + transmission. While reflectance only gives an image that is very close to what the unaided eye will see when inspecting the plate in white light, additional transmission brings out more details about week zones because also sample molecules from the region deeper inside the layer contribute to the image seen.



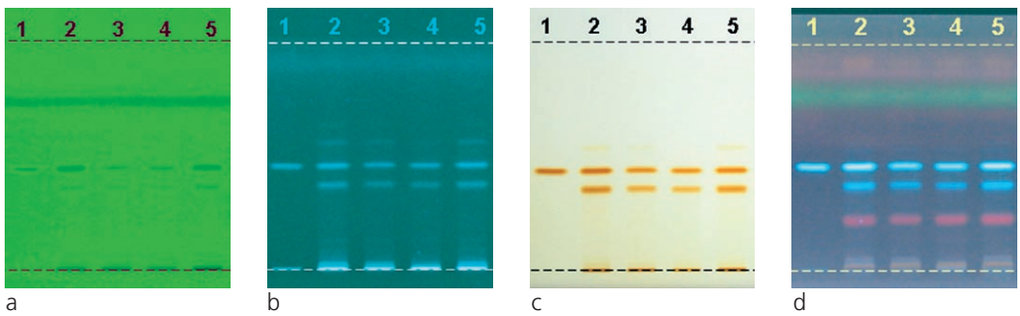
a b c

Fig. 2: Documentation using different illumination modes
a) HPTLC fingerprint of Chamomile oil at UV 254 nm (F_{254} left, F_{254s} right), b) HPTLC fingerprint of Crataegus spp. following derivatization with NP reagent at UV 366 nm (with cutoff filter left, without cutoff filter right) c) Chamomile oil derivatized with anisaldehyde reagent at white light (reflectance left, reflectance and transmission – middle, transmission right).

2. For documentation of a series of similar plates based on images of equal size it is essential that the plate is properly positioned and that zoom and focus of the camera are fixed. For all images to be compared the same resolution must be used. 640 x 480 pixels are fully sufficient for images displayed on screen. Better resolution such as 1024 x 768 or higher may be good for printing on photo-printers but generally such images use up too much space (>1-2MB) and become inconvenient to handle. When high-resolution images are to be displayed on screen one usually has to "zoom out" in order to fit the entire image into the current window, which often results in compression of the image and actual loss of fine details.
3. For accurate plate-to-plate comparison based on electronic images it must be ensured that all camera parameters, including white balance, aperture, exposure time, focus and zoom are kept constant. This requirement can be met when so-called configurations are used to control all functions of the digital camera. For GxP compliance of the documentation process all parameters must be traceable and saved with the image.
4. Although it may be very convenient, setting the digital camera on automatic mode, this is not recommended if images of several plates are to be compared. To bring out any differences between the plates all camera parameters should be known and fixed.

Multiple detection

In order to maximize the information that can be extracted from a given chromatogram, it is often useful to use multiple detections (Fig. 3) of the same plate without the need for repeating chromatography. In addition to different illumination modes also chemical derivatization can be utilized to bring out specific details of the sample.

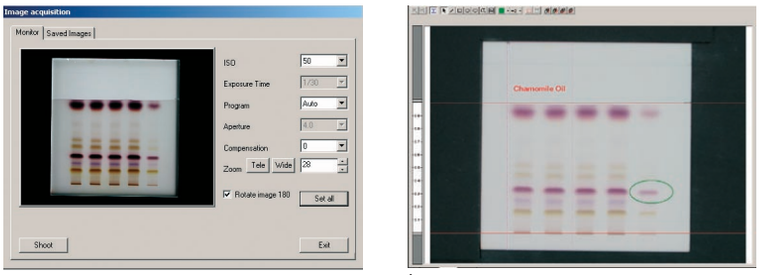


a b c d

Fig. 3: Multiple detection of Stephania separated on HPTLC silica gel prior to derivatization under a) UV 254 nm, b) UV 366 nm, and following derivatization with c) iodine under white light reflection, then with d) anisaldehyde reagent under UV 366 nm.

Software

winCATS – Planar Chromatography Manager (Fig. 4) features convenient tools for documentation of TLC plates. Either a digital or video camera can be controlled by the program (Fig. 5). All parameters necessary to obtain reproducible images are part of the analytical method file. For each plate multiple images captured under different conditions can be included in the analysis file. A great choice of annotation tools including a display of the R_F scale makes editing of images simple. Using the built-in export function images can be transferred to other applications such as word documents or powerpoint presentations. Documentation with winCATS is fully GxP compliant.



a b

Fig. 4: winCATS planar chromatography manager a) twain interface for camera control, b) annotation of captured image

Fig. 5: CAMAG DigiStore Documentation system with digital camera

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and used as samples for the B12 microbiological assay.

Food analysis, qualitative identification, vitamin B12 27

92 022 K. TYRPIEN et al., see section 3

28. Antibiotics, Mycotoxins

92 055 A. MORICZ, P. OTT, K.H. OTTA, E. TYIHAK, (Dept. of Chem. Techn. and Env. Chem., L. Eötvös Univ., P.O. Box 32, 1518 Budapest 112, Hungary): Separation and detection of some aflatoxins using overpressured layer chromatography and bioautography. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 319-327 (2003). OPLC of aflatoxins B1, B2, G1, G2 on silica gel, prewashed with methanol - acetone 1:9, with chloroform - acetone 22:3. Bioautography after drying by dipping in culture media containing Pseudomonas savastanoi pv. phaseolicola bacteria cells and incubation for 18 h at 100% rel. humidity at 28°C. For visualization bioautograms were stained with an aqueous solution of MTT (in 100 mL water 80 mg MTT and 100 mg Triton X-100 solution).

Qualitative identification, aflatoxins 28

92 056 A. MORICZ*, K.H. OTTA, E. TYIHAK, (*Dept. of Chem. Techn. and Env. Chem., L. Eötvös Univ., P.O. Box 32, H-1518 Budapest 112, Hungary): Separation and detection of aflatoxins using overpressured-layer chromatography and bioautography. J. Planar Chromatogr. **16**, 417-420 (2003). OPLC of aflatoxins B1, B2, G1, and G2 on silica gel, predeveloped with methanol - acetone 1:1, with chloroform - acetone 22:3. After drying bioautography of aflatoxins after drying by dipping into Pseudomonas savastanoi pv. phaseolicola cell suspensions and incubation for 18 h in a chamber at 100% relative humidity at 28°C. Visualization by staining with an aqueous solution of MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (80 mg MTT and 100 mg Triton X-100 in 100 mL water). Bioautography as a suitable detection procedure for aflatoxins separated by planar chromatography.

Toxicology, qualitative identification, OPLC, aflatoxins 28

92 057 E. TYIHAK*, P. OTT, A. MORICZ, G. KATAY, ZS. KIRALY-VEGHELY, (*Plant Prot. Inst., Hungarian Acad. of Sci., P.O. Box 102, 1525 Budapest, Hungary): Antibiosis, antibiotics, and formaldehyde cycle: Unique importance of planar chromatographic techniques in this progress direction. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 61-70 (2003). OPLC of trans-resveratrol on silica gel with chloroform - methanol 10:1. Detection by bioautography - dried plates were immersed for 20 s into a suspension of Pseudomonas savastanoi pv. phaseolocola and visualized by staining with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide).

Biochemistry, OPLC, trans-resveratrol 28

29. Insecticides, Pesticides and other Agrochemicals

92 002 M. JANICKA et al., see section 2

92 058 M. SOBER*, M. LEKIC, F. KORAC, B. IMAMOVIC, A. MARJANOVIC, (*Fac. of Pharm., Univ. of Sarajevo, Cekalusa 90, 71 000 Sarajevo, Bosnia and Herzegovina): Identification of chlorophenoxy herbicides by TLC in clinical toxicology. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 423-432 (2003). TLC of 2,4-dichlorophenoxyacetic acid, 2-methyl-4-chloro-phenoxypropionic acid, 2,4-dichloropropionic acid, and 2,4-dichlorobutyric acid on precoated silica gel plates and silica gel, prewashed, and impregnated with Cu(II),

Co(II) and Fe(III) salts with heptane - dioxane - acetic acid 60:40:1. Visualization of spots on precoated plates under UV 254 nm. The best results have been achieved with silica gel impregnated with 0.2 % $CuSO_4$. In addition to efficient separation, such impregnation makes possible visualization without further use of reagents or UV lamps. New method for separation and identification.

Toxicology, qualitative identification, chlorophenoxy herbicides 29

92 059 T. TUZIMSKI*, E. SOCZEWINSKI, (*Dept. of Inorg. and Anal. Chem., Med. Univ., Staszica 6, 20-081 Lublin, Poland): Correlation of retention data of pesticides in normal- and reversed-phase systems and utilization of the data for separation of a mixture of ten urea herbicides by two-dimensional thin-layer chromatography on cyanopropyl-bonded polar stationary phase and on a two-adsorbent-layer Multi-K SC5 plate. J. Planar Chromatogr. **16**, 263-267 (2003). Comparison of selectivities of TLC systems by use of correlations between RF(II) and RF(I) values (by analogy with two-dimensional TLC). 2D-TLC of 10 urea herbicides (monolinuron, linuron, metobromuron, chlorbromuron, chlorotoluron, diuron, metoxuron, isoproturon, chloroxuron, methabenzthiazuron) on cyanopropyl-modified silica gel, normal-phase with ethyl acetate - heptane 1:4 (step A) and reversed-phase with dioxane - water 2:3 (step B). Also separation by the 2D-TLC system comprising normal-phase chromatography on silica gel with tetrahydrofuran - heptane 2:3 and reversed-phase on RP-18 silica with methanol - water 3:2. Detection under UV 254 nm. One-dimensional HPTLC on RP-18 and cyanopropyl-modified silica gel.

Two-dimensional TLC, two-adsorbent layer Multi-K SC5 plate 29

30. Synthetic and Natural Dyes

92 060 D. MILOJKOVIC-OPSENICA*, K. LAZAREVIC, V. IVACKOVIC, Z.L. TESIC, (*Fac. of Chem., Univ. of Belgrade, P.O. Box 158, YU-11001 Belgrade, Yugoslavia): Reversed-phase thin-layer chromatography of some foodstuff dyes. J. Planar Chromatogr. **16**, 276-279 (2003). Study of the effects of eluent modifications TLC of 9 frequently used water-soluble food dyes (tartrazine, sunset yellow FCF, quinoline yellow FCF, amaranth, ponceau 6R, erythrosine, indigo carmine, brilliant blue FCF, brilliant black BN) on RP-18 with ammonium sulfate solutions (0.1, 0.5, and 1.0 mol/l) in water - organic modifier (ethanol or acetone) of widely variable composition.

Food analysis, qualitative identification, water-soluble food dyes 30

32. Pharmaceutical and Biomedical Applications

92 061 M. ALEKSIC*, J. ODOVIC, D. MILOJKOVIC-OPSENICA, Z. TESIK, N. PERISIK-JANJIC, (*Fac. of Pharm., Univ. of Belgrade, P.O. Box 146, 11000 Belgrade, Serbia and Montenegro): Reversed-phase thin-layer chromatography of several myorelaxants. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 181-184 (2003). TLC of myorelaxants (atracurium besylate, pancuronium bromide, rocuronium chloride, suxamethonium chloride, vecuronium bromide) on cellulose with water - ethanol and water - propanol, varying the concentration of the organic modifier from 30 to 70 vol % of ethanol, as well as from 10 to 90 vol % of propanol. Detection by exposure to iodine vapor.

Pharmaceutical research, qualitative identification, myorelaxants 32

92 062 B. BAGOCSI, A. LAUKO, S. MAHO, Z. VEGH, K. FERENCZI-FODOR, (Gedeon Richter Ltd., P.O. Box 27, H-1475 Budapest, Hungary): The role of OPLC as an in-process test in

total-synthesis of norsteroids. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 185-189 (2003). OPLC of norsteroids (e.g. 19-nortestosterone, estradiol-methylether-acetate) on silica gel with different mixtures of toluene, chloroform, butyl acetate, and hexane, i.e. toluene - chloroform - cyclohexane - butyl acetate 55:3:40:2.Visualization by spraying with 10% sulfuric acid in ethanol and heating at 120°C for 2 min. Also visual evaluation under UV 254 and 366 nm before and after spraying. Use of a video documentation system.

OPLC, norsteroids 32

92 063 B. BAGOCSI, G. RIPPEL, M. MEZEI, Z. VEGH, K. FERENCZI-FODOR*, (*Gedeon Richter Ltd.; Central Anal. Lab., H-1475 Budapest 10, P.O. Box 27, Hungary): OPLC, a method between TLC and HPLC, for purity testing of norethisterone bulk drug substance and tablet. J. Planar Chromatogr. **16**, 359-362 (2003). TLC of norethisterone and impurities (e.g. 17-epi-norethisterone, Δ^4-nordione, 6-ketonor-ethisterone) on silica gel with chloroform - acetone 9:1 and chloroform -methanol 19:1. Visualization by derivatization with 10% ethanolic sulfuric acid and heating at 120°C for 2 min. Visualization under UV 366 nm. OPLC on silica gel with hexane and butyl acetate - chloroform 17:3. Evaluation by videodensitometry. Comparison of selectivity and efficiency of TLC, OPLC, and HPLC.

Pharmaceutical research, quality control, qualitative identification, comparison of methods 32

92 064 B. BAGOCSI, G. RIPPEL, M. MEZEI, K. FERENCZI-FODOR, (Gedeon Richter Ltd., P.O. Box 27, H-1475 Budapest 10, Hungary): OPLC, a method between TLC and HPLC, in purity testing of norethisterone drug substance and tablet. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 191-196 (2003). OPLC and TLC of norethisterone and degradation products (6α-hydroxy-, 6ß-hydroxy-, 10-hydroxy-, 17-epi-, 6-ketonorethisterone, and Δ^4-nordione) on silica gel with chloroform -acetone 9:1 for TLC and n-hexane and butyl acetate - chloroform 17:3 for OPLC. Visualization by spraying with 10% ethanolic sulfuric acid solution and heating for 1 min at 120°C. Evaluation of the chromatograms under UV 366 nm. Quantitation by videodensitometry.

OPLC, norethisterone 32

92 065 V. BODIS, SZ. NYIREDY*, (*Res. Inst. for Med. Plants, Lupaszigeti út 4, 2011 Budakalász, Hungary): On-plate solid-liquid continuous extraction, a novel exhaustive technique. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 203-208 (2003). Description of the novel on-plate solid-liquid continuous extraction (SLCE) with preparative layers. The fine powdered sample to be extracted has to be introduced into a preparative layer on a 200 mm x 30 mm plate which is placed into a special device. The prepared preparative plate is covered with a glass plate, and with the help of a thick filter paper the extraction can be started with the appropriate extraction solvent. HPTLC of furocoumarins on silica gel under unsaturated conditions with ether - dichloromethane - tetrahydrofuran - hexane 10.71:9.68:7.5:72.11. Quantitation by densitometry at 313 nm.

Herbal, quantitative analysis, densitometry, furocoumarins 32

92 066 E. BRZEZINSKA*, G. KOSKA, (*Dept. of Anal. Chem., Med. Univ. of Lodz, Muszynskiego 1, 90-151 Lodz, Poland): TLC data in QSAR assay of thiazole and benzothiazole derivatives with H_1-antihistamine activity. Part 1. J. Planar Chromatogr. **16**, 451-457 (2003). TLC of derivatives of 2-[2-(phenylamino)thiazol-4-yl]ethanamine, 2-(2-benzyl-4-thiazolyl)ethanamine, 2-(benzhydrylthiazol-4-yl)ethanamine, 2-(1-piperazinyl)benzothiazole, and 2-(hexahydro-1H-1,4-diazepin-1-yl)benzothiazole on RP-2 impregnated with solutions of propionic acid, propionamide, and n-amylamine and their mixtures, with mobile phases comprising 4:1 mixtures of acetonitrile, methanol, or dichloromethane with 0.02 mol/l ammonium acetate buffer pH 7.4. Chromatographic data reported here were obtained by use of two mobile

phases: acetonitrile - methanol - buffer 2:2:1, and acetonitrile - methanol - dichloromethane - buffer 6:1:1:2. Detection under UV 254 nm. Investigation of quantitative structure-activity relationships (QSAR).

Pharmaceutical research, qualitative identification, thiazole and benzothiazole derivatives 32

92 067 A.N. CAMPBELL, J. SHERMA*, (*Dept. of Chem., Lafayette Coll., Easton, PA 18042, USA): Determination of famotidine in acid reduction tablets by HPTLC and videodensitometry of fluorescence quenched zones. J. Liq. Chrom. & Rel. Technol. **26**, 2719-2727 (2003). HPTLC of famotidine on silica gel (with concentrating zone) with ethyl acetate - methanol - toluene - NH_3 conc. 40:25:20:2 with 15 min chamber saturation. Quantitation by densitometry at 254 nm. Evaluation of precision (1.25 - 2.55% RSD) and accuracy.

Quality control, quantitative analysis, densitometry, famotidine 32

92 068 M. CAO (Cao Meifang)*, SH. LI (Li Shifang), (*Affil. Shuguang Hosp., Shanghai Univ. TCM, Shanghai 200021, China): (The quality standard for compound Bushen granules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(3), 193-196 (2003) TLC on silica gel with 1) toluene - chloroform - acetone - formic acid 40:25:35:3, 2) ethyl acetate - chloroform 4:1, 3) chloroform - methanol - water 13:7:2. Detection 1) by spraying with 5% $AlCl_3$ in ethanol and heating, 2) under UV, 3) by spraying with 5% sulfuric acid in ethanol and heating. Identification by finger-print technique.

Pharmaceutical research, quality control, herbal, qualitative identification, psoralen, isopsoralen 32

92 069 Y. CHEN (Chen Yunfeng), (Luoyang Municip. Inst. Drug Cont., Luoyang, Henan 471003, China): (An improved procedure for the identification of Shouwu pills by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 346-347 (2003). Improvement of a TLC procedure based on that in China Pharmacopoeia. TLC on silica gel by twice developing with benzene - ethanol 2:1 for the first and benzene - ethanol 4:1 for the second. Detection by 1) by spraying with 3% phosphomolybdic acid - H_2SO_4 solution and heating at 105ºC. Identification by finger print technique. Discussion of the advantages of the improved procedure in the quality control of the medicine.

Pharmaceutical research, quality control, herbal, qualitative identification, 32

92 070 I.M. CHOMA, (Dept. of Chem. Phys., Univ. of M. Curie-Sklodowska, M. Sklodowska Sq. 3, 20-031 Lublin, Poland): TLC separation of fluoroquinolones: Searching for better selectivity. J. Liq. Chrom. & Rel. Technol. **26**, 2673-2685 (2003). TLC of 6 veterinary fluoroquinolones (difloxacin, ciprofloxacin, norfloxacin, sarafloxacin, enrofloxacin, and flumequine) by 1- and 2-dimensional development on silica gel and on diol-, amino-, and cyanopropyl-modified silica gel with six different mobile phases like e.g. dichloromethane - methanol - 2-propanol - 25% NH_3 3:3:5:2 and 4:4:5:2. Detection under UV 254 or 366 nm. Optimization procedure.

Pharmaceutical research, qualitative identification, fluoroquinolones 32

92 071 C. CIMPOIU*, V. MICLAUS, G. DAMIAN, M. PUIA, D. CASONI, C. BELE, T. HODISAN, (*Fac. of Chem. and Chem. Eng., "Babes-Bolyai" Univ., 11 Arany Janos, 3400 Cluj-Napoca, Romania): Identification of new phthalazine derivatives by HPTLC-FTIR and characterization of their separation using some molecular properties. J. Liq. Chrom. & Rel. Technol. **26**, 2687-2696 (2003). Systematic investigation TLC of 9 phthalazines (e.g. 1-o-bromophenoxy-4-phenyl-phthalazine, 1-p-nitrophenoxy-4-phenyl-phthalazine, and 1-[3-(ß-diethylaminoethyl)-4-methyl-7-coumarinyloxy]-4-phenyl-phthalazine and their corresponding 4-tolyl- and -4-benzyl-compounds) on silica gel with toluene - chloroform- methanol 70:

20:1. The plates were developed twice with the same mobile phase at room temperature in a saturated N-chamber. Detection under UV 254 nm. The quality of HPTLC-FTIR spectra is sufficient for the identification of unknown substances.

Pharmaceutical research, qualitative identification, phthalazine derivatives 32

92 072 V.G. DONGRE*, V.W. KAMBIE, (*Dept. of Chem., Dr Babasaheb Ambedkar Marathwada Univ., Aurangabad-431 004, India): HPTLC detection and identification of heroin (diacetylmorphine) in forensic samples. Part III. J. Planar Chromatogr. **16**, 458-460 (2003). HPTLC of heroin, morphine, codeine, thebaine, papaverine, narcotine, paracetamol, benzodiazepines, methaqualone, phenobarbitone, and procaine hydrochloride on silica gel with chloroform - ethanol 9:1 with chamber saturation. Visualization by spraying with freshly prepared 1% aqueous ferric chloride, then with 1% acidified ethanolic 2,2-dipyridyl solution and heating at 100°C for 10 min.

Toxicology, qualitative identification, heroin, , 32

92 073 I. FECKA*, A. KOWALCZYK, W. CISOWSKI, (*Dept. of Pharmacogn., Wroclaw Med. Univ., pl. Nankiera 1, 50-140 Wroclaw, Poland): Optimization of the separation of flavonoid glycosides and rosmarinic acid from Mentha piperita on HPTLC plates. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 223-230 (2003). HPTLC of caffeetannins and flavonoid glycosides (eriocitrin, hesperidin, luteolin-7-O-rutinoside, diosmin and rosmarinic acid) on silica gel and chemically modified (amino-, cyano-, and RP-18) silica gel. The mobile phase acetone - acetic acid 17:3 was successful for the aminopropyl phase and water - methanol 3:1 for RP-18. Detection under UV 365 nm before and after spraying with 2% methanolic aluminium chloride solution or in visible light after spraying with bis-diazotized sulphanilamide. Simple and useful procedure.

Pharmaceutical research, herbal, qualitative identification, 32

92 074 H. FUJINO*, I. YAMADA, T. SAITO, S. SHIMADA, J. KOJIMA, (*Tokyo New Drug Res. Lab. I, Kowa Company Ltd. 2-17-43 Noguchicho, Higashimurayama, Tokyo, 189-0022 Japan): Metabolic interaction of several medicines examined using planar chromatogram-radioluminography. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 111-121 (2003). TLC of ^{14}C-labeled 6α-hydroxytaxol and 4-hydroxytolbutamide on silica gel with toluene - acetone - formic acid 60:39:1 in a horizontal chamber, separation of 6ß-hydroxytestosterone on silica gel with dichloromethane - acetone 4:1.After drying the plates were placed in contact with a phosphor imaging plate (IP) for 12 h. The amount of unchanged drug and metabolites were determined using a photo Film.

Radioisotope tracer technique 32

92 075 M. GLENSK, B. ZBIKOWSKA, W. CISOWSKI, (Dept. of Pharmacognosy, Univ. of Med., pl. Nankiera 1, 50-140 Wroclaw, Poland): TLC separation of Uncaria tomentosa alkaloids on chemically modified stationary phases. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 243-249 (2003). TLC of alkaloids on silica gel and diol-, cyano-, amino-, and RP-18 modified silica gel with ethyl acetate - methanol - water 100:13.5:10 (I), ethyl acetate - methanol - water - acetic acid 100:2.7:5:3 (II), ethyl acetate - methanol - water - formic acid 100:2.7:5:3 (III), and ethyl acetate - iso-propanol - NH_3 100:2:1 (IV) as mobile phases for silica gel and aqueous mobile phase containing methanol and ammonia for RP-chromatography in a horizontal developing chamber with sandwich configuration. 2-D TLC was performed on silica gel with (II) in the first direction and, after drying, with (III) in the second direction. Detection with Dragendorff or iodine reagent.

Herbal, qualitative identification, alkaloids 32

92 076 A. GUMIENICZEK*, A. BERECKA, H. HOPKALA, T. MROCZEK, (*Dept. of Med. Chem., Med. Univ. of Lublin, Chodzki Str. 6, 20-039 Lublin, Poland): Rapid HPTLC determination of rosiglitazone in pharmaceutical formulations. J. Liq. Chrom. & Rel. Technol. **26**, 3307-3314 (2003). HPTLC of rosiglitazone((+/-)-5-[4-[2-[N-methyl-N-(2-pyridinyl)amino]-ethoxy]benzyl]-2,4-thiazolidinedione maleate) on silica gel with chloroform - ethyl acetate - 25% NH_3 50:50:1. Detection and quantitation by densitometry in the reflectance/absorbance mode at 240 and 245 nm. Precision expressed as mean RSD was 3.58 and 2.76% for 240 nm, and 8.23 and 6.56% for 254 nm; mean recoveries from the fortified samples ranged from 89.48% to 99.38% for 240 nm, and from 89.05% to 100.89% for 254 nm. The mean recoveries from tablets were 101.95% to 103.2% for assays at 240 and 254 nm, respectively. New, simple, rapid method.

Quality control, quantitative analysis, densitometry, rosiglitazone 32

92 077 A. GUMIENICZEK*, H. HOPKALA, A. BERECKA, D. KOWALCZUK, (*Dept. of Med. Chem., Med. Univ. of Lublin, Chodzki Str. 6, 20-093 Lublin, Poland): Normal- and reversed-phase thin-layer chromatography of seven oral antidiabetic agents. J. Planar Chromatogr. **16**, 271-275 (2003). TLC of chlorpropamide, tolbutamide, glibenclamide, metformin, pioglitazone, rosiglitazone, and repaglinide on silica gel and oxide with mixtures of chloroform, ether, and ethyl acetate. For more effective resolution aqueous ammonia or acetic acid was added to the mobile phase. Silica enabled better separation than alumina. Reversed-phase chromatography was performed on RP-18 with mixtures of acetonitrile or 2-propanol with phosphate buffer. For the separation of these drugs RP chromatography was more effective than use of normal-phase mode. Determination under UV 254 nm. Systematic optimization of separation conditions.

Chlorpropamide, tolbutamide, glibenclamide, metformin, pioglitazone, rosiglitazone, and repaglinide biomedical application 32

92 078 G. HAN (Han Guiru)*, ZH. Zhao (Zhao Zhijun), R. XU (Xu Renliu), (*Hebei Prov. Inst. Drug Cont., Shijiazhuang, Hebei 050011, China): (Determination of ephedrine hydrochloride in Herba Ephdrae and its preparations by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(3), 203-205 (2003) TLC on silica gel with 1) chloroform - ethyl acetate - methanol - water 13:40:22:10. Detection by spraying with 0.5% ninhydrin in ethanol and heating at 105ºC for 10 min. Identification by comparison with the standard. Quantitation by densitometry at 525 nm.

Pharmaceutical research, quantitative analysis, densitometry, ephedrine hydrochloride 32

92 079 ZH. HAN (Han Zhangzhou)*, Y. LI (Li Yu), (*Shenzhen Sanjiu Pharm. Co., Ltd., Shenzhen, Guangdong 518029, China): (Determination of panaxatriol in Cuxuesheng granules by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(6), 459-461 (2003). TLC on silica gel with benzene - ether - ethyl acetate 5:3:3. Detection by spraying with 10% H_2SO_4 in ethanol and heating at 105 ºC. Identification by finger print technique. Quantitation of panaxatriol by densitometry at 530 nm. Discussion of use of the procedure for the quality control of the medicine.

Pharmaceutical research, quality control, quantitative analysis, densitometry, panaxatriol 32

92 080 W. HE (He Wei)*, R. RONG (Rong Rong), ZH. XIAO (Xiao Zhenliang), (Coll. TCM, Shandong Univ. TCM, Jinan 250014, China): (Study on the quality for Yishen Jiangya pills.) (Chinese). J. Chinese Trad. & Herb. Drugs, (Zhongcaoyao) **34**(4), 326-328 (2003). TLC on silica gel with 1) toluene (water saturated) - ethyl acetate - formic acid 5:4:1, 2) chloroform-acetone - formic acid 8:1:1, 3) chloroform - methanol - water 8:3.5:1. Detection 1) spraying with 5% $AlCl_3$ in ethanol and under UV365 nm, 2) by spraying with 2% $FeCl_3$ in ethanol, 3) under UV 365 nm. Identification by finger print technique.

Pharmaceutical research, quality control, herbal, qualitative identification, icariin 32

92 081 H. HOPKALA, A. POMYKALSKI*, T. MROZEK, M. OSTEP, (*Dept. of Med. Chem., Fac. of Pharm., Med. Univ. of Lublin, 6 Chodzki Str., 20-093 Lublin, Poland): Densitometric and videodensitometric TLC determination of timolol and betaxolol in ophthalmic solutions. J. Planar Chromatogr. **16**, 280-285 (2003). TLC of timolol (S-1[(1,1-dimethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol) and betaxolol (1-[4[2(cyclopropylmethoxy)ethyl]phenoxy-3-[(1-methylethyl)amino]-2-propanol) on silica gel with acetone - methanol 1:1, dichloromethane - methanol 1:1, dichloromethane - methanol - NH_3 (25%) 20:79:1, ethyl acetate - methanol - NH_3 (25%) 20:79:1 and 40:9:1 and on aluminium oxide with chloroform - 0.1 mol/l picric acid 97:3 or ethyl acetate - methanol - acetic acid 75:23:2. Also RP-TLC on RP-8 with tetrahydrofuran - 0.15 mol/l phosphate buffer pH 2.4 3:7. Detection after drying under UV 254 nm or after spraying with a 15% solution of iron(III)chloride in 5% HCl and then with 15% KI solution. Quantitation by densitometry of timolol at 300 nm and of betaxolol at 218 nm. Detection limits obtained for betaxolol and timolol were 0.025 and 0.05 μg, respectively; for UV irradiation at 254 nm were 2 and 0.5 μg for betaxolol and timolol, respectively.

32

92 082 Z. JANECZKO*, U. HUBICKA, J, KRZEK, I. PODOLAK, (*Dept. of Pharmacogn., Collegium Medicum, Jagiellonian Univ., Medyczna 9, 30-688 Krakow, Poland): Qualitative and quantitative analysis of diosmin in tablets by thin-layer chromatography with densitometric UV detection. J. Planar Chromatogr. **16**, 377-380 (2003). TLC of diosmin on silica gel with chloroform - methanol - water 23:12:2 with chamber saturation with 25% NH_3. Quantitation by densitometry at 344 nm. Repeatable and accurate results were obtained, limit of detection was 20 ng; satisfactory recovery (99.8 to 100.3%); linearity is given from 5 to 50 μg/mL. New and simple procedure.

Pharmaceutical research, quality control, quantitative analysis, densitometry, diosmin 32

92 083 H. KALASZ*, J. LENGYEL, T. SZARVAS, G. MOROVJAN, I. KLEBOVICH, (*Dept. of Pharmacol. and Pharmacotherapy, Semmelweis Univ., Nagyvárad tér 4, H-1089 Budapest, Hungary): Investigation of metabolism using TLC-DAR and reaction-displacement TLC. J. Planar Chromatogr. **16**, 381-385 (2003). TLC of (-)-deprenyl and metabolites (formaldehyde as dimedone adduct) on silica gel with chloroform - methanol - NH_3 100:10:1 and chloroform - methanol - water 7:5:1 in the first and second dimension, respectively. Also TLC of dimedone on silica gel with 5% triethanolamine in chloroform. Visualization under UV 254 nm. Detection of radiolabeled compounds by digital autoradiography (DAR) or on X-ray film after contact autoradiography. Two-dimensional separations have improved the evaluation.

Pharmaceutical research, quality control, quantitative analysis, densitometry, deprenyl 32

92 084 E. KEPCZYNSKA, J. BOJARSKI, A. PYKA*, (*Dept. of Anal. Chem., Fac. of Pharm., Silesian Acad. of Med., 4 Jagiellonska Street, 41-200 Sosnowiec, Poland): Lipophilicity of barbiturates determined by TLC. J. Liq. Chrom. & Rel. Technol. **26**, 3277-3287 (2003). TLC of 13 5,5-disubstitited barbiturates (e.g. barbital, pentobarbital, butabarbital, amobarbital, phenobarbital, aprobarbital, butalbital, talbutal, cyclopal) on RP-18 with methanol - water and methanol - Bates-Bower borate buffer mixtures. The methanol content was varied by 5% volume from 40 to 100%. After drying, visualization under UV 254 nm. Significant correlations were found between e.g. RM, RM0, log kIAM, log P, selected biological activity

values, and topological indices. TLC results as base for theoretical considerations.

Qualitative identification, 5,5-disubstitited barbiturates 32

92 085 Z. KIRALY-VEGHELY, G. KATAY, E. TYIHAK, (Res. Inst. for Viticulture and Enology of Agricultural Ministry, Experimental Wine Cellar, Maláta út 4, 1105 Budapest, Hungary): Separation of some stilbene isomers from red wine by overpressured layer chromatography. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 257-265 (2003). OPLC of cis-resveratrol, trans-resveratrol, cis-piceatannol, trans-piceatannol, cis-piceid, and trans-piceid on RP-18 with diluted acetic acid (pH 2.5) - acetonitrile 19:6. Evaluation by densitometry at 310 nm.

Food analysis, quantitative analysis, resveratrol, piceatannol, piceid 32

92 033 M. KOZYRA et al., see section 11

92 086 M. KOZYRA*, K. GLOWNIAK, A. ZADUBIEC, (*Dept. of Pharmacogn. with the Med. Plant Garden, Med. Univ., 1 Chodzki St, 20-093 Lublin, Poland): Phenolic acids in Peucedanum verticillare L. Koch ex Dc. J. Planar Chromatogr. **16**, 421-424 (2003). TLC and 2-D TLC of phenolic acids (p-coumaric, chlorogenic, hydroxybenzoic, caffeic, rosmarinic, syringic, isovanillic, vanillic, protocatechuic, ferulic, g-resorcylic, ß-resorcylic, and gentisic acid) on cellulose with toluene - ethyl formate - formic acid 5:4:1, sodium formate - formic acid - water 10:1:200, and 15% aqueous acetic acid for one-dimensional separation and after preconditioning with benzene - methanol - acetic acid 94:1:5 for 5 min with benzene - methanol - acetic acid - acetonitrile 16:2:1:1 in the first direction and sodium formate - formic acid - water 10:1:200 in the second direction. Detection under UV 254 and 366 nm. Visualization also by 3% methanolic solution of iron(III) chloride and 1:1 diazotized sulfanilic acid in 20% sodium carbonate solution.

Pharmaceutical research, herbal, phenolic acids 32

92 087 V.P. KUMAR, M.N. RAVISHANKARA, H. PADH, M. RAJANI*, (*Dept. of Pharmacogn. and Phytochem., B. V. Patel Pharm. Education and Res. Development Centre, Thaltej, Ahmedabad 380 054, India): High-performance thin-layer chromatographic method for estimation of rutin in medicinal plants. J. Planar Chromatogr. **16**, 386-389 (2003). TLC of rutin from medicinal plants (e.g. Tephrosia purpurea, Leptadenia reticulata, Ruta graveolens) on silica gel with ethyl acetate - butanol - formic acid - water 5:3:1:1 with chamber saturation for 15 min (25°C, relative humidity 40%). Quantitation by densitometry at 366 nm. The method was validated for precision (intra- and inter-day), repeatability, and accuracy. Simple, specific, and sensitive method.

Pharmaceutical research, quality control, herbal, quantitative analysis, densitometry, medicinal plants 32

92 088 J. LALLA, P. HAMRAPURKAR, T. WADHWA*, (*Dept. of Pharm. Anal., Principal K.M. Kundnani Coll. of Pharm., Worli, Mumbai 400 018, India): High-performance thin-layer chromatographic determination of satranidazole in its dosage form. J. Planar Chromatogr. **16**, 447-450 (2003). HPTLC of satranidazole and metronidazole on silica gel, prewashed with methanol, with chloroform - methanol 20:1 after equilibration for 10 min at 20°C. Visualization and quantitation by densitometry at 317 nm. Linearity of satranidazole between 200 and 1000 ng. Simple, specific, and sensitive TLC method.

Quality control, quantitative analysis, densitometry, satranidazole 32

92 089 J. LALLA*, P. HAMRAPURKAR, A. KULKARNI, H. MAMANIA, (*Flat No-701, Thakur Complex, Kandivli (E), Mumbai-400101, India): Quantitative analysis of homeopathic mother tincture of Boerhaavia diffusa Linn. by HPTLC employing the therapeutically active marker “Punarnavoside”. J. Planar Chromatogr. **16**, 465-468 (2003). HPTLC of punarnavoside on

prewashed silica gel with toluene - ethyl acetate - acetic acid 30:15:1 with chamber saturation for 10 min. Visualization by spraying with 0.2% aqueous $KMnO_4$ solution or with Liebermann-Burchard, Fiegel test, and ethanolic ferric chloride solution. Simple validated quantitative method.

Quality control, purnanavoside 32

92 090 J.K. LALLA*, P.D. HAMRAPUKAR, K. GAURI, (*Dept. of Pharm. Anal., Principal K. M. Kundnani Coll. of Pharm., Worli, Mumbai 400 018, India): Standardization of the homeopathic mother tincture of Calendula officinalis Linn. J. Planar Chromatogr. **16**, 298-302 (2003). HPTLC of rutin and quercetin on silica gel, pre-washed with methanol, with n-butanol - acetic acid - water 36:5:5 and toluene - chloroform - acetone - acetic acid 4:4:4:1, respectively. Visualization and quantitation by densitometry at 366 nm. The method was validated using calibration standards of rutin trihydrate and quercetin regarding specificity, limits of detection and quantitation, precision, accuracy and linearity.

Quality control, quantitative analysis, densitometry, rutin, quercetin 32

92 091 T. LI (Li Tong)*, ZH. ZHAO (Zhao Zhijun), L DING (Ding Liyu), C. YANG (Yang Caiqin), J. WANG (Wang Jing), Z. YAO (Yao Zihua), (*Coll. Chem. & Environ. Sci., Hebei Univ., Baoding, Hebei 071002, China): (Study on the quality for Tongjingping capsules.) (Chinese). J. Chinese Trad. & Herb. Drugs, (Zhongcaoyao) **34**(4), 328-329 (2003). TLC on silica gel with 1) chloroform - ethyl acetate - methanol - isopropanol - formic acid 150:75:50:10:1, 2) petroleum ether (60-90ºC) - ethyl acetate 20:17, 3) benzene - ethyl acetate - formic acid 10:6:1. Detection 1) by spraying with 5% vanillin - H_2SO_4 4:1 in ethanol and heating, 2) by spraying with 5% potassium iodobismuthate in water, 3) by spraying with 1% $FeCI_3$ - 1% $K_3[Fe(CN)_6$ 1:1. Identification by finger print technique.

Pharmaceutical research, quality control, herbal, qualitative identification, ferulic acid 32

92 092 A. LIU (Liu Anqiu)*, J. LI (Li Jianxiang), (*Hunan Xiangya Pharm. Co., Ltd., Changsha, Hunan 410013, China): (Study on the quality standard of Shenqinaoqing granules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(5), 360-362 (2003). TLC on silica gel with 1) butanol - ethyl acetate-water 4:1:5, 2) hexane - ethyl acetate 9:1, 3) petroleum ether (30-60ºC) - ethyl formate - formic acid 15:5:1, 4) chloroform - methanol - water 13:7:2. Detection 1) by spraying with 10% H_2SO_4 in ethanol and heating at 105ºC, 2) under UV 365 nm, 3) by exposure to ammonia vapor. Identification by finger print technique. Quantitation of astragaloside by densitometry at 530 nm. Discussion of use of the procedure for the quality control of the medicine.

Pharmaceutical research, quality control, herbal, densitometry, qualitative identification, astragaloside 32

92 093 L. LIU (Liu Lansheng)*, L. ZHANG (Zhang Li), (*Lanzhou Municip. Inst. Drug Cont., Lanzhou 730030, China): (Study of the quality standard for Banlong capsules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 288-290 (2003). TLC of isopsoralen on silica gel with 1) hexane - ethyl acetate 4:1, 2) toluene - ethyl acetate - formic acid 5:5:3, 3) petroleum ether (60-90ºC) - ethyl acetate 1:1. Detection under UV 365nm, UV 254 nm, under daylight. Identification by finger print technique. Quantitation of isopsoralen by densitometry at 250 nm. Discussion of use of the procedures for the quality control of the medicine.

Pharmaceutical research, quantitative analysis, densitometry, isopsoralen 32

92 094 Y. LIU (Liu Yarong), (Qinghai Inst. Drug Cont., Xining 810000, China): (Study of the quality standard for Tibetan Jingzhu Chongcao tablets.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(3), 201-202 (2003). TLC on silica gel with 1) chloroform - methanol - water 13:7:2, 2) chloroform - acetone 4:1. Detection 1) by spraying with 10% H_2SO_4 in ethanol and heating at 105ºC for 5 min, 2) under UV. Identification by finger-print

technique. Quantitation of astragaloside by densitometry at 520 nm. Discussion of use of the procedures for quality control of the medicine.

Pharmaceutical research, quality control, quantitative analysis, densitometry, astragaloside IV 32

92 047 M. LUCZKIEWICZ et al., see section 22

92 042 A. LUDWICZUK et al., see section 14

92 095 J. LUO (Luo Jie)*, W. LIN (Lin Weilan), D. WANG (Wang Deqing), W. ZHAO (Zhao Wenchang), (*Guangzhou Baiyunshan Pharm. Fact. Chinese Med., Guanzhou 510515, China): (Study on the quality standard of Yejuhua granules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(5), 363-365 (2003). TLC on silica gel with toluene - ethyl acetate - formic acid 20:10:3. Detection by spraying with 5% $AlCl_3$ in ethanol and heating at 105°C. Identification by finger print technique.

Pharmaceutical research, quality control, herbal, qualitative identification, chlorogenic acid 32

92 096 B. MALAWSKA*, K. KULIG, E. BENDIECK, (*Dept. of Pharm. Chem., Jagiellonian Univ. Med. Coll., Medyczna 9, 30-688 Krakow, Poland): Comparison of chromatographically determined values of the lipophilicity of anticonvulsant active N-substituted amides of α-arylalkylamine-γ-hydroxybutyric acid with values estimated by computational methods. J. Planar Chromatogr. **16**, 390-395 (2003). TLC of 5 series of anticonvulsant active N-substituted amides of α-arylalkylamine-γ-hydroxybutyric acid (37 compounds) on RP-18 with mixtures of methanol, 0.1 M tris buffer pH 7.4, and acetic acid with chamber saturation. Visualization under UV 254 nm. Rapid, easy, and convenient method for determination of lipophilicity.

Pharmaceutical research, qualitative identification, 32

92 097 L. MI (Mi Lili)*, SH. ZHANG (Zhang Shuwen), J. SUN (Sun Jiajin), ZH. WANG (Wang Zhihua), X. HONG (Hong Xiaoqun), (*Shanghai TCM Univ., Shanghai 200032, China): (Study of nucleotides in Cordyceps and its mycelia by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(5), 402-405 (2003). TLC on silica gel with chloroform - isopropanol - methanol - ethyl acetate - NH_3 conc. 80:30:30:30:9. Detection under UV 254 nm. Identification of by finger print technique. Quantitation by densitometry at 270 nm. Publication of the determination results of adenosine, uridine and guannosine in 14 drugs cultivated in different regions. Discussion of the discrepancy among the drugs from different sources, and use of the procedure for the screening and the quality control of the drugs.

Pharmaceutical research, quality control, herbal, quantitative analysis, densitometry, adenosine, uridine, guannosine 32

92 098 K. MIGLECZI, I. KLEBOVIC*, E. MINCSOVICS, I. HAZAI, (*EGIS Pharmaceuticals Co. Ltd., Dept. of Pharmacokinetics, Keresztúri út 30-38, 1106 Budapest, Hungary): Application of planar radiochromatography in metabolic research. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 139-147 (2003). TLC of ^3H- or ^{14}C-labeled drugs on silica gel with e.g. n-butanol - acetic acid - water 4:1:1 or chloroform - hexane - ethanol - NH_3 conc. 75:15:9:1. Also OPLC of glyceryl trinitate and metabolites (glycerol di- and mononitrate) on silica gel with di-n-butylether. Detection by densitometry with phosphor imager or with DAR (digital autoradiography). Today three principal methods for detection of radioactivity are recognized, namely film autoradiography, radioluminography (i.e. storage phosphor imaging) and electronic radiography (linear analyzers, digital autoradiography, and ß-imager).

Pharmaceutical research, quantitative analysis, qualitative identification, autoradiography 32

92 099 G. MISZTAL*, L. KOMSTA, (*Dept. of Med. Chem., Med. Univ., 6 Chodzki, 20-093 Lublin, Poland): The retention behavior in normal-phase chromatographic systems of some fibrate-type antihyperlipidemic drugs. J. Planar Chromatogr. **16**, 351-358 (2003). TLC of bezafibrate, ciprofibrate, clofibrate, clofibric acid, fenofibrate, and gemfibrozil on silica gel, aluminium oxide, amino-, diol-, and cyano-modified silica gel and polyamide 11 with mobile phases containing hexane as weakly polar diluent and five polar modifiers: acetone, dioxane, methyl ethyl ketone, ethyl acetate, and tetrahydrofuran. Visualization under UV 254 nm. Quantitation by videodensitometry and densitometry.

Pharmaceutical research, quantitative analysis, densitometry, fibrate-type antihyperlipidemic drugs 32

92 100 G. MISZTAL* B. PAW, R. SKIBINSKI, l. KOMSTA, J. KOLODZIEJCZYK, (*Dept. of Med. Chem., Med. Acad., 6 Chodzki, 20-093 Lublin, Poland): Analysis of non-selective calcium-channel blockers by reversed-phase TLC. J. Planar Chromatogr. **16**, 433-437 (2003). TLC of prenylamine, lidoflazine, bepridil hydrochloride, and fendiline hydrochloride on RP-8 and RP-18 altering the pH and the concentration of organic modifier (methanol, ethanol, tetrahydrofuran, acetonitrile) in the aqueous phase. On RP-8 the best separation was achieved with 50% acetonitrile in pH 2.06 phosphate buffer and on RP-18 with 50% ethanol in pH 2.06 phosphate buffer. Detection under 254 nm and videodensitometric determination. New TLC procedure.

Pharmaceutical research, qualitative identification, prenylamine, lidoflazine, bepridil hydrochloride, fendiline hydrochloride 32

92 101 S. NARASIMHAN, M. VIJAYAKUMAR, S. MEHROTRA*, (*Pharmacogn. and Ethnopharmacology Div., Nat. Bot. Res. Inst., Rana Pratap Marg, P.O. Box 436, Lucknow-226 001, India): A new spray reagent for detection and differentiation of sulfur compounds in plant extracts. J. Planar Chromatogr. **16**, 468-469 (2003). HPTLC of plant extracts with different sulfur-containing groups (e.g. allicin and disulfides) on silica gel with toluene - ethyl acetate 10:3 for Allium extracts and toluene - ethyl acetate 7:3 for Ferule foetida extracts. Visualization by spraying with a solution of 3 g bismuth nitrate in 5 mL concentrated nitric acid and dilution to 100 mL with water. Alternatively, a solution of 3 g bismuth nitrate in 100 mL acetone can be used; in addition observation under UV 254 and 366 nm. Densitometry at 417 nm for allicin and at 392 nm for disulfides.

Qualitative identification, herbal, biochemistry, allicin, disulfides 32

92 102 T. PENG (Peng Tuohua)*, F. ZENG (Zeng Fan), H. ZHONG (Zhong Honglan), X. FANG (Fang Xiaotang), (*Guangdong Prov. Inst. Pharm., Guangzhou 510440, China): (Determination of valine in Dilong injections by thin-layer chromatography.) (Chinese). TLC on silica gel with butanol - acetic acid - water 8:1:2. Detection by spraying with ninhydrin reagent and heating. Identification by finger print technique. Quantitation of valine by densitometry at 487 nm.

Pharmaceutical research, quality control, quantitative analysis, densitometry, valine 32

92 053 N.U. PERISIC-JANJIC et al., see section 24

92 103 N.U. PERISIC-JANJIC*, T.L. DJAKOCIC-SEKULIC, K. POPOV-PERGAL, (*Dept. of Chem., Fac. of Sci., Univ. of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia and Montenegro): Effect of the stationary phase and the mobile-phase modifier on the retention of some thiazoles. Correlation with the lipophilicity of the compounds. J. Planar Chromatogr. **16**, 363-368 (2003). Study of structure-activity relationship TLC of 12 differently substi-

tuted 2,4-dioxotetrahydro-1,3-thiazoles (4'-dimethylamino-benzylidene-, 1'-naphthylidene , 2'-oxybenzylidene-, 3',4'-methylenedioxybenzylidene-, benzylidene-, 3'-thienylidene-, 3',4'-dimethoxybenzylidene-, 4'-bromobenzylidene-, 5'-methyl-2'-furfurylidene-, 4'-benzyloxy-benzylidene-, 4'-ethoxybenzylidene-) on silica gel with hexane - ethyl acetate, hexane - acetone, and hexane - dioxane and on paraffin oil-impregnated silica gel with NH_3 conc. - methanol, NH_3 conc. - dioxane, and NH_3 conc. - acetone in different ratios. Visualization under UV 254 nm.

Qualitative identification, thiazoles 32

92 004 N.U. PERISIC-JANJIC et al., see section 2

92 104 N.U. PERISIC-JANJIC*, B.Z. JOVANOVIC, N.J. JANJIC, O.S. RAKOVIC, D.G. ANTONOVIC, (*Dept. of Chem., Fac. of Sci., Trg Dositeja Obradovica 3, 21 000 Novi Sad, Serbia and Montenegro): Study of the retention behavior of newly synthesized s-triazine derivatives in RP TLC systems, and the lipophilicity of the compounds. J. Planar Chromatogr. **16**, 425-432 (2003). HPTLC of 9 new s-triazines (e.g. 2,4-bis(cyclopropylamino)-6-chloro-s-triazine, 2,4-bis(cyclobutylamino)-6-chloro-s-triazine etc.) on RP-18, cyano- and amino-modified silica gel with water - tetrahydrofuran, water - dioxane, and water - acetonitrile mixtures. Detection under UV 254 nm.

Pharmaceutical research, qualitative identification, s-triazine derivatives 32

92 005 N.U. PERISIC-JANJIC et al., see section 2

92 105 N.U. PERISIC-JANJIC, B. LUCIC, N.J. JANJIC, D. AGBABA*, (*Inst. of Pharm. Chem. and Drug Anal., Fac. of Pharm., Vojvode Stepe 450, P.O. Box 146, 11000 Belgrade, SCG.): Study of the lipophilicity and retention behavior of some beta-adrenoceptor antagonists. J. Planar Chromatogr. **16**, 347-350 (2003). HPTLC of beta-adrenoceptor antagonists (e.g. betaxolol HCl, propanolol HCl, celipronol HCl, oxprenolol, carvedilol, metoprolol tartrate, atenolol) on RP-18 with water - acetonitrile and water - dioxane mixtures in different ratios. Visualization under UV 254 nm.

Pharmaceutical research, qualitative identification, beta-adrenoceptor antagonists 32

92 106 R. SLAVESKA-RAIKI, V. RAFAJLOVSKA*, V. RIZOVA, I. SPIREVSKA, (*Fac. of Techn. and Metallurgy, Rudjer Boskovic St 16, Skopje, R. Macedonia): HPTLC determination of gallic acid and tannin in extracts of bearberry leaves. J. Planar Chromatogr. **16**, 396-401 (2003). HPTLC of gallic acid and tannin on cellulose with iso-butanol - acetic acid - water 14:1:3.5 and on silica gel with chloroform - ethanol - formic acid 5:4:1 in a saturated chamber. After drying visualization under UV light at 254 nm. Quantitation by densitometry at 280 nm in absorbance mode. The validated method was found to be simple, reliable, and convenient for routine analysis. Establishment of a validated procedure.

32

92 107 T. SLAWIK*, B. PAW, (*Dept. of Med. Chem., Med. Univ. of Lublin, 6 Chodzki Street, 20-093 Lublin, Poland): RPTLC determination of the lipophilicity of 1,2-benzisothiazol-3(2H)-one derivatives substituted in the heterocyclic ring. J. Planar Chromatogr. **16**, 442-446 (2003). TLC of 8 new substituted 1,2-benzisothiazol-3(2H)-one derivatives on RP-18 with methanol - water, methanol - pH 1.95 glycine buffer and methanol - pH 10.0 glycine buffer at 20°C. Visualization under UV 254 nm.

Pharmaceutical research, qualitative identification, 1,2-benzisothiazol-3(2H)-one derivatives 32

92 108 B. TANG (Tang Bing), (Beihai Municip. Inst. Drug Cont., Beihai, Guangxi 536000, China): (Identification of ingredients in Kangji Xiaoke pills by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 339-340 (2003). TLC on silica gel with 1) chloroform - ethyl acetate - methanol - water 3:8:4:2, 2) butanol - acetic acid-water 7:1:2, 3) toluene - ethyl acetate 9:1. Detection by 1) by spraying with 10% H_2SO_4 in ethanol and heating at 105ºC, 2) under UV 365 nm, 3) by spaying with 10% phosphomolybdic acid in ethanol and heating at 105ºC. Identification of ginsenosides Re, Rg1, berberine hydrochloride, Wuweizi ester by finger print technique.

Pharmaceutical research, herbal, qualitative identification, ginsenosides Re, Rg1, berberine hydrochloride, Wuweizi ester 32

92 109 I. VOVK*, M. KOVAC, B. SIMONOVSKA, H. VUORELA, P. VUORELA, (*Lab. for Food Chem., Nat. Inst. of Chem., Hajdrihova 19, 1001 Ljubljana, Slovenia): Cellulose HPTLC plates in the separation of selected flavan-3-ols using aqueous eluents. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 25-39 (2003). TLC and HPTLC of selected flavan-3-ols [e.g. (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, (+)-catechin gallate, (-)-epicatechin gallate, epicatechin-(4ß®8)-catechin, epicatechin-(4ß®8)-epicatechin] on cellulose. The developing solutions consisted of aqueous solutions containing acetone, acetic acid, tetrahydrofuran, acetonitrile, ethyl acetate, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, and 2-butanol as organic modifiers in various concentrations. TLC in twin-trough chambers (saturated and unsaturated) and in horizontal developing chambers, used in tank and sandwich configuration. Separations were performed at ambient temperature and humidity (20-24°C, 46-56%). Visualization after drying by immersion for 1 s into vanillin/phosphoric acid reagent. The final optimized HPTLC method with water - 1-propanol - acetic acid 80:20:1 provided a fast separation of the flavan-3-ols studied with satisfactory resolution. Ready-to-use cellulose plates must be prewashed with water. In most cases the development in horizontal chamber with sandwich configuration gave the best resolution.

Food analysis, herbal, flavan-3-ols 32

92 030 I. VOVK et al., see section 10

92 110 G. WANG (Wang Guihua)*, S. HUANG (Huang Siyuan), (*Leshan Municip. Inst. Drug Cont., Leshan, Sichuan 614000, China): (Identification of Zhishu Yangwei pills by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 340-341 (2003). TLC on silica gel with 1) chloroform - ethyl acetate - methanol 9:1:2 with traces of formic acid, 2) benzene - ethyl acetate 9:1, 3) butanol - acetic acid - water 7:1:2. Detection by 1) by spaying with 5% vanillin in H_2SO_4 solution in ethanol and heating, 2) under day light, 3) under UV 365 nm. Identification by finger print technique. Discussion of using the procedures as the simple method for the quality control of the medicine.

Pharmaceutical research, quality control, herbal, qualitative identification 32

92 111 W. WEI (Wei Wenpeng), (Inst. Zhanjian Municip. Drug Cont., Zhanjiang, Guangdong 524037, China): (Identification of Yigan Fuzheng granules by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(5), 428-429 (2003). TLC on silica gel with 1) toluene - ethyl acetate - formic acid 15:2:1; 2) petroleum ether (60-90ºC) - ethyl acetate 4:1; 3) petroleum ether (60-90ºC) - ethyl acetate 7:3; 4) benzene - ethyl acetate - acetic acid 18:1:1. Detection 1) by exposure to ammonia vapor; 2) under UV. Identification of by finger print technique. Discussion of use of the procedure for the rapid quality control of the medicine.

Pharmaceutical research, quality control, herbal, qualitative identification, Chinese traditional medicine 32

92 112 H. WEN (Wen Hongmei)*, W. LI (Li Wei), A. ZHANG (Zhang Aihua), G.PENG (Peng Guoping), ZH. ZHANG (Zhang Zhengxing), (*Nanjing Univ. TCM, Nanjing 210029, China): (Study on the quality standards for Zhixuan granules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(6), 454-456 (2003). TLC on silica gel with 1) benzene - acetone 5:1, 2) petroleum ether (60-90ºC) - ethyl acetate 3:1. Detection 1) by spraying with 2% H_2SO_4 in ethanol and heating at 105º for 10 min, 2) by spraying with 4% H_2SO_4 in ethanol and heating at 105ºC for 10 min. Identification by finger print technique.

Pharmaceutical research, quality control, herbal, qualitative identification, 23-acetate alisol 32

92 113 SH. WU (Wu Shaojie)*, ZH. YANG (Yang Zhijuan), L. ZHU (Zhu Lihua), F. JIN (Jin Fengxie), (*North China Coal Med. Coll., Tangshan, Hebei 114001, China): (Study on the biotransformation of glycyrrhizin.) (Chinese). J. Chinese Trad. & Herb. Drugs, (Zhongcaoyao) **34**(6), 516-518 (2003). TLC of the zymolysed glycyrrhin and the fermentation solution on silica gel with butanol - acetic acid-water 4:1:2. Detection under UV 365 nm. Identification of monoglucuronyl-glycyrrhetinic acid (MGGA) by comparison with the standard. Quantitation by densitometry at 360 nm. Calculation of the transformation rate of the zymolysed and fermented products. Optimization of the condition for the biotransformation of the title compound. Discussion of use of the procedure in preparation of MGGA and the study of its physiological activity.

Pharmaceutical research, herbal, densitometry, qualitative identification, glycyrrhizin, monoglucuronyl-glycyrrhetinic acid (MGGA) 32

92 114 L. WULANDARI, G. INDRAYANTO*, (Dept. of Nat. Prod., Fac. of Pharm., Airlangga State Univ., Jl. Dharmawangsa dalam, Surabaya 60286, Indonesia): HPTLC determination of betamethasone in tablets and its validation. J. Liq. Chrom. & Rel. Technol. **26**, 2709-2717 (2003). HPTLC of betamethasone on silica gel with chloroform - methanol - water 36:10:1. Quantitation by densitometry at 245 nm. The method was tested for linearity, homogeneity, detection limit, accuracy, and working range. Mean recovery 99.2-100.1%. Selective, precise, and accurate procedure.

Quality control, quantitative analysis, densitometry, betamethasone 32

92 115 L. WULANDARI, G. INDRAYANTO*, (*Plant BioTechn. Res. Group, Fac. of Pharm., Airlangga Univ., Jl. Dharmawangsa dalam, Surabaya 60286, Indonesia): Densitrometric determination of betamethasone dipropionate and salicylic acid in lotions, and validation of the method. J. Planar Chromatogr. **16**, 438-441 (2003). TLC on silica gel with ethanol (96%) - toluene - chloroform - acetic acid 12:40:28:1 after saturation for at least 2 h at ambient temperature (25°C). Densitometry in absorbance mode between 200 and 400 nm. Quantification at 250 nm for betamethasone dipropionate and at 310 nm for salicylic acid. The method was validated for linearity, homogeneity, detection limit, accuracy, and working range. A cheap, rapid, and simple validated TLC method.

Quality control, quantitative analysis, densitometry, betamethasone dipropionate, salicylic acid 32

92 116 H. YANG (Yang Hongwu)*, ZH. WANG (Wang Zheng), M. XIN (Xin Mintong), P. WANG (Wang Ping), (*Liaoning Nothern Bio. Pharm. Group Ltd., Shenyang 110003, China): (Determination of cholic acid in artificial calculus bovis and its preparation by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 290-293 (2003). TLC of cholic acid on silica gel with 1) hexane - ethyl acetate - acetic acid - methanol 20:25:2:3. Detection by spraying with 10% H_2SO_4 in ethanol and heating at 105ºC, and under UV 365 nm. Identification by comparison with the standard. Quantitation by densitometry at 380 nm.

Pharmaceutical research, quality control, quantitative analysis, densitometry, cholic acid 32

92 117 X. YE (Ye Xiaoqiang), (Hechuan Inst. Drug Cont., Wuzhou, Guangxi 543001, China): (Study on the quality standard for Jinzhitai capsules.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(4), 284-288 (2003). TLC on silica gel with 1) cyclohexane - ethyl acetate - formic acid 60:20:1, 2) chloroform - ethyl acetate - formic acid 12:7:1, 3) chloroform - methanol - NH_3 90:10:1, 4) petroleum ether (60-90ºC) - hexane - ethyl acetate - formic acid 10:20:24:1. Detection 1) by exposure to ammonia vapor, 2) by spraying with 10% H_2SO_4 in ethanol and heating at 105ºC, 3) under daylight. Identification by finger print technique.

Pharmaceutical research, quality control, herbal, quantitative analysis, emodin, chrysophenol, physcion 32

92 118 T. YRJÖNEN, I. VOVK, B. SIMONOVSKA, O. MOUSA, R. HILTUNEN, H. VUORELA, P. VUORELA*, (*Dept. of Pharm., Vikki Drug Discovery Techn. Center, Univ. of Helsinki, P.O. Box 56, Viikinkaari 5E, Fin-00014 Finland): Comparison of medium pressure solid-liquid extraction and rotation planar extraction of Ficus leaves with reference to optimum operating parameters. J. Liq. Chrom. & Rel. Technol. **26**, 3289-3305 (2003). TLC of extracts from Ficus sycomorus L. on silica gel with hexane - ether - 1,4-dioxan - ethanol 39:5:3:3 optimized by use of the "PRISMA" system. Visualization under UV 254 and 366 nm. Quantitation by densitometry.

Pharmaceutical research, qualitative identification, herbal, extracts from Ficus sycomorus L 32

92 119 M. ZHANG (Zhang Mingxu)*, J. LI (Li Jian), (*Dept. Pharm., Guiyang Coll. TCM, Guiyang, Guizhou 550002, China): (*Determination of matrine in Meilupijiling by thin-layer chromatography.) (Chinese). J. Chinese Trad. Patent Med., (Zhongchengyao) **25**(6), 518-520 (2003). TLC on silica gel with benzene - acetone - ethyl acetate - NH_3 conc. 10:15:20:1. Detection by spraying with 5% potassium iodobismuthate solution and 5% sodium nitrite solution. Identification by finger print technique. Quantitation of matrine by densitometry at 480 nm.

Pharmaceutical research, quality control, herbal, quantitative analysis, matrine 32

92 120 SH. ZHU (Zhu Shanyin)*, H. XU (Xu Honhjie), B. XU (Xu Bengen), (*Jiaxing Municipal Inst. Drug Cont., Jiaxing, Zhejiang Prov. 314001, China): (Comparing identification between Cassia obtusifolia L and C. sophera L.) (Chinese). J. Chinese Trad. & Herb. Drugs, (Zhongcaoyao) **34**(4), 378-380 (2003). TLC on silica gel with petroleum ether (30-60ºC) - ethyl formate - formic acid 15:5:1. Detection under UV 365nm. Identification by finger-print technique. Comparison also by microscopy and UV spectroscopy, as well as visual characters.

Pharmaceutical research, quality control, herbal, quantitative analysis 32

92 121 W. ZHU (Zhu Wenrong), (Jiangyin Tianjiang Pharm. Co., Ltd., Jianyin, Jiangsu 214429, China): (Study on the quality control for compound Chuipencao granules.) (Chinese). TLC on silica gel with 1) benzene - butanone - methanol 3:1:1, 2) chloroform - methanol 4:1. Detection 1) under UV 365 nm, 2) by spraying with a solution of 1% iodine - 3% sodium azide 1:1 and heating. Identification by finger print technique.

Pharmaceutical research, qualitaty control, isorhamnetin-3-7-D-glucoside 32

33. Inorganic Substances

q92 122 R.M. BAOSIC, D.M. MILOJKOVIC-OPSENICA, Z.L. TESIC*, (*Fac. of Chem., Univ. of Belgrade, Studentski trg 16, P.O. Box 158, 11001 Belgrade, Serbia and Montenegro): The effect of substituents in ß-ketoiminato ligand of copper(II) and nickel(II) complexes on their retention in polyacrylonitrile thin layer. Proc. Intern. Symp. on Planar Separations Plan. Chrom. 197-202 (2003). Investigation of structure-retention relation TLC of 24 copper(II) and nickel(II) complexes on a polyacrylonitrile sorbent with 12 different solvents (six mono-, four two-component and two aqueous solvents) in a horizontal developing chamber after presaturation for 30 min. After development, the colored spots of the complexes were readily visible.

Qualitative identification, copper(II) and nickel(II) complexes 33

92 123 A. ORINAK*, M. ADAMOVA, L. HALAS, P. TOMCIK, M. JUSTINOVA, (*Univ. of P.J. Safárik, Inst. of Chem., Dept. of Phys. and Anal. Chem., Moyzesova 11, 041 54 Kosice, Slovak Republic): Reagents for the detection of carboxylate anion ligands after chromatographic separation of zinc carboxylato complexes. J. Planar Chromatogr. **16**, 286-288 (2003). TLC of zinc(II) butyrate, zinc(II) isobutyrate, and zinc acetate on silica gel, prewashed with water – methanol, with 2-propanol - water 2:3 and pure redistilled water and on anion exchange layers with dioxane - water 3:2. Detection by derivatization with bromophenol blue, bromocresol green, and thymol blue indicator solutions, directly on the wet layer. Bromocresol green (0.5% solution + 5 mL 0.1 M NaOH) resulted in most sensitive detection of the carboxylates tested. The detection limit for this reagent and butyrate anion was established as 0.03 mg/L. New detection procedure.

Zinc(II) butyrate, zinc(II) isobutyrate, zinc acetate 33

35. Other Technical Products and Complex Mix

92 124 T. CSERHATI*, E. FORGACS, Z. ILLES, (*Inst. of Chem., Chem. Res. Center, Hungarian Acad. of Sci., P.O. Box 17, 1525 Budapest, Hungary): TLC study of the binding of nonionic surfactants to the corn protein zein. J. Liq. Chrom. & Rel. Technol. **26**, 2751-2761 (2003). TLC of nonionic surfactants (Arkopal N40, 50, 60, 80, 90, 100, 130, 150, 230, 300, Sapogenate T60, 100, 110, 130, 138, 180, 300, 500) on zein-coated aluminium oxide prepared by dissolving 0.5 g zein in a mixture of 160 mL propanol and 40 mL water and adding to 20 g aluminium oxide, removal of the solvents at 70°C in vacuum and coating of plates with a suspension of 5 g of stationary phase and 14 mL water. Bidistilled water and water - methanol mixtures containing 5, 10, and 15 vol.% methanol were used as stationary phases. Developments were also carried out in aqueous solutions of 0.5, 1.0, 2.0, 3.5, 4.0, and 5.0 M LiCl, as well as e.g. aqueous solutions of 4 M NaCl, 4 M KCl, 4 M RbCl, 4M NH4Cl etc. After drying at 105°C, the surfactants were detected by exposure to iodine vapor.

Qualitative identification, nonionic surfactants 35

How international was the HPTLC Symposium in Lyon 2003?

14

▲ *The organizers (left to right) L. Vicard, P. Bernard-Savary, E. Tavel, J. Wajsman, "Les Quatre Mousquetaires" of the Symposium*

15

▲ *Snapshot from the dinner cruise: International scientists ready to check some French fizzy soft drug (left to right) Anna Pelander (Fin), Illka Ojanperä (Fin), Susan Mayfield (GB), Michael Lancaster (GB), Andrew Handford (GB)*

The International Symposium for HPTLC, Lyon 15–18 October has been a real success. 107 scientists from 16 countries attended. The scientific sessions were chaired by a pantheon of the trade, Friedrich Geiss, Klaus Burger, and A.M. Siouffi. True high lights were the papers by Ms. Iuliana Popa on immuno-staining TLC of lipids and by Nico Vervoort on shear-driven chromatography. Traditional interest centers of pharmaceutical industry such as clean-up validation, natural substances validation methods, and toxicology were also addressed. One might regret that lipids, where HPTLC shows its superiority over other methods, were under represented.

A poster session was held in parallel with the exhibition where the major TLC manufacturers were represented.

Most of the symposium participants took advantage of two workshops preceding the symposium, led by Gerda Morlock. Even real specialists of the "well known" method were enthusiastic to get many new hints and explanations of observed phenomena during these two workshop sessions.

A manufacturers session on the morning of the last day was attended by many motivated symposium participants.

The Symposium web site will be maintained. Meanwhile most of the lectures and poster abstracts are available on line.

Social events included a Rhone River dinner cruise aboard the "Hermes" late Friday night and visits to Lyon, the vineyards, or the Grand Chartreuse monastery and distillery on Saturday afternoon.

Conversion of a gradient from an AMD1 to an AMD2 system

16

Dr. Ursula Wippo

As part of her doctoral thesis on determination of thermically unstable and nonvolatile pesticide residues in vegetable foodstuffs, Ursula Wippo* developed a process for conversion of AMD 1 gradients into a gradient suitable for AMD2. The thesis was performed in the research group of Prof. H.-J. Stan, Institute for Food Chemistry at the Technical University Berlin.

Scope

The technical improvement of the AMD system lead to the more user-friendly, software controlled AMD2, which allows full control of all processes. The solvent mixer of the AMD2 was designed in a way that offers free choice of developing solvent for each developing step, whereas in the AMD1 system, developing solvent was successively mixed with the previously used solvent. As an additional effect, less solvent was needed. Also the accuracy of measurement of the solvent front was improved significantly by monitoring the migration distance instead of calculating it from the developing time.

Calculation of conversion

The conversion of an AMD 1 gradient is helpful, if an analytical procedure, developed for AMD 1 should be applied to AMD 2. For adaptation of the gradient, a calculation program was created, using EXCEL 97 SR-2 (German version) which transforms the solvent composition of an AMD1 gradient into the composition of an AMD2 gradient. It is acknowledged that the developing solvent in the AMD1 system is gradually mixed and only a percentage change (following change of bottle 19% and without change of bottle 35%) of the effective solvent composition can be achieved.

The applied formula asks[1]:

- Whether the mixer was emptied prior to the run. If yes, the solvent composition is set to 100% of the new bottle.
- Whether bottles were changed.
  If yes, the solvent composition is calculated according to the following formula:
  = 0,81 x solvent composition of the last run + 0,19 x solvent composition of the current bottle.

  If no, the solvent composition is calculated according to the following formula:
  = 0,65 x solvent composition of the last run + 0,35 x solvent composition of the current bottle.

For the calculations, first the given AMD1 gradient is entered into the excel sheet.

[1] The following colors mark the respective parts of formula 1.

	A	B	C	D	E	F
9		Step	Bottle 1	Bottle 2	Bottle 3	Bottle 4
10		1	100			
11		5	50	50		
12		11		100		
13		17			100	
14		21			50	50
15		26				100
16						
17	Mixer emptied before step	31				

Excel sheet with previous AMD1 gradient
Bottle 1: Methanol – dichloromethane – HCOOH 12:88:0,1
Bottle 2: Dichloromethane – HCOOH 100:0,1
Bottle 3: Dichloromethane
Bottle 4: n-Hexane

In a second step, the formulas defined in the following table are executed (in the same excel sheet) and the program automatically calculates the AMD2 gradient.

	J	K	L	M	N	O
1	Step	=C9	=D9	=E9	=F9	...
2	1	**=C10**	=D10	=E10	=F10	...
3	**2**	**Formula 1**	Formula 2	Formula 3	Formula 4	...
4	3	Formula 1 copied	Formula 2 copied	Formula 3 copied	Formula 4 copied	...
	...	...	...	...	...	...

For better orientation in formula 1 the different cells were marked with colors.

Formula 1:

```
=IF($J3=$B$17;VLOOKUP($J3;$B$9:$H$15;2;True);IF(OR($J3=$B$10;$J3=$B$11;$J3=$B$12;$J3=$B$13;$J3=$B$14;$J3=$B$15);0.81*K2+0.19*VLOOKUP($J3;$B$9:$H$15;2;True);0.65*K2+0.35*VLOOKUP($J3;$B$9:$H$15;2;True)))
```

In formulas 2 to 4 formula 1 is copied and only column number 2 (underlined in formula 1) is replaced by 3, 4 or 5 respectively.

The result of the conversion of the selected AMD gradient can be transferred from the excel sheet directly into the AMD2 instrument.

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CAMAG AMD System
(Automated Multiple Development)

Ms. Wippo was intensively engaged in the AMD System during her doctorial thesis. AMD is used when the desired resolution is unattainable over the available separation distance by one step isocratic development. This is often the case for complex samples with high or differing matrix content, mixtures of components with a wide polarity range, or for multi-component mixtures.

For separation of samples with components covering a wide polarity range, a universal gradient reaching from high elution strength to very low elution strength is employed. A shallow gradient is suitable for the separation of closely related substances.

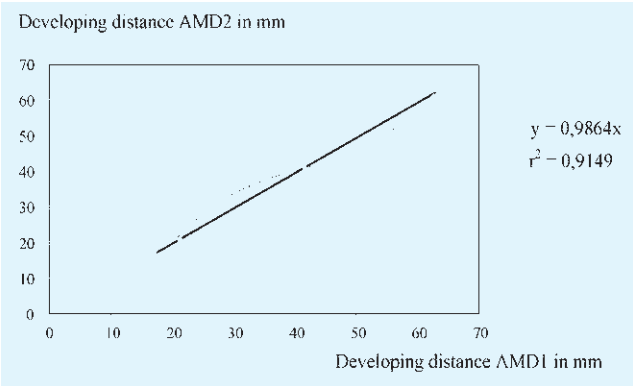
AMD allows reproducible gradient elution on silica gel because the plate is used only once. The time-consuming conditioning step needed in normal phase column chromatography is skipped in HPTLC. The AMD gradient starts with the strongest (polar) and ends with the weakest (non-polar) solvent on silica gel phases. The most polar solvent is run over the shortest developing distance, the most non-polar over the longest. The combination of multiple and gradient development leads to a focusing effect of the zones and peak sharpness is improved. Reproducibility of the migration distance is largely independent of the sample matrix.

Trial

The transferability of gradients by these calculations was tested for three gradients. For the experiments, pesticides were selected that cover the entire range of migration distances. 500 ng of each pesticide was applied onto HPTLC plates and developed with different gradients, each in both AMD systems. Detection was performed via multi-wavelength scan from 200 to 300 nm.

The migration distances obtained in both AMD systems were used to evaluate the test series by plotting and comparing the migration distances of the individual analytes in a diagram. Upon good conformity, the migration distances obtained for the individual analytes with the two gradients should be the same. In this case, the calculated regression curve should have a slope of 1 and a correlation better than 0,99.

The figure below shows an example for the comparison of the migration distances for selected pesticides obtained with a gradient of the AMD1 and AMD2 system. Assuming linear relationship the equation of the curve and the correlation were calculated and plotted in excel. In addition a trend line was calculated under the assumption of a polynomial relationship.



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▲ *Comparison of migration distances of selected analytes following development with a 30 step gradient on the basis of dichloromethane in the AMD1 and AMD2 system*

Results

The conversion of AMD1 gradients into AMD2 gradients with the presented mathematical system is feasible. Significant differences in migration distances only occur if the drying times in the existing AMD1 gradient are not sufficient, that means too short.

Through additional experiments (not presented here) it was shown that drying of HPTLC plates is improved in the AMD 2 system compared to the AMD1 system. This difference in drying may be seen as an explanation for possible deviations in the migration distances in both systems.

Further information and the Excel-calculations mentioned above are available on request from CAMAG or Dr. Wippo.

* Dr. Ursula Wippo, Federal Institute for Drugs and Medical Devices (BfArM), Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, Germany, Phone +49-(0)228-2074182, wippo@bfarm.de

Quality control of *Stephania tetrandra*

19 *Anne Blatter*

20 *Dr. Eike Reich*

The following article is a summary of a paper published in the Journal of Liquid Chromatography & Related Technologies by Anne Blatter and Eike Reich. You surely know the two experts in the field of herbal drugs. Ms. Blatter was already introduced in CBS 88 and joined the CAMAG laboratory after her diploma 2001. Dr. Reich is head of the laboratory since 1998.

Scope

While Traditional Chinese Medicine (TCM) is gaining more and more acceptance also in Western countries, safety concerns with some plant species have caused authorities worldwide to look into analytical methods for proper identification and quality control of botanicals including *Stephania tetrandra*. Two cost efficient, reliable and rapid HPTLC methods are presented, which may be used by industry and regulatory agencies in a meaningful quality control of Stephania. HPTLC offers qualitative and quantitative results using the same chromatographic system:

1. A specific fingerprint of the plant focusing on the main alkaloid tetrandrine as chemical reference to ensures identity of the plant material.
2. Quantitative determination of the marker tetrandrine establishes whether a given batch meets established acceptance criteria.

Sample preparation

1. For qualitative identification 200 mg powdered drug are sonicated with 10 mL methanol – water – formic acid 40:9:1 for 10 min, then centrifuged. As reference 1 mg tetrandrine is dissolved in 1 mL methanol.
2. For quantitative determination 50 mg of powdered drug are extracted in a soxhlet extractor for 2 h with 50 mL methanol containing 5% concentrated ammonia. After cooling to room temperature the volume of the extract is adjusted to 50 mL with methanol. As calibration standard a solution containing 0.025 mg/mL tetrandrine in methanol is used.

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm

Sample application

With Automatic TLC Sampler 4, max. 18 tracks, band length 8 mm, application of varying amounts of test and standard solutions, distance from lower edge 8 mm, distance from the side at least 15 mm, track distance at least 10 mm.

Chromatography

Twin-Trough Chamber saturated for 30 min (filter paper soaked with 10 mL developing solvent) with toluene – ethyl acetate – methanol – ammonia 25% 10:10:50:3, migration distance 70 mm from lower edge

Documentation and derivatization

DigiStore using UV 254 nm, and UV 366 nm prior to derivatization. Following derivatization with iodine (0.05 g in 10 mL ethanol 96%) documentation is performed under white light.

Densitometric evaluation

TLC Scanner 3 with winCATS Software, absorption measurement at 210 nm, calibration with linear regression by peak height

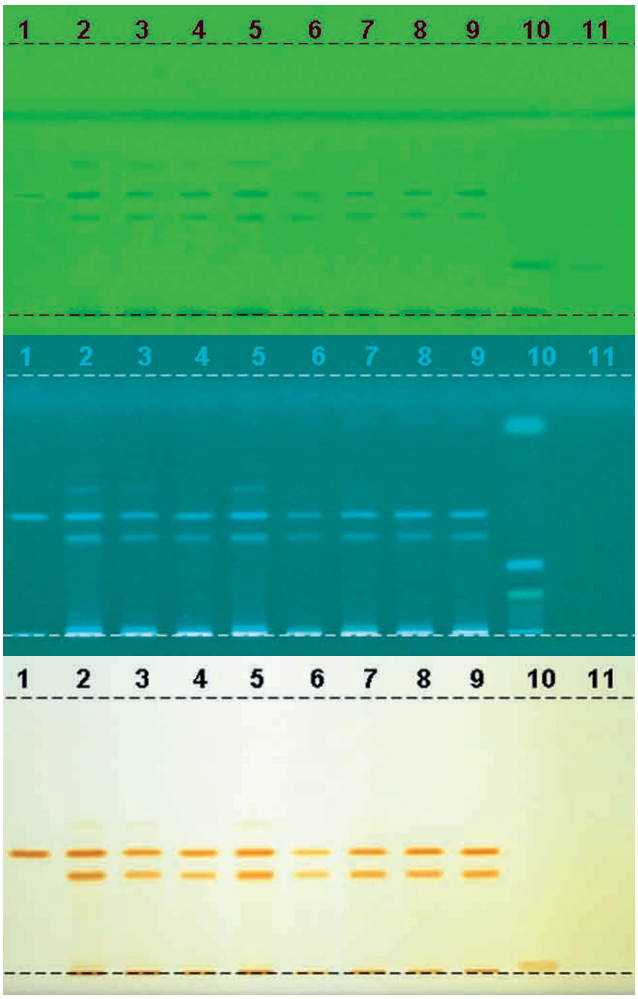
Results and discussion

1. Identification of *Stephania tetrandra*

The proposed method allows quick, convenient and specific identification of *Stephania tetrandra* based on the main alkaloid tetrandrine (hR_F ~ 50). Tetrandrine is well separated from two other alkaloids, which can be detected just above and below it at hR_F ~ 60 respectively hR_F ~35. The compound at higher R_F is only present in low amounts. The alkaloids absorb UV light, fluoresce under UV 366 nm and react with iodine to derivatives visible under white light as yellowish zones.

2. Quantitation of tetrandrine in *Stephania tetrandra*

The mobile phase optimized for identification purpose achieves baseline resolution for tetrandrine, therefore the same system is used for quantitative measurements. One of the principal requirements of any quantitative analysis is its specificity, which ensures that no component of the samples interferes with detection of the target compound. Specificity of the proposed method was established by comparison of the UV spectra of the reference substance and that of the corresponding zone in the extract.



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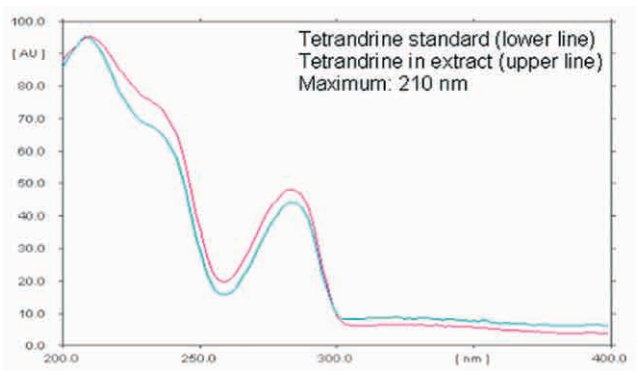
▲ *Identification of* Stephania tetrandra *root: Documentation under UV 254, 366 nm, and after derivatization with iodine under white light.*

Track 1: tetrandrine, track 2–9: different Stephania tetrandra *root samples, track 10: Aristolochia fangji root (common toxic adulterant), track 11: aristolochic acids mixture, toxic marker of adulteration (only a faint band is seen under UV 254 nm at hR_F ~20).*



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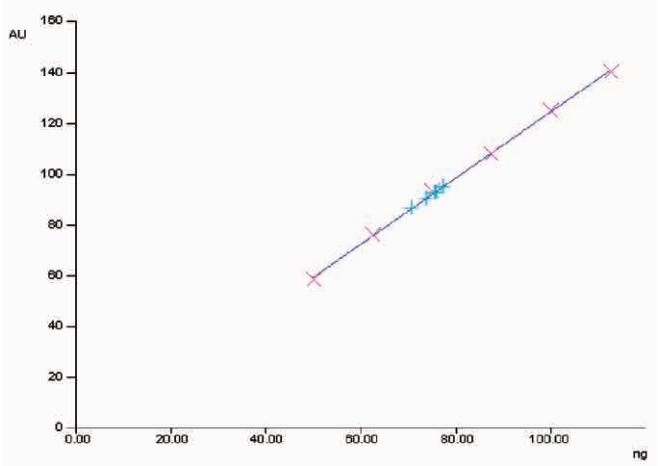
▲ *Comparison of UV spectra of standard and sample*

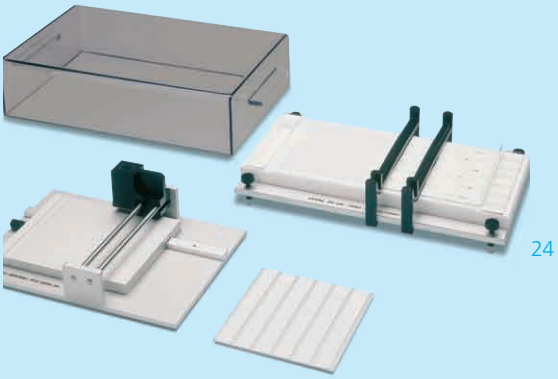
Linear regression was used to fit a set of 6 calibration points. Linearity was established in the range from 50 to 112.5 ng. The relative standard deviation (RSD) for 6 sample replicates in the middle of the working range (75 ng) is 0.45%. At the lower and upper limits of the calibration function, the RSD are higher, i.e. 2.4% and 5.2% respectively.

The repeatability measuring 6 replicates of the same sample on one plate varies between 1.6% and 3.7%, while repeatability of the means from 3 different plates (6 measurements each) is 1.2%.



23

▲ *Calibration function for tetrandrine measured at 210 nm (peak height, r = 0.9997, sdv 0.84%)*



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HPTLC Vario System

The HPTLC Vario Chamber is unsurpassed in an economic point of view and in flexibility for optimization of development conditions. It is well-suited for efficient method development.

A great advantage of the HPTLC Vario Chamber is the possibility of testing six different solvents side by side on a 10 × 10 cm plate in one run, making solvent selection during method development very convenient. Another advantage is the low consumption of solvent (1 mL each), i. e. 90% of solvent are saved compared to a flat bottom chamber.

The developing conditions in tank and sandwich configuration can be simulated side by side, making results directly comparable. Further on six different conditions of pre-equilibration of the layer can be tested. For example the influence of up to six different relative humidities of the ambient air can be compared side by side.

Literature

Monograph Radix *Stephaniae Tetrandrae* (Fangji), Pharmacopoeia of the People's Republic of China, English Edition, Chemical Industry Press, Beijing, 2000, Vol. I.

Please contact CAMAG for a reprint of the article published in the Journal of Liquid Chromatography & Related Technologies

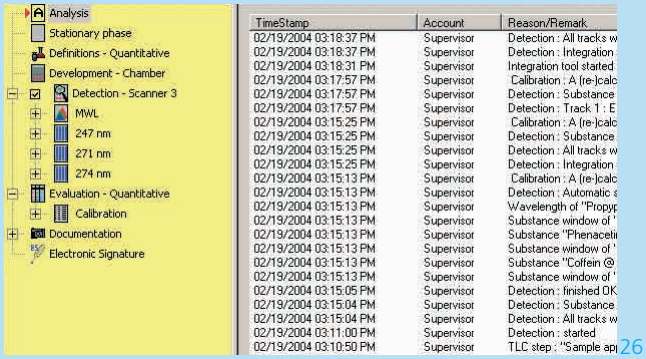
New in

winCATS

Planar Chromatography Manager

winCATS supports 21 CFR part 11

Beginning with Version 1.3.0 the CAMAG software winCATS – Planar Chromatography Manager supports regulation 21 CFR part 11 "Electronic Records, Electronic Signatures" of the US FDA (Food and Drug Administration). For a growing number of TLC users regulation 21 CFR part 11 is binding. They need the program option "21 CFR Part 11 compliance ready".



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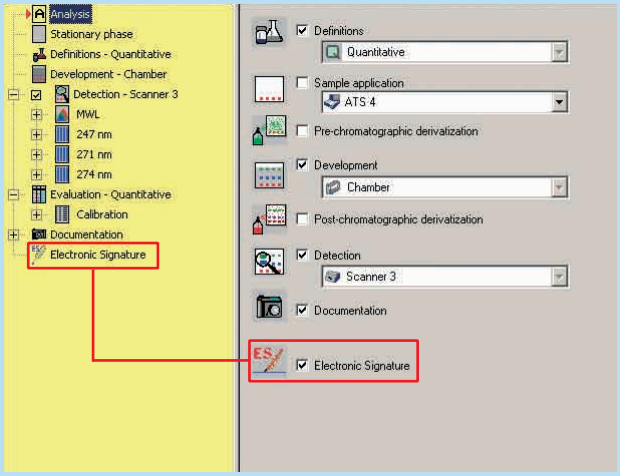
Audit Log: each change of a file is documented with date, time and user



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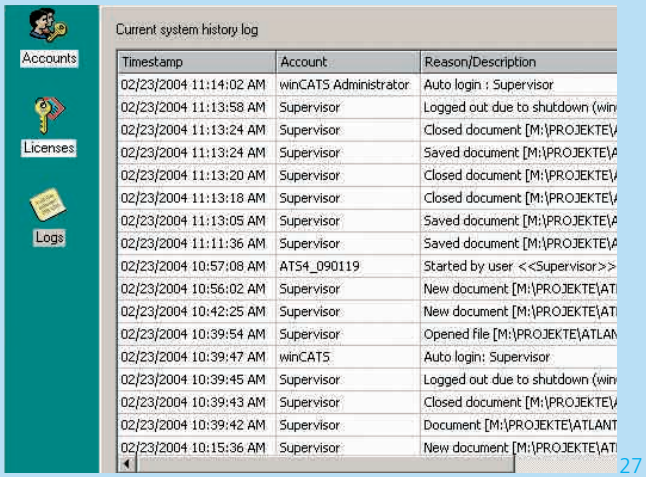
TLC Steps: the electronic signature is activated and appears in the current flow chart of the analysis



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System Log: daily a file is generated, which records all system processes

Access management, tracking of changes and storage of data are central elements of the regulation. The winCATS program option "21 CFR Part 11 compliance ready" allows:

- Acquisition and handling of data through password protected user identification
- Safe storage of all results including raw data ensuring full integrity and traceability of data
- Documentation of all activities in the "System/ Audit Log" for traceability of electronic signatures.