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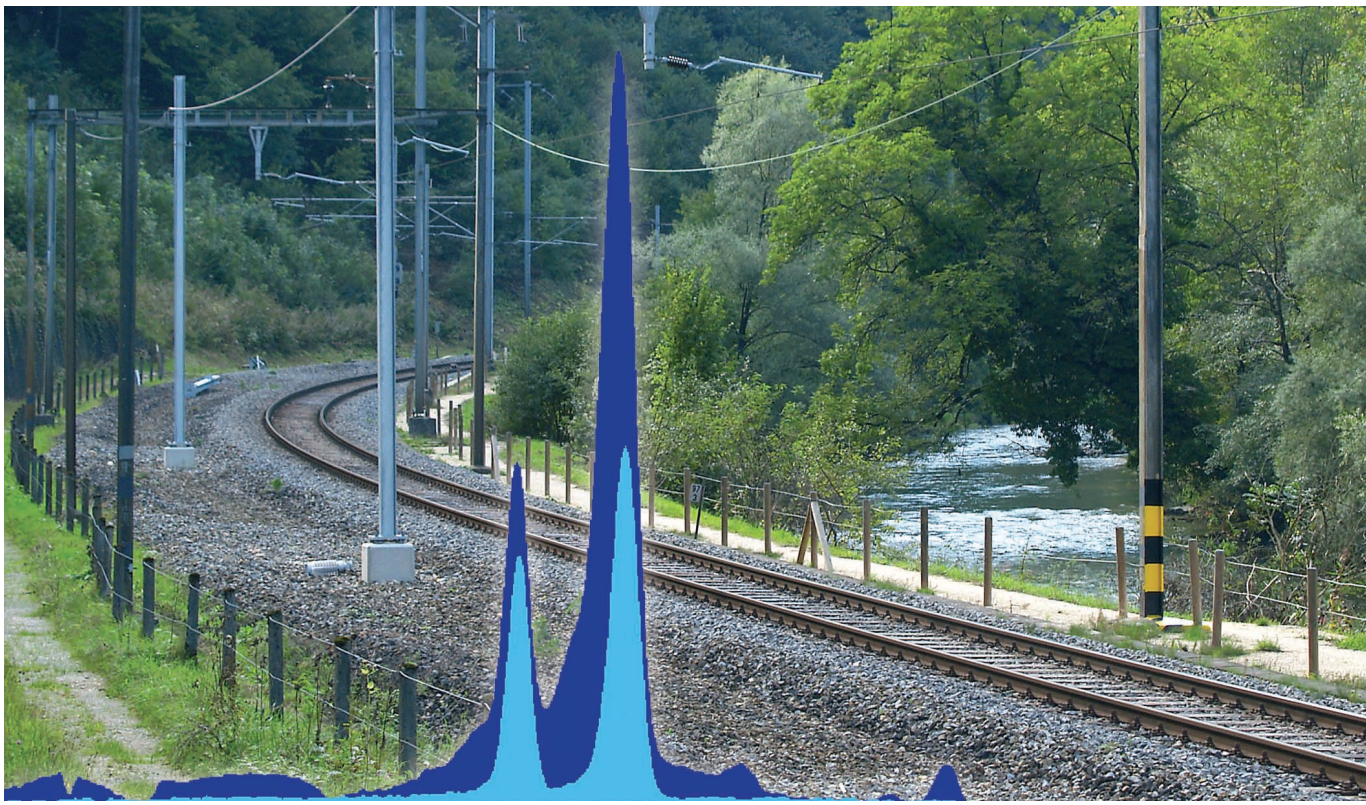
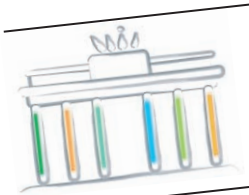


Planar-Chromatographie – eine vorteilhafte Alternative zur HPLC

(siehe Seite 2–7)



INTERNATIONAL SYMPOSIUM ON
PLANAR CHROMATOGRAPHY –
INSTRUMENTAL THIN-LAYER CHROMATOGRAPHY

Berlin (Germany), 9–11 October 2006

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95

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IN DIESER AUSGABE

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Bestimmung von Histamin und weiteren mittels Dünnschicht-Chromatographie

▲ *LUA Standort Dresden (von links nach rechts): Sven Kretzschmar, Sibylle Neugebauer, Dr. Dieter Hübner*

▲ *Prof. Dr. Karl Speer*

Bereits im CBS 83 (September 1999) haben wir über die quantitative Bestimmung von Histamin mittels Planar-Chromatographie im Institut für Veterinär-Pharmakologie und Toxikologie (IVPT), Bernau berichtet. Damals wurde eine Trennung auf RP-18-Phasen durchgeführt und postchromatographisch detektiert mit Pauly´s Reagenz.

Die nachfolgende Methode zum Screening von 7 biogenen Aminen in Fisch wurde an der Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen (LUA), Fachbereich Lebensmittelchemie in Dresden entwickelt und mit anderen analytischen Verfahren verglichen. Die Basis für die verwendete prächromatgraphische Derivatisierung mit Dansylchlorid lieferte eine Veröffentlichung des Food Technology and Dairy Science Department in Dokki, Ägypten [1]. Betreut wurde Herr Kretzschmar bei der Anfertigung seiner Diplomarbeit [2] seitens des Instituts für Lebensmittelchemie der Technischen Universität Dresden durch Herrn Professor Speer*.

Einleitung

Bei unsachgemässer Lagerung werden in Fischen und Fischprodukten verstärkt biogene Amine, besonders Histamin, gebildet. Zum Schutze der Verbraucher vor Vergiftungen mit biogenen Aminen sind in §16 der Fischhygieneverordnung für bestimmte Fischarten Grenzwerte festgesetzt worden, allerdings bislang nur für den Gehalt an Histamin.

Da auch andere biogene Amine physiologisch wirksam sind, sollten auch diese analytisch erfasst werden. Hierzu wurde eine Screeningmethode entwickelt, die kostengünstig ist und die es ermöglicht, eine hohe Probenzahl in kurzer Zeit zu analysieren. Zur Überprüfung des für Histamin vorgegebenen Grenzwertes ist die vorgestellte DC-Methode sehr gut geeignet und sie liefert vergleichbare Ergebnisse zur ebenfalls eingesetzten HPLC-, ELISA- und fluorimetrischen Methode bei erheblich geringerem Analysenaufwand.

Aus der Probenmatrix wird zunächst mit 10%iger Trichloressigsäure ein Extrakt hergestellt, der alkalisiert und mit Dansylchlorid versetzt wird. Aus diesem Extrakt werden die derivatisierten Amine ex-

biogenen Aminen in Fisch

trahiert, auf einer Kieselgelschicht getrennt und entweder visuell (halbquantitativ) oder densitometrisch bei UV 365/>400 nm quantitativ ausgewertet.

Probenvorbereitung

10 g der homogenisierten Probe werden mit 80 mL Trichloressigsäure (10%ig) versetzt und 90 s bei 9000 U/min homogenisiert. Nachdem mit TCA auf 100 mL aufgefüllt und filtriert wurde, kann das Filtrat direkt für die Derivatisierung eingesetzt oder bei –20 °C gelagert werden.

Prächromatographische Derivatisierung

Zur prächromatographischen Derivatisierung wird 1 mL Filtrat tropfenweise mit 4N NaOH auf pH 8 eingestellt. Nach Zugabe von 1 mL Borat-Puffer und 2 mL Dansylchloridlösung (0,5% in Aceton) wird die Lösung 30 s geschüttelt und 1 h bei 40 °C im Wasserbad inkubiert. Anschliessend wird mit Wasser auf 10 mL aufgefüllt, nach Zugabe von 5 mL Diethylether kräftig geschüttelt und die Lösung zentrifugiert. Die organische Phase wird abgenommen und das Ausschütteln mit Diethylether noch zweimal wiederholt. Nach Vereinigen und Einengen der organischen Phasen wird der Rückstand in 5 mL Acetonitril aufgenommen. Je 1 mL der Standardlösungen wird ebenso derivatisiert.

Standardlösungen

Die Hydrochloride der biogenen Amine Putrescin, Cadaverin, Spermidin, Spermin, Histamin, Tyramin und β-Phenylethylamin (0,5 mg/mL in Wasser) sind im Kühlschrank 2 Wochen haltbar, tiefgekühlt mindestens 6 Monate. 5 mL jeder Stammlösung werden mit TCA auf 100 mL verdünnt (25 µg/mL). Standardlösungen zur Kalibration, die ebenfalls prächromatographisch derivatisiert werden, sind jeweils frisch herzustellen, indem 200, 400, 600 und 800 µL jeweils mit TCA auf 1 mL aufgefüllt werden.

Schicht

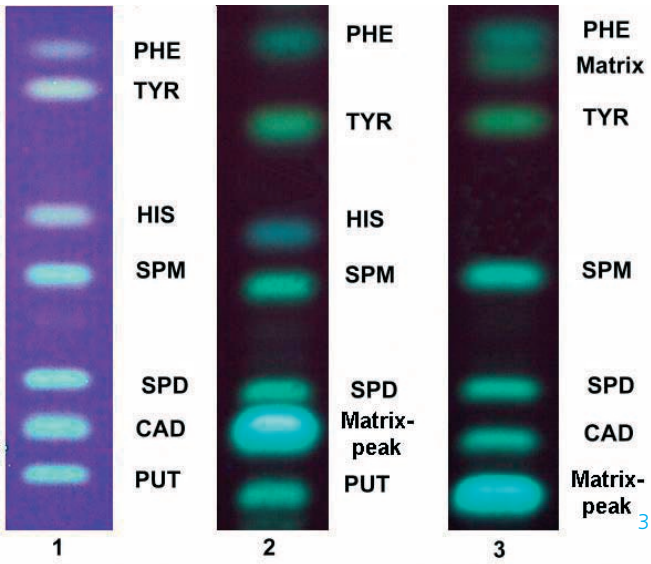
DC-Platten Kieselgel 60 (Merck), 20 × 10 cm, Schichtdicke 0,25 mm, vorgewaschen mit dem Fliessmittel (durch Chromatographie).

Probenauftragung

Bandförmig mit DC-Probenautomat, 15 Bahnen, Auftragevolumen 10 µL, Bandlänge 6 mm, unterer Randabstand 8 mm, seitlicher Randabstand mind. 15 mm, Bahnabstand 12 mm

Chromatographie

In der Horizontalentwicklungskammer mit Benzen – Chloroform – Triethylamin 10:6:7; Laufstrecke vom unteren Plattenrand 90 mm. Bei einigen Fischproben wird Cadaverin von einem Matrixpeak überlagert. Durch Änderung der Fliessmittelzusammensetzung in 10:6:2 lässt sich Cadaverin jedoch ebenfalls bestimmen.



▲ *Chromatogramme ausgewählter Bahnen unter UV 365/>400 nm; Bahn 1: Standardgemisch der 7 biogenen Amine β-Phenylethylamin, Tyramin, Histamin, Spermin, Spermidin, Cadaverin und Putrescin; Bahn 2: unbelastete Fischprobe dotiert mit Standardgemisch; Bahn 3: wie Bahn 2, nur entwickelt mit der Fliessmittelzusammensetzung 10:6:2*

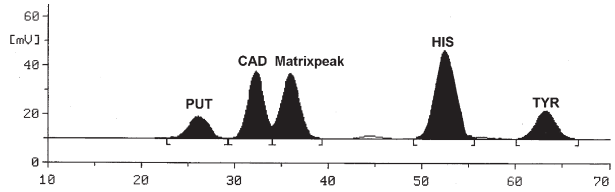
Densitometrische Auswertung

Im UV-Cabinet bei UV 365 nm sind aufgetragene Substanzmengen von 10 ng noch gut zu erkennen. Die quantitative Auswertung erfolgt im TLC-Scanner mittels Fluoreszenzmessung bei UV 365/>400 nm, die lineare Kalibration über die Peakfläche.

Ergebnisse und Diskussion

Mit der entwickelten Methode können Histamingehalte zwischen 50–250 mg/kg bestimmt werden. Bei höherem Gehalt ist die Probeneinwaage zu reduzieren oder der Extrakt zu verdünnen. Nachfolgend Validierungsdaten für Histamin:

Linearität	50–250 mg/kg
NG $_{\text{DIN 32645}}$	17,5 mg/kg
BG $_{\text{DIN 32645}}$	56 mg/kg
Wiederfindung (200 mg/kg)	108 %
Vertrauensbereich	± 9,9 mg



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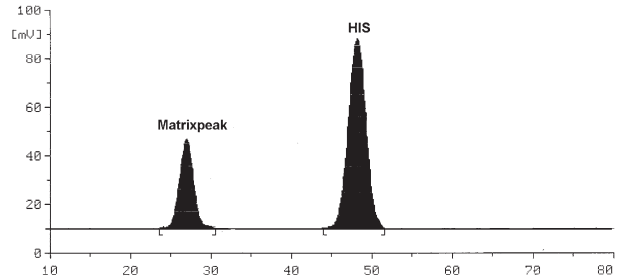
▲ *Densitogramm der Fischprobe »Makrele geräuchert« bei UV 365/>400 nm*



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▲ *Densitogramm einer Thunfischprobe in Öl (1:10 verdünnt) bei UV 365/>400 nm*

Die beiden Fischproben wurden sowohl mit der entwickelten DC-Screening-Methode als auch mit einer HPLC-Methode sowie mit einem ELISA- und einem fluorimetrischen Verfahren der LUA Sachsen vergleichend analysiert. Die Ergebnisse sind in der Tabelle gegenübergestellt. Deutlich wird, dass zur Überprüfung des für Histamin vorgegebenen Grenzwertes die vorgestellte DC-Methode sehr gut geeignet ist: sie liefert vergleichbare Ergebnisse bei erheblich geringerem Analysenaufwand.

Gehalte an biogenen Aminen in den Proben Makrele und Thunfisch, analysiert mit verschiedenen Methoden

Methode	Probe	Histamin [mg/kg]	Putrescin [mg/kg]	Cadaverin [mg/kg]	Spermin [mg/kg]	Spermidin [mg/kg]	Tyramin [mg/kg]
DC	Makr.	180	(20)	60	nn	nn	(30)
	Thun.	2805	nn	nn	nn	nn	nn
HPLC	Makr.	175	10	70	<5	<5	40
	Thun.	2540	25	75	<5	<5	35
ELISA (Histamin)	Makr.	250					
	Thun.	2895					
Fluori-metrie	Makr.	170					
	Thun.	3100					

nn = nicht nachweisbar; Gehalte in Klammern liegen unterhalb der Bestimmungsgrenze

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

[1] A. R. SHALABY, Food Chemistry 65 (1999) 117-121.

[2] S. Kretzschmar: Entwicklung einer dünnschicht-chromatographischen Screening-Methode für Histamin und weitere biogene Amine in Fisch. Diplomarbeit, TU Dresden 2004.

* Prof. Dr. Karl Speer, Institut für Lebensmittelchemie, TU Dresden, Bergstr. 66, D-01062 Dresden, Tel. +49-351-463-33603, Karl.Speer@chemie.tu-dresden.de

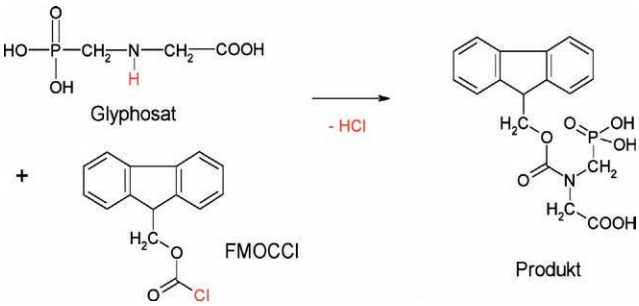
Ultraspurenanalytik von Glyphosat und AMPA in Wasser mittels HPTLC

▲ *Dr. Walter Weber**

▲ *Wolfram Seitz und Anna Aichinger*

Im Betriebs- und Forschungslaboratorium des Zweckverbandes Landeswasserversorgung (LW) unter der Leitung von Dr. Weber werden neben der chemischen, physikalisch-chemischen und mikrobiologischen Überwachung der Trinkwassergewinnung und -verteilung an über drei Millionen Verbraucher umfangreiche analytische und wasserchemische Forschungsvorhaben bearbeitet.

Neben GC/MS- und HPLC/MS-Verfahren wird auch die moderne Planar-Chromatographie eingesetzt. In der letzten Ausgabe des CBS wurde bereits die luminographische Toxizitätsdetektion mit Vibrio fischeri (Leuchtbakterien) vorgestellt. Die Analytik des weit verbreiteten Totalherbizides Glyphosat und dessen Abbauprodukt AMPA werden ebenfalls mittels HPTLC durchgeführt, da sich diese Methode im Labor der LW gegenüber der HPLC-Bestimmung als robuster und bei Vergleichs- und Ringversuchen als überaus geeignet erwiesen hat.



▲ *Derivatisierung von Glyphosat mit FMOCCl (9-Fluorenylmethyl-chlorformiat)*

Einleitung

Glyphosat (N-(Phosphonomethyl)glycin) ist eines der am häufigsten eingesetzten Totalherbizide weltweit. Es findet seit über 30 Jahren Anwendung als systemisch wirkendes Pflanzenschutzmittel gegen Wildkräuter/-gräser, z.B. bei der Vegetationskontrolle im Schienenverkehr. Das Hauptabbauprodukt von Glyphosat ist Aminomethylphosphonsäure (AMPA), wobei dieses auch durch den Abbau verschiedener Phosphonsäuren entstehen kann.

In der Trinkwasserverordnung (TrinkwV) wurde für Pflanzenbehandlungs- und Schädlingsbekämpfungsmittel ein Grenzwert von 0,1 µg/L für den Einzelstoff bzw. 0,5 µg/L in der Summe festgelegt. Eine sichere Bestimmung von Glyphosat ist deshalb unabdingbar. Bei der Untersuchung von Oberflächenwasser wurde Glyphosat gelegentlich bis in den µg/L-Bereich detektiert. Für die Substanz AMPA konnte z.B. in der Ruhr eine Grundbelastung von 0,73 µg/L festgestellt werden [1].

Die Analyse im ng/L-Bereich in Wasser erfordert eine Anreicherung, die durch die hohe Polarität der Substanzen erschwert wird. Ausserdem macht das Fehlen von analytisch wichtigen Molekülgruppen wie Chromo- und Fluorophoren eine Derivatisierung zur Detektion notwendig.

Bisher wurden analytische Verfahren mittels HPLC und Vor- bzw. Nachsäulenderivatisierung sowie mittels Gaschromatographie beschrieben. Dabei wird zur Trennung aufgrund des ionischen Charakters der Substanzen die Flüssigchromatographie vorgezogen. Meist werden die Analyten mittels eines Kationenaustauschers aus dem Wasser extrahiert und an einem Anionenaustauscher gereinigt. Nach der Trennung erfolgt eine spezielle Derivatisierung mit anschliessender Fluoreszenz-Detektion.

Bei der beschriebenen Methode zur Bestimmung von Glyphosat und dessen Abbauprodukt AMPA mittels Planar-Chromatographie werden die Analyten in Wasser derivatisiert, um sie einer Flüssig-flüssig-Extraktion und der Fluoreszenz-Detektion zugänglich zu machen [2]. Durch die erfolgreiche Teilnahme an einem Ringversuch der AQS-BW, bei dem unterschiedliche Methoden

zur Bestimmung von Glyphosat eingesetzt wurden, konnte die Leistungsfähigkeit der planar-chromatographischen Methode bereits unter Beweis gestellt werden. Das Herbizid Glufosinat lässt sich ebenfalls auf diese Weise nachweisen [3].

Probenvorbereitung

50 mL Wasserprobe werden mit 8 mL Boratpuffer (pH = 9) und 50 mL FMOCCl-Reagenz (16 mg/100 mL Aceton) versetzt. Nach 30 min Reaktionszeit wird die Mischung dreimal mit je 30 mL Dichlormethan – 2-Propanol (3:1 v/v) ausgeschüttelt, wobei die organische Phase verworfen wird. Die Analyten werden nach dem Ansäuern mit Schwefelsäure zweimal mit je 30 mL Dichlormethan – 2-Propanol (3:1 v/v) extrahiert. Das Extrakt wird im Rotationsverdampfer eingeengt und in Methanol gelöst.

Schicht

HPTLC-Platte Kieselgel 60 F_{254} (Merck) 20 × 10 cm, Schichtdicke 0,1 mm, vorgewaschen in 2-Propanol (Eintauchen über 24 h) und getrocknet bei 100 °C für 30 min auf dem DC-Plattenheizer III im Stickstoffstrom.

Probenauftragung

Bandförmig mit DC-Probenautomat 4, 18 Bahnen, Auftragevolumen 80 µL, Bandlänge 5 mm, unterer Randabstand 12 mm, seitlicher Randabstand mind. 15 mm, Bahnabstand 10 mm

Chromatographie

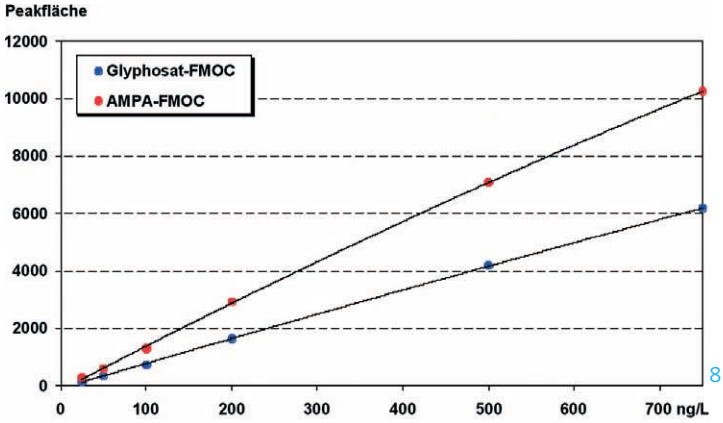
In der Doppeltrogkammer mit n-Butanol – Wasser – Eisessig 5:4:1. Das Fliessmittel wird nach Phasentrennung aus der organischen Phase abgenommen. Laufstrecke vom unteren Plattenrand 70 mm

Densitometrische Auswertung

TLC-Scanner 3 mit winCATS Software, Fluoreszenzmessung mit der Deuteriumlampe bei UV 268/Sekundärfilter M 360 nm, lineare Kalibration über die Peakfläche

Ergebnisse

Das Verfahren erwies sich als sehr linear im getesteten Konzentrationsbereich ($r^2 > 0{,}999$). Die Bestimmungsgrenze wird auf 50 ng/L über das Gesamtverfahren einschliesslich Flüssig-flüssig-Extraktion festgelegt.



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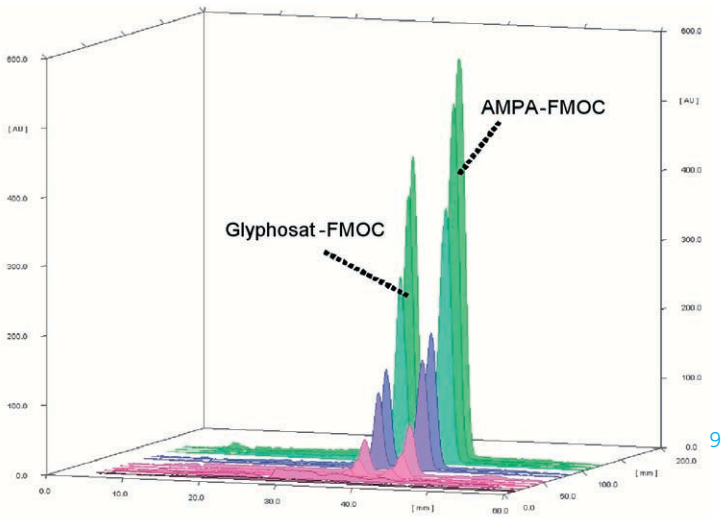
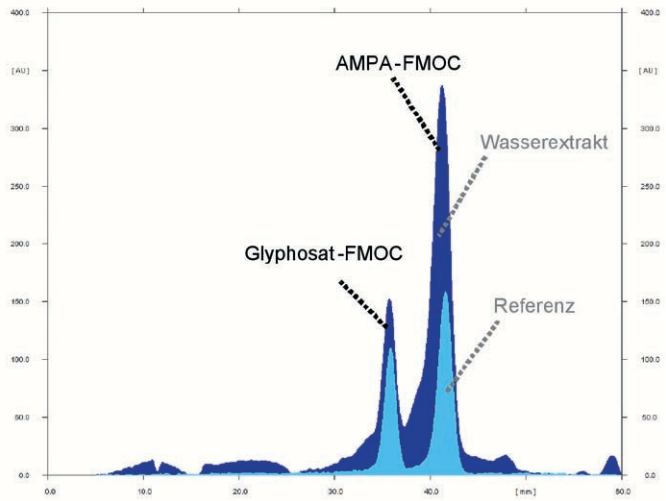
▲ *Lineare Regression von Glyphosat-FMOC und AMPA-FMOC nach Flüssig-flüssig-Extraktion aus Wasser und HPTLC-Analyse mit Fluoreszenzdetektion ($r^2 > 0{,}999$)*



9

▲ *Fluoreszenzauswertung bei UV 268/M 360 nm eines HPTLC-Chromatogramms von je 25 bis 750 ng/L Glyphosat-FMOC (36 mm) und AMPA-FMOC (42 mm) berechnet als Glyphosat und AMPA*

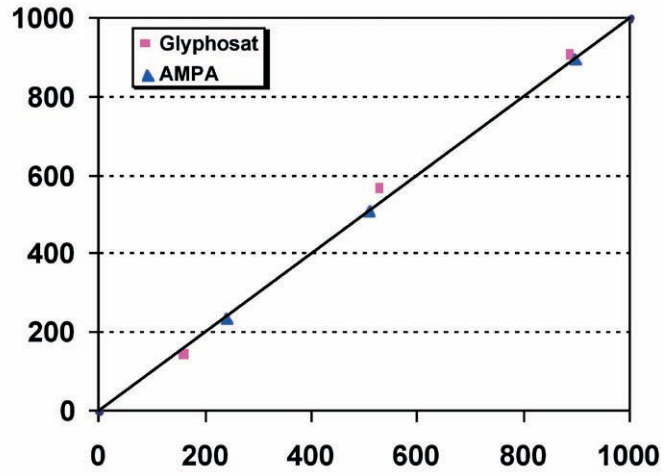
Untersuchungen von realen Proben ergaben, dass eine ausreichend hohe Selektivität zur Bestimmung der Analyten vorliegt. Die HPTLC stellt somit eine gute Alternative zur HPLC bei der Ultraspurenanalytik von Glyphosat und AMPA in Wasser dar.



10

▲ *Vergleich der Analyse eines Wasserextrakts auf Glyphosat-FMOC und AMPA-FMOC mit einer Standardbahn von 200 ng/L*

Die Messwerte von Glyphosat und AMPA zeigen im Ringversuch AQS-BW 2005 eine sehr gute Korrelation ($r^2 = 0{,}986$) mit den Vorgabewerten. Der Z_u-score liegt für die drei Konzentrationsniveaus zwischen 0,05 und 0,55.



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▲ *Korrelation der Vorgabewerte mit den Messwerten HPTLC (RV AQS-BW 2005)*

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

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[1] R. Reupert, C. Schlett, gwf Wasser Abwasser 138 (1997) 559-563.

[2] R. Gauch, U. Leuenberger, U. Müller, Z. Lebensm. Unters. Forsch. 188 (1989) 36-38.

[3] Unveröffentlichte Untersuchungsergebnisse des Zweckverbandes Landeswasserversorgung

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CAMAG TLC-Scanner 3

Dank moderner Densitometrie ist die Planar-Chromatographie im Methodenvergleich nicht weniger leistungsfähig, wie die erfolgreiche Teilnahme des Betriebs- und Forschungslaboratoriums des Zweckverbandes Landeswasserversorgung am Ringversuch belegt. Der CAMAG TLC-Scanner 3 wird zur bestmöglichen Genauigkeit der quantitativen Auswertung eingesetzt. Die Verwendung der optimalen Messwellenlänge ist unverzichtbar, wenn spektrale Selektivität und Empfindlichkeit gefordert werden (siehe auch Mittelteil, Parameter der Planar-Chromatographie).

Das Betriebs- und Forschungslaboratorium des Zweckverbandes Landeswasserversorgung setzt zur Fluoreszenzmessung im Anregungs-/Emissionsoptimum der FMOC-Derivate die Deuteriumlampe bei 268 nm mit einem Monochromatfilter bei 360 nm zum Ausfiltern der Anregungswellenlänge ein. Dr. Hegewald, Leiter von Lacrome in Evora (S. 9), zieht jedoch die Quecksilberlampe bei UV 265 nm vor, welche eine etwas empfindlichere Detektion aufgrund der höheren Strahlungsintensität der Quecksilberlampe erlaubt.

CAMAG
Summer Meeting 2005

13

In der Woche vom 22. bis 28. August trafen sich die Geschäftsführer der fünf umsatzstärksten Ländervertretungen wie jedes Jahr bei CAMAG in Muttenz zum Erfahrungsaustausch. Im Zentrum standen dabei die Wünsche unserer Kunden, welche wir in neue Produktideen und Marktstrategien umsetzen wollen.

14

Dilip Charegaonkar von Anchrom, unserem Partner in Indien, wurde für seinen ausserordentlich guten Einsatz im Kampf um eine Ausschreibung über 11 komplette Systeme mit dem erstmals verliehenen »Distributor of the Year« Preis ausgezeichnet.

15

Ein besonderes Highlight war die Besichtigung der Firma Hakama, ein führender Schweizer Feinblechverarbeiter und seit 1979 Lieferant von Gehäuseteilen für CAMAG-Geräte. Wir konnten dort zum ersten Mal life verfolgen, wie Bleche zu anspruchsvollen Produkten, z.B. zur Haube vom CAMAG TLC-Scanner 3, veredelt werden.

16

Den entspannten Teil des Summer Meetings verbrachten wir in Gruyère, einem malerischen und geschichtsträchtigen kleinen Ort, inmitten der für ihre Käseproduktion weltweit bekannten Region. Neben dem Besuch einer Alpkäserei (traditionell – nicht GMP-konform) gehörte die Besichtigung des Schlosses von Gruyère zum Programm an diesem Wochenende. Bei einer längeren Wanderung konnten wir die Gegend zwischen Les Rosalys, Dent de Lys und Le Moleson geniessen und dabei den Teamgeist fördern.

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Liebe Freunde

Die Planar-Chromatographie wird weit verbreitet als Screening-Methode eingesetzt. Nicht so oft wird sie in einem Methodenvergleich anderen Verfahren gegenübergestellt. Ein Methodenvergleich ist aufwendig und oft sind in einem Labor nur begrenzt Möglichkeiten für eine alternative Analytik vorhanden.

Aus diesem Grund heben wir Anwendungen in diesem Kontext hervor. Zum Beispiel hat die Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Fachbereich Lebensmittelchemie in Dresden, in Zusammenarbeit mit dem Institut für Lebensmittelchemie der TU Dresden einen Methodenvergleich zur Bestimmung von Histamin in Fisch durchgeführt (S. 2–4).

Die planar-chromatographische Ultraspurenanalytik von Glyphosat und AMPA in Wasser, die im Betriebs- und Forschungslaboratorium des Zweckverbandes Landeswasserversorgung in Langenau durchgeführt wird, konnte ihre Leistungsfähigkeit in einem Ringversuch der AQS-BW unter Beweis stellen (S. 5–7). Interessant hierzu ist auch die Bestimmungsvariante auf Seite 9.

Ein reger Austausch in der Planar-Chromatographie fand auch dieses Jahr statt, sei es auf dem »International Symposium on Planar Chromatography« in Siofok, Ungarn, oder bei den halbjährlichen Treffen des französischen Clubs (vorgestellt im CBS 90). Informieren möchte ich Sie schon heute über das nächste Symposium zur Planar-Chromatographie 2006 in Berlin (siehe letzte gelbe Seite).

Herzlichst Ihre

Gerda Morlock

Gerda Morlock

Dear friends

Planar chromatography is widely used as a screening method, however, not so often confronted with a comparison to other methods. A method comparison is time-consuming and many laboratories have only limited possibilities for alternative confirmation of the results obtained.

For this reason, we lay emphasize on applications in such a context. For example the Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Saxony, Department of Food Chemistry, Dresden, in cooperation with the Institute of Food Chemistry, TU Dresden, conducted a method comparison for the determination of histamine in fish (p. 2-4).

Planar chromatographic ultra-trace analysis of glyphosate and AMPA in water performed in the operations and research laboratory of the Landeswasserversorgung in Langenau assured its competence in an interlaboratory trial of the AQS-BW (p. 5-7). Of interest might also be the alternative derivatization technique on page 9.

Active exchange of ideas in planar chromatographic research was also fruitful this year, either on the International Symposium on Planar Chromatography in Siofok, Hungary, or at the semi-annual meetings of the French Club CCCM (introduced in CBS 90). Thus, I would like to focus your interest on the next International Symposium for Planar Chromatography/ Instrumental HPTLC 2006 in Berlin (see last yellow page).

Sincerely,

Gerda Morlock

Gerda Morlock

CAMAG

SEPTEMBER 2005 95

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

95 001 J. QU (Qu Jianbo)*, H. LOU (Lou Hongxiang), P. FAN (Fan Peihong) (*School Pharm., Shandong Univ., Jinan 250012, China): (Application of TLC-bioautography in drug screening) (Chinese). J. Chinese Trad. and Herb. drugs 36 (1), 132-137 (2005). A review with 22 references on TLC-bioautography, including the screening of natural compounds with antibacterial and/or antifungal activity, cholinesterase inhibitors, free radical eliminators, and antioxidants. Discussion of the advantages of the technique compared to other related techniques.

Traditional medicine, pharmaceutical research, herbal, qualitative identification, autoradiography, review, TLC-bioautography, antibacterial activity, antifungal activity, cholinesterase inhibition, free radical, antioxidation 1, 32

2. Fundamentals, theory and general

95 002 M. FILIP, Virginia COMAN*, R. GRECU, K. Albert, Z. MOLDOVAN (*`Raluca Ripan' Institute for Research in Chemistry, 30 Fantanele Street, P. O. Box 702, RO-400294 Cluj-Napoca, Romania): Characterization of some chemically modified acidic alumina T samples for TLC. J. Planar Chromatogr. 17, 424-430 (2004). Chemically modified acidic alumina T stationary phases have been prepared by organosilylation with the trifunctional organosilicon compounds n-octadecyltrichlorosilane, 3-mercaptopropyltrimethylsilane, and N-(2-aminomethyl)-3-aminopropyltrimethoxysilane. These chemically modified phases were characterized by elemental analysis, measurement of specific surface area, FTIR spectroscopy, 13C CP/MAS NMR spectroscopy, mass spectroscopy, and thermal analysis. The TLC behavior has been tested by separation and identification of some dyes and benzo[a]pyrene derivatives.

Stationary phases 2a

95 003 H. KALÁSZ (Semmelweis University, Department of Pharmacology and Pharmacotherapy, H-1089 Budapest, Nagyvárad tér 4, Hungary): Planar displacement chromatography. J. Planar Chromatogr. 17, 464-467 (2004). Displacement chromatography (DC) has been widely used to separate metabolites with similar chemical characteristics. DC works with highly overloaded sample sizes, which are normal for samples not subjected to clean-up. DC successfully handles samples which contain high concentrations of salts and/or proteins, and results in consecutive steps of displaced compounds rather than Gaussian curves. Planar displacement chromatography (DTLC) is suitable for seeking new metabolites in excreted body fluids. Transfer of the radio labeled methyl group can easily be proven using spacer-displacement planar chromatography. Displacement TLC of L-deprenyl and 14C-L-deprenyl on silica gel with chloroform - triethanolamine 19:1. Detection by X-ray film with an exposure time of 120 h.

Pharmaceutical research, autoradiography 2a, 32a

95 004 E. REICH*, A. SCHIBLI (*CAMAG Laboratory, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland): A standardized approach to modern high-performance thin-layer chromatography (HPTLC). J. Planar Chromatogr. 17, 438-443 (2004). Proposals for general standardized HPTLC methodology: 1. Plate material (consistent material, prewashing, direction of development, activation of plates, influence of relative humidity). 2. Sample application (precise and accurate volume, solvent, position, spot or bandwise application). 3. Preparation and storage of mobile phases (stability, possible reaction). 4. Development (saturation, use of a twin-trough chamber, influence of the vapor phase, distance, drying). 5. Derivatization (dipping, spraying, heating). 6. Documentation of plates. 7. Labeling (plates, images). 8. Quantitative evaluation 8. Documentation of work.

HPTLC, standardization 2a

95 028 Irena BARANOWSKA et al., see section 17a

95 102 R. M. BAOSIC et al., see section 33a

95 041 Malgorzata JANICKA et al., see section 29d

95 036 M. NATIC et al., see section 24

95 044 Nada U. PERISIC-JANJIC et al., see section 29e

3. General techniques

95 005 V. G. BEREZKIN*, A. O. BALUSHKIN, E. B. NEPOKLONOV, A. V. TOPCHIEV (*Institute of Petrochemical Synthesis, Russian Academy of Sciences, Leninski pr. 29, 119991 Moscow, GSP-1, Russia): Principles of electroosmotic circular thin-layer chromatography. J. Planar Chromatogr. 17, 476-479 (2004). Compared with traditional linear thin-layer chromatography, circular TLC is known to have three advantages: substantially better resolution, lower limits of detection (because of the concentration of the zones), and lower solvent consumption. The results obtained indicate that use of circular electroosmotic TLC (EO-TLC) made the chromatographic process both faster and more efficient. Traditional circular TLC and circular electroosmotic TLC of dyes (rhodamin 6G, brilliant green, sudan III, crystal violet) with DMSO.

Electroosmotic circular thin-layer chromatography 3d

95 006 T. H. DZIDO*, J. MRÓZ, G. W. JÓZWIAK (*Department of Physical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Adaptation of a horizontal DS chamber to planar electrochromatography in a closed system. J. Planar Chromatogr. 17, 404-410 (2004). Highly reproducible retention was achieved when the adsorbent layer of the plate was pre-wetted and equilibrated with the mobile phase after adaptation of a horizontal DS chamber for electrochromatography in a closed (pressurized) system. The disadvantages of the open system, evaporation of the mobile phase from the plate and excessive flow of mobile phase to the surface of the adsorbent layer during development, were eliminated. Separation of a test dye mixture on pre-wetted RP-8 and RP-18 phases with acetonitrile - buffer and application of a potential of 2 kV to create the electric field. Detection by scanning in reflectance mode at 420 or 254 nm.

HPTLC, planar electrochromatography 3d

95 008 E. MINCSOVICS (OPLC-NIT, Andor u. 60, 1119 Budapest, Hungary): Flowing eluent wall processing OPLC: Using segmentation of non-segmented adsorbent layer for single and parallel separations. J. Planar Chromatogr. 17, 411-419 (2004). A new concept, the flowing eluent walls (FEW) process, for segmentation of a non-segmented adsorbent bed, has been used for single- and multi-channel on-line overpressured-layer chromatography, which leads to active and non-active regions on the adsorbent layer during the separation process. Mobile phase only is introduced to the non-active part of the layer whereas mobile phase and sample can be admitted to the active parts. The FEW concept provides the possibility of real multichannel liquid chromatographic separation on a non-segmented layer and column shaped adsorbent bed. Separation of chamomille oil, dye mixtures, ascorbigen standards, and cabbage extracts, were used as examples. The FEW configuration is suitable for rapid isolation of relatively large amounts of a substance.

Flowing eluent walls process, OPLC 3d

95 010 Y. WANG (Yuping Wang), D. WANG* (Dongyuan Wang), J. WANG (Jie Wang), Z. XIONG (Zhili Xiong), H. ZHANG (Hongxia Zhang), G. SHE, (Gaohong She) J. LI (Jian Li), S. XIAO (Shengtao Xiao) (*Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016 P. R. China): A new instrument for automated multiple development in thin-layer chromatography. J. Planar Chromatogr. 17, 290-296 (2004). Description of a new AMD instrument. Its main advantages are very low cost both of construction and in use. In comparison

with ascending development in conventional instruments, a laboratory-made horizontal sandwich chamber is used for development. With the help of a series of special accessories no obvious mobile phase remains in the distributor after each step thus saving a large amount of solvent. All the components of the instrument are easy to obtain, so the average worker in the laboratory could construct the entire instrument except the control unit. An application of the instrument is described; the results obtained were satisfactory. Compared with the commercial instrument the main differences are 1) a horizontal sandwich chamber with funnel distributor is used as development chamber, 2) the most expensive component, a motor-driven valve, is omitted, 3) a micro air pump (normally used to supply oxygen for goldfish) is used to deliver mobile phase to the chamber. AMD separation of 13 dyes with first acetone - ethyl acetate 1:1 to compress the spots to slim bands, then seven steps with ethyl acetate - chloroform 4:21 to1:9 were completed; then seven steps with chloroform - cyclohexane 17:3 to 67:33. After these fifteen steps of AMD the mixture was separated into eighteen visible spots.

AMD 3d

95 007 S. KHAWAS, D. PANJA, S. LASKAR* (*Natural Products Laboratory, Chemistry Department, University of Burdwan, Burdwan-713104, W. Bengal, India): A new reagent for identification of amino acids on thin-layer chromatography plates. J. Planar Chromatogr. 17, 314-315 (2004). Separation of 22 amino acids on silica gel with n-propanol - water 7:3. Detection by spraying with 1) 5 % 4-hydroxyacetophenone in acetone, followed by drying in air until all solvent had completely evaporated, and heating in an oven at 110 °C for 10 min, and, after cooling, spraying with 2) 0.4 % isatin-5-sulfonic acid (sodium salt) in ethanol - water 4:1, followed by drying in air and heating for 10 min at 110 °C. Detection limits were between 0.1 and 2 µg.

3e, 18

95 009 B. SPANGENBERG*, M. WEYANDT-SPANGENBERG (*University of Applied Sciences Offenburg, Badstrasse 24, 77652 Offenburg, Germany): Fluorescence evaluation using the Kubelka-Munk formula. J. Planar Chromatogr. 17, 164-168 (2004). HPTLC of flupirtine maleate on silica gel with ethyl acetate - methanol - 25 % ammonia 17:2:1 in a saturated developing chamber. Then the developed plate must be dipped for 2 s in 1:3 paraffin - hexane. Dipping increases the fluorescence tenfold and preserves the fluorescence stability for hours. Presentation of a new formula for transforming fluorescence measurements in accordance with Kubelka-Munk theory. The fluorescence signals, the absorption signals, and data from a selected reference are combined in one expression. Only diode-array techniques can measure all the required data simultaneously. The fluorescence calibration curve was linear over the range 300 to 5000 ng per spot.

Quality control, HPTLC, densitometry, quantitative analysis, flupirtine maleate 3f, 32a

95 013 K. L. BUSCH et al., see section 4e

4. Special techniques

95 013 K. L. BUSCH (Wyvern Associates, 4201 Wilson Blvd, 110-440 Arlington, VA 22203, USA): Planar separations and mass spectrometric detection. J. Planar Chromatogr. 17, 398-403 (2004). Review divided into several sections: ‚Summaries' contains a review of some recent research results in TLC-MS and PC-MS (use of a diode IR laser to desorb samples from a thin-layer chromatogram, with ionization of the desorbed gaseous molecules via a corona discharge; description of an interface between TLC and an electrospray ionization (ESI) mass spectrometer; on spot matrix-assisted laser desorption ionization (MALDI) mass spectrometry for TLC-MS); in ‚Assessments and perspectives' new results are detailed, precedent and new instrumental developments are previewed; in the section ‚Interconnections' synergies between mass spectrometry and different approaches to planar separations are explored. Finally, in ‚Forecasts' expectations and future developments, in addition to recent techniques, are described. 11 references.

Review, mass spectrometry, TLC-MS 4e, 3g

95 011 I. HAZAI (IVAX Drug Research Institute, Department for Pharmacokinetics, H-1325 Budapest, P. O. Box 82, Hungary): Use of multiple readings to increase the sensitivity of phosphor image detection in TLC. J. Planar Chromatogr. 17, 449-453 (2004). In thin-layer radiochromatography the high sensitivity of the phosphor imaging analyzer can be further increased by multiple reading of the image plates, because the latent image is not lost quantitatively in the reading process. Summing of the chromatograms obtained in successive readings results in increased signal-to-noise ratio. Thus, by use of this approach either the exposure time can be shortened or higher sensitivity can be achieved. TLC of a 14C-labeled test substance in rat serum on silica gel with chloroform - n-hexane - ethanol - ammonia 75:15:9:1. Detection by radioluminography.

Radioscanning, quantitative analysis 4e

95 012 G. HORVÁTH*, L. G. SZABÓ, É. LEMBERKOVICS, L. BOTZ, B. KOCSIS (*Department of Botany, Faculty of Natural Sciences, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary): Characterizaton and TLC-bioautographic detection of essential oils from some Thymus taxa, determination of the activity of the oils and their components against plant pathogenic bacteria. J. Planar Chromatogr. 17, 300-304 (2004). TLC of essential oils and thymol, carvacrol, geraniol as standards and streptomycin and gentamycin as positive controls on silica gel with toluene - ethyl acetate 93:7. Detection with ethanolic vanillin sulfuric acid. Quantitative determination at 500 nm. For bioautography the developed plates were dipped for 10 s in approximately 50 mL culture medium containing the test organism followed by drying for 2 min. After storage of the plates at 26 - 28 °C for 17 h they were dipped for 10 s in an aqueous solution (0.1 g/60 mL) of 3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide (MTT) the layers were incubated at 28 °C for 2 h and then dipped in 70 % ethanol and dried at room temperature.

Herbal, qualitative identification, quantitative analysis, densitometry, Thymus 4e, 32e

95 014 H. LUFTMANN*, D. HOPPE, J. BECKER (*Organisch-Chemisches Institut, Westfälische Wilhelms-Universität, Corrensstr. 40, 48149 Münster, Germany, luftman@uni-muenster.de): Hyphenated HPTLC-MS as rapid method for elucidation of synthesis mixtures. CBS 94, 5-7 (2005). HPTLC of synthesis reaction products on silica gel with pentane - tert. butyl methyl ether 3:1 with chamber saturation. Detection by spraying with phosphomolybdic acid reagent (0.4g in 100 mL ethanol), followed by drying in hot air. Identification by online hyphenation with mass spectrometry. For online extraction with the ChromeXtract device the substance zones are eluted with methanol - chloroform 1:1 (flow rate 0.1mL/min). The outlet capillary is connected directly to the electrospray mass spectrometer (ESI-MS). Measurement in 2 s cycles in a mass range from m/z 100-600.

Qualitative identification, HPTLC, ChromeXtract, TLC-MS online coupling, synthesis 4e

95 015 M. PROSEK*, L. MILIVOJEVIC, M. KRIZMAN, M. FIR (*National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia): On-line TLC-MS. J. Planar Chromatogr. 17, 420-423 (2004). A new on-line TLC-MS interface, with computer-controlled extraction of substances from selected spots on a TLC or HPTLC plate, has been constructed. The controlled collection of the sample and its programmed injection into the mass spectrometer is the advantage of this type of interface. It has been tested and validated with a standard solution of caffeine as test substance. HPTLC of caffeine on silica gel with dichloromethane - methanol 9:1. Quantification with a video-documentation system.

HPTLC, TLC-MS interface 4e

95 016 W. WEBER*, W. SEITZ, Anna AICHINGER, R. ALBERT (*Zweckverband Landeswasserversorgung, Betriebs-und Forschungslaboratorium, Am Spitzigen Berg 1, D-89129 Langenau, Germany, weber.w@lw-online.de): Luminographic detection of toxicity with Vibrio fischeri (luminescent bacteria). CBS 94, 2-4 (2005). HPTLC-AMD of four pharmaceuticals and extracts of surface water on silica gel prewashed with 2-propanol (immersion for 24 h) with a 25-step gradient based on acetonitrile - formic acid - dichloromethane. Luminographic detection at ng-

level by immersion of the developed HPTLC plates into Vibrio fischeri bacteria suspension. Visual evaluation with CCD-camera, exposure time 40 s, inversion and scaling of exposure in pseudocolors. To remove matrix (humic acids) from surface water samples size exclusion chromatography is recommended.

Environmental, toxicology, AMD, HPTLC, qualitative identification, Vibrio fischeri, Bioluminex, luminographic detection, water analysis 4e, 37c

95 104 L. WILLIAMS et al., see section 35b

8. Substances containing heterocyclic oxygen

95 017 T. HOFMANN*, L. ALBERT, T. RÉTFALVI (*University of West Hungary, Institute for Chemistry, Ady Endre u. 5, 9400 Sopron, Hungary): Quantitative TLC analysis of (+)-catechin and (-)-epicatechin from Fagus Sylvatica L. with and without red heartwood. J. Planar Chromatogr. 17, 350-354 (2004). TLC of (+)-catechin and (-)-epicatechin on silica gel with diisopropyl ether - formic acid 9:1. Detection by spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min. Quantitative determination by absorbance measurement at 513 nm.

Quantitative analysis, densitometry, Fagus Sylvatica, catechin, epicatechin 8a

95 018 B. LAPORNIK, Alenka GOLC WONDRA*, M. PROSEK (*National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia) : Comparison of TLC and spectrophotometric methods for evaluation of the antioxidant activity of grape and berry anthocyanins. J. Planar Chromatogr. 17, 207-212 (2004). HPTLC of malvidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, peonidin 3-glucoside, and petunidin 3-glucoside on silica gel with ethyl acetate - formic acid - twice distilled water 17:2:3 in an unsaturated twin trough chamber. After drying detection with methanolic 2,2-diphenyl-1-picrylhydrazyl reagent. Quantitative determination by videodensitometry.

Food analysis, HPTLC, qualitative identification, quantitative analysis, densitometry, anthocyans 8a

95 019 Z. MALES*, M. PLAZIBAT, V. B. VUNDAC, I. ZUNTAR, K. H. PILEPIC (*Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, 10000 Zagreb, Croatia): Thin-layer chromatographic analysis of flavonoids, phenolic acids, and amino acids in some Croatian Hypericum taxa. J. Planar Chromatogr. 17, 280-285 (2004). TLC of flavonoids (quercetin, I3,I8-biapigenin, quercitrin, isoquercitrin, hyperoside, rutin) and phenolic acids (caffeic and chlorogenic acid) on silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:26 and ethyl acetate - formic acid - water 8:1:1. Detection by spraying with natural products - polyethylene glycol reagent and observation under UV light at 365 nm. Detection limit for flavonoids was 2.5 µg. Quantitative determination by spectrophotometry, calculated as quercetin. Also TLC of 16 amino acids on cellulose.

Herbal, quantitative analysis, qualitative identification, Hypericum 8a, 11a, 18a, 32e

95 020 Marica MEDIC-SARIC*, I. JASPRICA, A. MORNAR, A. SMOLCIC-BUBALO, P. GOLJA (*Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10000 Zagreb, Croatia): Quantitative analysis of flavonoids and phenolic acids by two-dimensional thin layer chromatography. J. Planar Chromatogr. 17, 459-463 (2004). TLC of standard solutions of nine flavonoids and six phenolic acids (cinnamic, o-coumaric, m-coumaric, p-coumaric, caffeic, ferulic acid, galangin, quercetin, pinocembrin, naringenin, apigenin, chrysin, kaempferol, morin, acacetin) on silica gel in pre-saturated developing chambers with 1) n-hexane - ethyl acetate - glacial acetic acid 31:14:5, or 2) chloroform - methanol - formic acid 88:7:5. After drying, bands were visualized under short- and long-wavelength UV light. Detection by spraying with 1 % aluminium trichloride solution and evaluation under long-wavelength UV light. Standards were chromatographed again with a propolis extract. First,

plates were developed with mobile phase 1 (or 2), the eluent was evaporated, standard solutions were applied again, and the plate was rotated through 90 ° and chromatographed again with mobile phase 2 (or 1). The presence (or absence) of all standards was determined according to their Rf values and fluorescence colors. Quantitative determination by absorbance measurement at 254 and 366 nm.

Herbal, densitometry, quantitative analysis, qualitative identification, flavonoids, phenolics 8a

95 021 E. SOCZEWINSKI*, M. WOJCIAK-KOSIOR, G. MATYSIK (*Department of Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Analysis of glycosides and aglycones of flavonoid compounds by double-development thin-layer chromatography. J. Planar Chromatogr. 17, 261-263 (2004). HPTLC of aglycones and glycosides (flavone, hesperetin, naringenin, apigenin, kaempferol, quercetin, myricetin, tiliroside, apigenin 7-glucoside, myricitrin, kaempferol 3,7-dirhamnoside, hyperoside, hesperidin, rhoifolin, rutin, naringin) on silica gel by using mixtures of dichloromethane and ethyl acetate for aglycones and mixtures of ethyl acetate and formic acid for glycosides. The plates were conditioned for 15 min and developed face down in a horizontal chamber. To separate mixtures of flavonoids two-step gradient development was used. In the first step (for glycosides) the plates were developed to a distance of 65 mm with ethyl acetate - formic acid - water 170:30:1. In the second step (for aglycones) the plates were developed to a distance of 95 mm with dichloromethane - ethyl acetate - formic acid 170:30:1. Quantitation by densitometry at 254 nm.

Herbal, HPTLC, densitometry, quantitative analysis, glycosides, aglycones 8a

95 022 M. WÓJCIAK-KOSIOR*, G. MATYSIK, A. SKALSKA (*Department of Chemistry, Medical Academy, Staszica 6, 20-081 Lublin, Poland): Densitometric determination of kinetics of hydrolysis of flavonoid glycosides. J. Planar Chromatogr. 17, 286-289 (2004). HPTLC of isoquercitrin, avicularin, rutin, apigenin 7-glucoside, naringin, and hesperidin on silica gel with ethyl acetate - methanol - formic acid 90:10:1. Detection under UV light at 254 nm and by spraying with 1 % methanolic diphenylboric acid beta-ethylamine ester, followed by spraying with 5 % ethanolic polyethylene glycol 4000. Quantitative determination by densitometry at 254 nm. Report of the possibilities and advantages of HPTLC for investigation of hydrolysis.

Herbal, pharmaceutical research, HPTLC, densitometry, quantitative analysis, hydrolysis, flavonoid glycosides 8a

95 032 M. PROSEK et al., see section 20

10. Carbohydrates

95 023 Gerda MORLOCK*, Shashi PRABHA (*University of Hohenheim, Institute of Food Chemistry 170, Garbenstr. 28, 70599 Stuttgart, Germany, gmorlock@uni-hohenheim.de): Amino phases for derivatization of sucralose in milk-based confection. CBS 94, 14-15 (2005). HPTLC of sucralose on amino phases with acetonitrile - water 4:1 over 70 mm. Stability of sucralose in Burfi, a milk-based confection, was determined over a defined time period. The 2 products of hydrolysis have been monitored as well. Detection by heating for 20 min at 190° C. Quantitative determination by fluorescence measurement under UV 366. Limit of quantitation in the lower ng range.

Food analysis, quantitative analysis, HPTLC, densitometry, sucralose 10a

11. Organic acids and lipids

95 019 Z. MALES et al., see section 8a

95 024 J. A. JARUSIEWICZ, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA 18042, USA): High-performance thin-layer chromatographic analysis of neutral li-

pids and phospholipids in the apple snail Pomacea bridgesii. J. Planar Chromatogr. 17, 454-458 (2004). HPTLC of lipids and phospholipids (tricylglycerols, free sterols, free fatty acids, steryl esters, phosphatidylcholine, phosphatidylethanolamine) on silica gel with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 in a presaturated twin-trough chamber. Detection by spraying with 5 % phosphomolybdic acid in ethanol, followed by heating for 10 min at 115 °C. The neutral lipids are visible as blue spots on a yellow background. For polar lipid analysis, plates were developed with chloroform - methanol - water 65:25:4 and sprayed with 10 % cupric sulfate, followed by heating for 10 min at 140 °C, to detect phospholipids as brown spots on a white background. Quantitative determination by densitometric analysis at 610 nm for neutral lipids and at 370 nm for polar lipids.

Agricultural, HPTLC, quantitative analysis, densitometry 11c

95 025 Iuliana POPA*, Marie-Jeanne DAVID** (*EA 37-32, Laboratory of Dermatology, Pav. R, and ** Laboratory of Biochemistry, Edouard Herriot Hospital, 69437 Lyon Cx 03, France, popa@lyon.inserm.fr. Permanent adress of I. Popa: Institute of Macromolecular Chemistry, Aleea Gr. Ghica Voda 41A, Iassy, Romania): Immunoassay detection of gangliosides by specific antibodies. CBS 94, 11-13 (2005). HPTLC of gangliosides extracted from tissues on silica gel with chloroform - methanol - 0.2% aqueous CaCl2 11:9:2 over 60 mm. Immunoassay detection by dipping in polyisobutylmethacrylate solution and bovine serum albumine solution, followed by immersion in anti-body containing supernatant or patient‘s sera at 4° C overnight. After washing with phosphate-buffered saline detection of Mab binding by stepwise incubation with biotinylated chain-specific anti-mouse immunoglobulin, followed by steptavidin-horsradish peroxidase complex. Visualization with chloro-4-naphtol reagent. Immuno detection is better than chemical derivatization with resorcinol-HCl reagent and has advantages over detection on ELISA microtiter plates.

HPTLC, gangliosides, immunoassay 11e

13. Steroids

95 026 Mária BÁTHORI*, A. HUNYADI, G. JANICSÁK, I. MÁTHÉ (*Department of Pharmacognosy, University of Szeged, Szeged, Eötvös u. 6, H-6720 Hungary): TLC of ecdysteroids with four mobile phases and three stationary phases. J. Planar Chromatogr. 17, 335-341 (2004). TLC and HPTLC of 29 ecdysteroids (e. g. 20-hydroxyecdysone, polypodine B, 2-deoxyintegristerone, ajugasterone C, isovitexirone, muristerone A, turkesterone, makisterone C, rubrosterone, poststerone, ecdysone, herkesterone) on silica gel, RP-18, and cyano phase with four mobile phases enabling separation of all the ecdysteroids from each other in at least one system. Detection under UV light at 254 nm or by use of vanillin-sulfuric acid spray reagent. After spraying the spots were either observed in daylight or at 366 nm. Quantitative determination by reflectance-absorbance measurement at 254 nm.

Herbal, HPTLC, densitometry, quantitative analysis, qualitative identification, ecdysteroids 13e

14. Steroid glycosides, saponins and other terpenoid glycosides

95 027 R. T. SANE, M. SASIKUMAR, A. Y. DESHPANDE*, A. A. MENEZES, G. GUNDI (*TDM Laboratory, Plot No. 194, Scheme No, 6, Sion East, Mumbai 400022, India): Quantitation of protodioscin in Tribulus terrestris L. fruit powder by reversed-phase high-performance thin-layer chromatography. J. Planar Chromatogr. 17, 379-382 (2004). HPTLC of protodioscin and fruit powder extracts on RP-18 (prewashed with methanol) with 0.1 M potassium dihydrogenphosphate - acetonitrile - methanol - triethylamine 50:40:10:1 in a twin-trough chamber previously saturated with the mobile phase for 10 min. Detection by dipping in 0.1 M sulfuric acid, drying and heating for 10 min at 80 °C. Densitometric evaluation at 366 nm. Detection and quantitation limits were 0.03 µg and 0.05 µg, respectively. Response was linearly dependent on amount of protodioscin in the range 0.05 to 1.00 µg.

Food analysis, HPTLC, densitometry, quantitative analysis, protodioscin, Tribulus terrestris
14

17. Amines, amides and related nitrogen compounds

95 028 Irena BARANOWSKA*, M. ZYDRON (*Department of Analytical and General Chemistry, Silesian University of Technology, 7 M. Strzody Str., 44-100 Gliwice, Poland): Retention-mobile phase relationships for methylxanthines and biogenic amine metabolites in adsorption thin-layer chromatographic systems. J. Planar Chromatogr. 17, 233-237 (2004). TLC of biogenic amines (dopamine, adrenaline, noradrenaline, 3-methoxytyramine, methanephrine, normethanephrine, vanillylmandelic acid, homovanillic acid, 3,4-dihdroxyphenylacetic acid, 3,4-dihydroxyphenylethyleneglycol, 3-methoxy-4-hydroxyphenylethyleneglycol) and methylxanthines (1-methylxanthine, 7-methylxanthine, theophylline, paraxanthine, theobromine, caffeine) on silica gel with mobile phases comprising a non-polar or weakly polar diluent (chloroform, heptane, or hexane) and a polar modifier (tetrahydrofurane, dioxane, acetone, or ethyl acetate). Examination under UV light or detection with a solution of iron(III) chloride (5 g) and iodine (2 g) in 50 mL of 20 % aqueous tartaric acid. Relationship between Rf values and mobile phase composition was investigated.

Clinical chemistry research, qualitative identification, biogenic amines 17a, 23, 2c

95 030 K. LESKÓ, Livia SIMON-SARKADI*, É. STFANOVITS-BÁNYAI, Z. VÉGH, G. GALIBA (*Budapest University for Technology and Economics, Department of Biochemistry and Food Technology, 1111 Budapest, Müegyetem rkp. 3, Hungary): OPLC analysis of polyamines in wheat seedlings under cadmium stress. J. Planar Chromatogr. 17, 435-437 (2004). OPLC of agmatine, spermine, spermidine, putrescine, cadaverine, histamine, and tyramine and homogeneous fresh plant tissue extracts (as dansyl derivatives) on silica gel with 1) hexane - n-butanol - triethylamine 900:100:91, and 2) hexane - n-butanol 4:1 with overrun development and a stepwise gradient. Off-line quantitative evaluation of the dansyl amines by fluorodensitometry at 313/>400 nm.

Agricultural, quantitative analysis, densitometry, OPLC 17a

95 031 Gertrud MORLOCK* (*Institute of Food Chemistry, University of Hohenheim, D-70599 Stuttgart, Germany): New HPTLC method, with systematic mobile-phase optimization, for determination of six apolar heterocyclic aromatic amines. J. Planar Chromatogr. 17, 431-434 (2004). HPTLC of six heterocyclic amines (2-amino-9H-pyrido[2,3-b]indole, 2-amino-3-methyl-9H-pyrido[2,3-b]indole, norharmane, harmane, 2-aminodipyrido[1,2-alpha:3‘,2‘-d]imidazole, 2-amino-6-methyldipyrido[1,2-alpha:3‘,2‘-d]imidazole) on silica gel by multiple development with diethylether. The mobile phase was selected by using a practical and systematic four-level optimization scheme based on the solvent classification system according to Snyder and the PRISMA model of Nyiredy et al. Quantitative determination by fluorescence measurement at 366/>400 nm was performed immediately after development.

Food analysis, HPTLC, quantitative analysis 17a

18. Amino acids and peptides, chemical structure of proteins

95 007 S. KHAWAS et al., see section 3e

95 019 Z. MALES et al., see section 8a

20. Enzymes

95 032 M. PROSEK*, A. SMIDOVNIK, M. FIR, M. STRAZISAR (*National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia): TLC identification and quantification of coenzyme

Q10-beta-cyclodextrin complex. J. Planar Chromatogr. 17, 181-185 (2004). HPTLC of an inclusion complex of coenzyme Q10 with beta-cyclodextrin on silica gel by one-dimensional, two-dimensional, and multi-dimensional separation with 1) dioxane - water 1:1 and 2) chloroform - methanol 11:9. Detection by spraying with 5 % phosphomolybdic acid in ethanol and drying at 110 °C, followed by spraying with 50 % sulfuric acid and heating at 120 °C for 5 min.

Food analysis, HPTLC, quantitative analysis, densitometry, coenzyme Q10 20, 8b

22. Alkaloids

95 033 T. A. LÓPEZ*, M. L. DE LA TORRE, M. S. CID (*Estación Experimental Agropecuaria Balcarce, Instituto Nacional de Tecnologia Agropecuaria (INTA), Animal Toxicology Laboratory, C. C. 276, Balcarce (7620), Buenos Aires, Argentina): An efficient TLC method for the analysis of gamma-coniceine and coniine in Conium maculatum L. foliage. J. Planar Chromatogr. 17, 218-223 (2004). TLC of gamma-coniceine and coniin on silica gel with chloroform - ethanol 13:7. Detection and quantification by spraying with Dragendorff‘s spray reagent and visual comparison of the intensity of the color of the sample spots with that of the spots of the corresponding standards. Detection limits were 1.7 and 0.7 µg per spot for coniine and gamma-coniceine, respectively.

Toxicology, qualitative identification, quantitative analysis, gamma-coniceine, coniin 22

23. Other substances containing heterocyclic nitrogen

95 028 Irena BARANOWSKA et al., see section 17a

95 035 Joanna NOWAKOWSKA (University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen Hallera 107, PL 80-416 Gdansk, Poland): Use of HPTLC with non-aqueous binary mobile phases for determination of selected porphyrins. J. Planar Chromatogr. 17, 388-390 (2004). HPTLC of uroporphyrins I and III, coproporphyrins I and III, and protoporphyrin on silica gel with binary mobile phases prepared by mixing pure esters, ketones, and xylenes with DMSO in proportions from 0 to 100 %. Detection under UV light at 254 nm. The separation of the porphyrins required the presence of DMSO in the mobile phase.

Pharmaceutical research, HPTLC, qualitative identification, porphyrins 23a

95 034 J. D. VELICKOVIC, D. ANDRIC, G. ROGLIC, Z. L. TESIC, Dusanka M. MILOJKOVIC-OPSENICA* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): Planar chromatography of some 1-arylpiperazines behaving as dopaminergic ligands. J. Planar Chromatogr. 17, 255-260 (2004). TLC of fourteen 1-arylpiperazine derivatives on silica gel with monocomponent and binary non-polar mobile phases and on RP-18 with binary mobile phases comprising mixtures of methanol, acetone, or dioxane (as organic modifiers) and water in a horizontal chamber after equilibration for 15 min. Detection under UV light at 254 nm.

Clinical chemistry research, qualitative identification 23e, 32a

24. Organic sulfur compounds

95 036 M. NATIC, R. MARKOVIC, K. ANDELKOVIC, D. MILOJKOVIC-OPSENICA, Z. TESIC* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): Reversed-phase thin-layer chromatography of stereodefined 2-alkylidene-4-oxothiazolidines and 1,2-dithiols. J. Planar Chromatogr. 17, 323-327 (2004). TLC of three 1,2-dithioles and five 2-alkylidene-4-oxothiazolidines on RP-18 with binary mixtures of methanol and water, tetrahydrofuran and water, and acetone and water in different proportions. Detection with iodine vapor. Good correlation of chromatographically obtained lipophilicity data with calculated log P values.

Clinical chemistry research, qualitative identification 24, 2d

27. Vitamins and various growth regulators

95 037 G. KÁTAY*, Z. NÉMETH, S. SZANI, O. KÖCK, L. ALBERT, E. TYIHÁK (*Plant Protection Institute, Hungarian Academy of Sciences, P. O. Box 102, H-1525 Budapest, Hungary): Overpressured-layer chromatographic determination of ascorbigen (bound vitamin C) in Brassica vegetables. J. Planar Chromatogr. 17, 360-364 (2004). Analytical OPLC of ascorbigen (ASC, 2-C-[(indol-3-yl)methyl]-alpha-l-threo-l-glycero-3-hexulofuranosonic acid lactone) on silica gel by means of two-step development: the first step (n-hexane) served for elimination of the total wetness front, the second (chloroform - methanol 9:1) for the separation. Detection by spraying with 10 mL Procházka's reagent (reaction with formaldehyde), then heated for 5 min at 105 °C. Quantitative determination by densitometry at 460 nm.

Food analysis, agricultural, qualitative identification, quantitative analysis, densitometry, OPLC 27

28. Antibiotics, Mycotoxins

95 039 Joanna NOWAKOWSKA (Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, Al. Gen. J. Hallera 107, PL 80-416, Gdansk, Poland): Analysis of selected macrocyclic antibiotics by HPTLC with non-aqueous binary mobile phases. J. Planar Chromatogr. 17, 200-206 (2004). HPTLC of macrocyclic antibiotics (erythromycin, troleandomycin, tylosin, vancomycin, rifamycin B, and rifampicin) on LiChrospher silica gel. A wide range of mixtures of alcohols and ketones with hexamethyldisiloxane in proportions of 0 to 100 % and with dimethyl sulfoxide in proportions from 0 to 50 % were used as mobile phases. Separations were performed in chromatographic chambers after presaturation with mobile phase vapor. Detection by spraying with a 1:4 mixture of concentrated sulfuric acid and methanol followed by heating for approximately 10 min at 120 °C.

Quality control, qualitative identification, HPTLC, antibiotics 28a

95 038 C. MARUTOIU*, S. PUIU, M. I. MOISE, L. SORAN, O. F. MARUTOIU, L. BOBOS (* „Lucian Blaga" University from Sibiu, Department of Chemistry, 7-9 Ion Ratiu Street, RO-2400 Sibiu, Romania): Optimization of the separation of some aflatoxins by thin-layer chromatography. J. Planar Chromatogr. 17, 372-374 (2004). TLC of aflatoxins B1, B2, G1, and G2 on silica gel with chloroform - acetone 23:2 in unsaturated chambers. Detection under UV light at 366 nm. Selection of the optimum mobile phase composition by software programs.

Food analysis, toxicology, qualitative identification, aflatoxins 28b

29. Pesticides and other agrochemicals

95 043 T. TUZIMSKI* (*Department of Physical Chemistry, Medical University, Staszica 6, 20-081 Lublin, Poland): Separation of a mixture of eighteen pesticides by two-dimensional thin-layer chromatography on a cyanopropyl-bonded polar stationary phase. J. Planar Chromatogr. 17, 328-334 (2004). HPTLC of eighteen pesticides (propaquizafob, quizalofop-P, triadimefon, triadimenol, dimethomorf, quinoxyfen, cyromazine, oxyfluorfen, fluoroglycofen, acetochlor, metazachlor, imazapyr, furalaxyl, triclopyr, buprofezin, pyriproxyfen, fenoxycarb, piperonyl butoxide) on cyano phase. The greatest spread of separated compounds was obtained by combining nonaqueous normal-phase mobile phases (tetrahydrofuran or ethyl acetate in n-heptane 1:4 in the first direction and aqueous reversed phases mobile phases (methanol - water 7:3 or acetonitrile - water 1:1) in the second dimension. Detection under UV light at 254 or 366 nm. Videoscanning and densitometry at 254 nm.

Agricultural, densitometry, quantitative analysis, HPTLC, pesticides 29

95 041 Malgorzata JANICKA*, B. OSCIK-MENDYK, B. TARASIUK (*Department of Planar Chromatography, Faculty of Chemistry, M. Curie-Sklodowska University, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland): Planar chromatography in studies of the hydrophobic properties of some new herbicides. J. Planar Chromatogr. 17, 186-191 (2004). TLC of sixteen new herbici-

des (7 2-(chlorophenoxy)acyl derivatives like e.g.methyl 2,4-dichlorophenoxyacetate, methyl 2-(2,4,5-trichlorophenoxy)propionate and 9 N-aryltrichloroacetamides like e.g. N-(4-chlorophen yl)trichloroacetamide, n-trichloroacetanilide, trichoroacetanilide) on RP-18 with aqueous buffer - methanol mixtures in saturated sandwich chambers. A Reprostar 3 video camera and Videostore software were used for visualization and evaluation of chromatograms.

Agricultural, qualitative identification, herbicides 29d, 2d

95 042 Malgorzata JANICKA*, N. U. PERISIC-JANJIC, J. K. RÓZYLO (*Faculty of Chemistry, Department of Planar Chromatography, Maria Curie-Sklodowska University, Maria Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland): Thin-layer and overpressured-layer chromatography for evaluation of the hydrophobicity of s-triazine derivatives. J. Planar Chromatogr. 17, 468-476 (2004). HPTLC of nine s-triazines on RP-18 and cyano phases with aqueous solutions of different organic modifiers (acetone, acetonitrile, tetrahydrofuran, and dioxane). After development the dried plates were examined under UV light at 254 nm. OPLC on RP-8 and RP-18 with water - acetonitrile. The plates were preconditioned in acetonitrile before development.

Qualitative identification, HPTLC 29d

95 044 Nada U. PERISIC-JANJIC*, T. L. DJAKOVICS-SEKULIC, K. POPOV-PERGAL (*Department of Chemistry, Faculty of Science, University of Novi Sad, Trg D, Obradovica 3, 21000 Novi Sad, Serbia and Montenegro): Correlation between the structure of some 2,4-dioxotetrahydro-1,3-thiazoles and TLC retention data. J. Planar Chromatogr. 17, 192-199 (2004). TLC of 3-ethyloxycarbonyl-5-substituted-2,4-dioxotetrahydro-1,3-thiazole derivatives (2‘-fluoro-6‘-chlorobenzylidene, 1‘-naphthylidene, 4‘-methoxybenzylidene, 2‘-oxybenzylidene, 2‘-thienylidene, 4‘-dimethylaminobenzylidene, 1‘-carboethoxy-3‘-indolylidene, 3‘,4‘-dimethoxybenzylidene, 4‘-isopropylbenzylidene, benzylidene) on silica gel with non-polar diluent (hexane) - polar modifier (ethyl acetate, acetone, or dioxane) and on reversed-phase systems of the type rice starch with polar diluent (aqueous ammonia) - polar modifier (methanol, acetone, or dioxane). Examination of the dried plates after development under UV light at 254 nm.

Agricultural, pharmaceutical research, qualitative identification 29e, 2c

95 040 S. GE (Ge Shimei), F. TANG (Tang Feng), Y. YUE* (Yue Yongde), R. HUA (Hua Rimao), R. ZHANG (Zhang Rong) (* International Center for Bamboo and Rattan, 100102 Beijing, China): HPTLC determination of pyrethroid residues in vegetables. J. Planar Chromatogr. 17, 365-368 (2004). HPTLC of deltamethrin, fenpropathrin, bifenthrin on silica gel (prewashed with chloroform - methanol 1:1) with toluene - petroleum ether 8:3 or cyclohexane - chloroform 1:1 in a twin-trough chamber and in a horizontal chamber. Quantitative determination by densitometric scanning at 254 and 366 nm.

Food analysis, HPTLC, densitometry, quantitative analysis, qualitative identification, pyrethroid residues 29f

30. Synthetic and natural dyes

95 045 Temenushka N. KONSTANTINOVA*, A. S. NEICHEVA, A. Y. VENKOVA (*Organic Synthesis Department, University of Chemical Technology and Metallurgy, 8 Ohridsky str., Sofia 1756, Bulgaria): TLC and HPLC studies of new 9-phenylxanthene dyes. J. Planar Chromatogr. 17, 369-371 (2004). TLC of 9-phenylxanthene derivatives (fluorescein, erythrosine, eosin, rhodamine B, and their allyloxy-derivatives) on silica gel with benzene - methanol 5:1, toluene - ethanol 7:1, acetonitrile - water 7:1, toluene - ethyl acetate - methanol 1:5:2. Detection under UV light at 254 nm or with iodine vapor. Quantitation by densitometric scanning.

Food analysis, cosmetics, densitometry, quantitative analysis 30a

95 046 Temenushka N. KONSTANTINOVA*, R. A. LAZAROVA, P. P. MILADINOVA (*Organic Synthesis Department, University of Chemical Technology and Metallurgy, 8 Ohridsky Str., Sofia

1756, Bulgaria): Thin-layer chromatographic study of some dyes and fluorescent brighteners for polymers. J. Planar Chromatogr. 17, 444-448 (2004). TLC of 16 naphthalimide dyes, 17 benzanthrone dyes, 20 triazine dyes, and 17 fluorescent brighteners on (mainly) silica gel with n-heptane - acetone 1:1 and 3:1, chloroform - methanol 1:1 and 2:1, n-heptane - benzene - chloroform 3:2:1, n-heptane - chloroform - acetone 2:2:1, n-butanol - pyridine - 25 % ammonia 1:1:1, chloroform - methanol - 25 % ammonia 11:5:1, n-propanol - 25 % ammonia 1:1 and 2:1, n-butanol - acetic acid - water 4:1:5. Quantitative determination by UV-scanning densitometry.

Environmental, qualitative identification, quantitative analysis, densitometry, dyes 30a

95 047 J. SHERMA*, B. FRIED (*Department of Biology, Lafayette College, Easton, PA 18042, USA): Separation and determination of chloroplast pigments from spinach by thin-layer chromatography: a student laboratory experiment. J. Planar Chromatogr. 17, 309-313 (2004). TLC of extracted pigments from spinach (carotene, chlorophyll a, lutein, chlorophyll b, violaxanthin, neoxanthin) on silica gel with isooctane - acetone - diethyl ether 3:1:1 or on RP-18 with petroleum ether (35 - 60 °C) - acetonitrile - methanol 1:2:2 in a twin- trough chamber covered with aluminum foil, lined with a saturation pad, and equilibrated with the mobile phase for 15 min before insertion of the plate. Quantitative determination by densitometry at 429 nm. A TLC experiment with great value for students.

Food analysis 30b

32. Pharmaceutical and biomedical applications

95 049 S. B. AGARWAL, N. D. GRAMPUROHIT*, A. S. PAREKAR (*C. U. Shah College of Pharmacy, S.N.D.T. Women‘s University, Santacruz (W), Mumbai 400 049, India): Standardization of herbal formulations containing kurchi (Holarrhena antidysenterica). IPC 56th 2004, Abstract No. D-9. HPTLC for the standardization of the alkaloid conessine in several market formulations containing kurchi bark, on silica gel with toluene - methanol - chloroform 1:2:1. Detection by spraying with Dragendorff‘s reagent. Densitometric evaluation at 460 nm. The method was validated for accuracy, precision, linearity range, specificity, LOD, LOQ and found suitable for routine analysis of herbal formulations containing Kurchi as main ingredient.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, conessine 32a

95 050 Danica AGBABA*, D. NOVOVIC, K. KARLJIKOVIC-RAJIC, V. MARINKOVIC (*Institute of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P. O. Box 146, 11000 Serbia and Montenegro): Densitometric determination of omeprazole, pantoprazole, and their impurities in pharmaceuticals. J. Planar Chromatogr. 17, 169-172 (2004). HPTLC of omeprazole and pantoprazole and their impurities omeprazole sulfone and N-methylpantoprazole on silica gel with chloroform - 2-propanol - 25 % ammonia - acetonitrile (108:12:3:40). Detection under UV light at 254 nm. Quantitation of omeprazole and omeprazole sulfone at 300 nm and of pantoprazole and N-methylpantoprazole at 295 nm in reflectance-absorbance mode. Regression coefficients ($r > 0.998$), recovery (90.7 - 120.0 %), and detection limits (0.025 - 0.05 %) were validated and found to be satisfactory.

Quality control, HPTLC, densitometry, quantitative analysis, omeprazole, pantoprazole 32a

95 053 Mugdha BHOSALE*, A. R. PARADKAR**, K. R. MAHADIK**, K.S. JAIN* (*Sinhgad College of Pharmacy, Vadgaon (Bk), Pune 411 041, India) (** Bharati Vidyapeeth Deemed University‘s, Poona College of Pharmacy, Erandwane, Pune 410038, India): Stability indicating HPTLC determination of cefuroxime axetil as bulk drug and in pharmaceutical formulations. 56th IPC 2004, Abstract No. GP-46. Stability indicating HPTLC determination of cefuroxime axetil in bulk drug and in formulations on silica gel with chloroform - methanol 23:2. Quantitative determination by scanning at 278 nm. The sample was subjected to acidic, and alkali hydrolysis, oxidation and photo degradation. The degraded products were well separated. The method

was validated for accuracy, precision, linearity, specificity, ruggedness, and recovery (98–100 %).

Pharmaceutical research, quality control, quantitative analysis, densitometry, postchromatographic derivatization, comparison of methods, HPTLC, cefuroxime axetil 32a

95 055 Mira CAKAR*, G. POPOVIC, S. VLADIMIROV (*Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, P. O. Box 146, 11000 Belgrade, Serbia and Montenegro): Simultaneous HPTLC determination of imidazole antimycotics and parabens in creams. J. Planar Chromatogr. 17, 177-180 (2004). HPTLC of bifonazole, econazole nitrate, methyl- and propylparaben on silica gel with ethyl acetate - n-hexane - methanol - ammonia - diethylamine 5:40:8:4:20 in a twin-trough chamber. Quantitation by scanning in reflectance/absorbance mode at 230 nm (econazole nitrate), 250 nm (bifonazole), and 300 nm (parabens).

Quality control, densitometry, HPTLC, quantitative analysis, qualitative identification, antimycotics, parabens 32a

95 083 M. S. CHARDE*, M. J. UMEKAR, S. B. JOSHI, A. V. KASTURE (*Department of Pharmaceutical Sciences, Nagpur University, Nagpur 440033, India): Estimation of ranitidine HCl and domperidone in combined dosage form using HPTLC. IPC 56th 2004, Abstract No. GP-8. Simultaneous HPTLC determination of ranitidine and domperidone on silica gel with methanol - 1, 4-dioxan 2:3. Quantitative determination by densitometric scanning at 282 nm. Rf values were 0.33 for ranitidine and 0.78 for domperidone. Linearity range was 0.5 - 2.5 mg/mL for both of the drugs. The recovery was in the range of 100.25 - 100.78 %. The method is suitable for the analysis of both drugs in combined dosage form.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, ranitidine, domperidone 32a

95 059 Shruti DHURU*, Pratima TATKE, K. K. SINGH (*C.U.Shah College of Pharmacy, S.N.D.T. Women‘s University, Santacruz (West) , Mumbai 400 049, India): Standardization and evaluation of Neem oil in pharmaceutical formulations by HPTLC. IPC 56th 2004, Abstract No. G-28. Neem Oil obtained from the seed kernels of Azardirachla indica (Meliaceae) is a fixed oil known as oil of Margosa. An HPTLC method is reported for the analysis of Neem oil as a bulk drug and formulations containing oil. TLC of neem oil extracted with chloroform, on silica gel with chloroform - n-hexane - methanol 18:2:1. Quantitative determination by scanning at 254 nm. The linearity range was 100 - 500 mg/mL. Formulations were found to contain 0.35 g/g of Neem Oil.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, neem oil 32a

95 051 S. B. GAICA, D. M. OPSENICA, B. A. SOLAJA, Z. L. TESIC, Dusanka M. MILOJKOVIC-OPSENICA* (*Faculty of Chemistry, University of Belgrade, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): The effect of the structure of mixed tetraoxanes on their chromatographic behavior on different adsorbents. J. Planar Chromatogr. 17, 342-349 (2004). TLC of 29 1,2,3,4-tetraoxanes on silica gel, cyano phase, and RP-18. The binary mobile phases ethyl acetate - petroleum ether and ethyl acetate - toluene were used under normal-phase conditions, and water - organic modifier (methanol, acetone, dioxane) under reversed-phase conditions. Chromatography was performed using a HPTLC horizontal developing chamber equilibrated for 15 min with the vapor of the mobile phase. Detection by spraying with 50 % sulfuric acid and heating until the spots became visible.

Pharmaceutical research, qualitative identification 32a

95 061 M. GANDHIMATHI, S. C. VIJAY KUMAR*, T. K. RAVI, Shaise JACOB, Lekha MATHEW, S. MALATHI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS,395, Sarojini Naidu Road, Coimbatore 614044, India): Simultaneous estimation of Loratadine and Ambroxol from formulation by HPTLC. IPC 56th 2004, Abstract No. G-20. Simultaneous HPTLC

determination of loratadine and ambroxol in combined dosage form on silica gel with n-hexane - dichloromethane - triethanolamine 11:8:1.The Rf value of loratadine and ambroxal was found to be 0.40 and 0.16 respectively. Quantitative evaluation by scanning at 254 nm. The method was linear in the range of 0.2 -1 mg/spot for loratadine and 1.2 - 6 mg/spot for ambroxol with recovery of 98.2 - 98.5 %. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, loratadine, ambroxol 32a

95 082 K. R. GUPTA*, A. N. MALIYE, M. R. TAJNE, S. G. WADODKAR (*Department of Pharmaceutical Sciences, Nagpur 440033, India): Stability indicating HPTLC determination of indapamide in tablets. IPC 56th 2004, Abstract No. GP-11. Stability indicating HPTLC determination of indapamide in tablets on silica gel with toluene - methanol 7:3. Quantitative determination by scanning at 246 nm. Optimization of experimental parameter such as band size, chamber saturation, and slit width. The method was linear in the range of 1.4 - 3.72 g, recovery was 100.01 %. The drug was subjected to stress conditions according to ICH guidelines, degradation products were separated from the pure drug. The method was validated for accuracy, precision, linearity, and specificity.

Pharmaceutical research, quality control, quantitative analysis, densitometry, postchromatographic derivatization, comparison of methods, HPTLC, indapamide 32a

95 066 H. HOPKALA, A. POMYKALSKI* (*Department of Medicinal Chemistry, Faculty of Pharmacy, Prof. Skubiszewski Medical Universit y of Lublin, 6 Chodzki St., 20-093 Lublin, Poland): TLC analysis of non-steroidal anti-inflammatory drugs and videodensitometric determination of fenbufen in tablets. J. Planar Chromatogr. 17, 383-387 (2004). TLC of fenbufen, ibuprofen, ketoprofen, diclofenac sodium, mefenamic acid, and tiaprofenic acid on silica gel by ascending and horizontal techniques, and on RP-18 in horizontal chambers. Good separation was achieved on silica gel by horizontal development with chloroform - methanol - 25 % ammonia 67:25:8; reversed phase chromatography on RP-18 with 0.15 mol/L phosphate buffer, pH 5.73 - 10 % CTMA-Br (N-cetyl-N,N,N-trimethylammonium bromide) in methanol 7:13 enabled better separation of the six drugs. Detection under UV light at 254 nm - for ibuprofen detection was best achieved after normal phase chromatography with 20 % aqueous sodium carbonate solution. A simple videodensitometric procedure was developed and validated. RSD for quantitation of fenbufen was 2.44 - 3.10 %.

Quality control, densitometry, quantitative analysis, non-steroidal anti-inflammatory drugs 32a

95 068 A. JASHIDI (Department of Novel Drug-delivery Systems, Iran Polymer and Petrochemical Institute, P. O. Box 14185/458, Tehran, Iran): A convenient and high throughput HPTLC method for determination of progesterone in release media of silicon-based controlled-release drug-delivery systems. J. Planar Chromatogr. 17, 229-232 (2004). HPTLC of progesterone on silica gel in an automatic multiple development chamber (AMD) with toluene - 2-propanol 9:1 without chamber saturation and with 10 min drying time. Visual inspection under UV light at 254 nm. Quantitative determination in reflectance mode at 252 nm. Limits of quantitation and detection were 25 and 5 ng/zone.

Quality control, AMD, HPTLC, densitometry, quantitative analysis, progesterone 32a

95 069 N.S. JEGANATHAN*, M. RAJ MOHMED, R.MANAVALAN (*Dept.of Pharmacy, Annamalai University, Annamalai Nagar -608002 TN, India): Quantitative determination of piperine in Trikatukuc Curanam by HPTLC. IPC 56th 2004, Abstract No. DP-33. An HPTLC method is reported for the standardization of Trikatukuc Curanam, an Ayurvedic preparation with Piper nigrum and Piper longum, both containing piperine as major alkaloid. HPTLC of piperine on silica gel with toluene - ethyl acetate 7:3. Extraction with methylene chloride, the evaporated residue of

the organic layer was taken in ethyl acetate and subjected to the analysis. The band corresponding to piperine was scanned at 338 nm. Linearity was 8-40 ng with recovery of 99.03 %.

Pharmaceutical research, quality control, postchromatographic derivatization, densitometry comparison of methods, quantitative analysis, piperine 32a

95 003 H. KALÁSZ et al., see section 2a

95 070 N. KAUL, H. AGRAWAL, A. R. PARADKAR, K. R. MAHADIK* (*Department of Quality Assurance Techniques, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane, Pune-411038, Maharashtra State, India): Stability-indicating high-performance thin-layer chromatographic determination of zidovudine as the bulk drug and in pharmaceutical dosage forms. J. Planar Chromatogr. 17, 264-274 (2004). HPTLC of zidovudine (3'-azido-3'-deoxythymidine) and degradation products on silica gel with toluene - carbon tetrachloride - methanol - acetone 35:35:20:1. Quantitative determination by absorbance measurement at 270 nm. The method was validated for precision, robustness, and recovery. Limit of detection was 20 ng per spot, limit of quantitation 40 ng.

HPTLC, densitometry, quantitative analysis, zidovudine 32a

95 071 Amandeep KAUR*, Prateek K. JAIN* and R. K. AGRAWAL (*Pharmaceutical Chemistry, Research Laboratory, Department of Pharmaceutical Science, Dr. Hari Singh gour University Sagar m.p. 470003, India): TLC densitometric method for the quantification of conessine in Holarrhena antidysenterica. IPC 56th 2004, Abstract No. G-5. HPTLC of conessine in Holarrhena antidysentrica, an important ayurvedic drug, on silica gel with toluene - ethyl acetate - diethyl amine 13:5:2. Detection by spraying with Dragendorff's reagent. Quantitative determination by densitometric scanning at 520 nm. Different market samples of the drug were found to contain 0.30 - 1.46 % of conessine with recovery of 95.18 - 102.70 %

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, conessine 32a

95 060 M. G. PAI*, Dattesh VEREKAR, K. VENKATESHWAR RAO (*Goa College of Pharmacy, Panaji, Goa, India): Development and validation of a new sensitive method for the simultaneous estimation of amlodipine - atenolol in tablets by HPTLC. IPC 56th 2004, Abstract No. GP-5. Simultaneous HPTLC determination of amlodipine and atenolol on silica gel with ethyl acetate - methanol - ammonia 60:40:3. Quantitative determination by scanning at 254 nm. The method was found linear in the range of 0.5 - 5.0 mg/mL amlodipine and 5.0 mg - 50 mg/mL atenolol. Recovery was 98.11 - 101.5 % for both of the compounds. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, quantitative analysis, postchromatographic derivatization, comparison of methods, densitometry, HPTLC, amlodipine, atenolol 32a

95 078 R. PIETRAS*, H. HOPKALA, D. KOWALCZUK, A. MALYSZA (*Department of Medicinal Chemistry, Medical University, Chodzki 6, 20-093 Lublin, Poland): Normal-phase TLC separation of some antiarrhythmics. Densitometric determination of mexiletine hydrochloride in capsules. J. Planar Chromatogr. 17, 213-217 (2004). TLC of disopyramide, flecainide, mexiletine, tocainide, and verapamil on aluminium oxide and silica gel in horizontal chambers. The best mobile phase for separation on the alumina plates was tetrahydrofuran - hexane - 25 % ammonia 25:24:1 and on silica chloroform - tetrahydrofuran - ethanol - 25 % ammonia 81:19:20:1. Detection under UV light at 210 nm and by use of different reagents. Quantification of mexiletine hydrochloride in capsules was performed densitometrically at 254 nm. Correlation coefficient in the concentration range 20 - 45 µg per band was 0.9974, with RSD of 5.23 %.

Quality control, quantitative analysis, densitometry, antiarrhythmic drugs 32a

95 080 P. N. PRESANNAKUMARAN, Ann Mary ISAAC* (Thejus Tharu. College of Pharmaceutical Sciences, Medical College, Trivandrum, India): Estimation of rabeprazole using HPTLC. 56th IPC 2004, Abstract No. GP-48. HPTLC of rabeprazole sodium in tablet dosage form on silica gel with ethyl acetate - methanol 9:1. Optimization of experimental parameters such as bandwidth, chamber saturation time, solvent front migration, and mobile phase composition. Quantitative determination by scanning at 260 nm. The Rf value was 0.59. The method was linear with a correlation coefficient of 0.99, recovery was 98.81 %.

Pharmaceutical research, quality control, comparison of methods, postchromatographic derivatization, quantitative analysis, densitometry, HPTLC, rabeprazole 32a

95 081 Alina PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Silesian Academy of Medicine, 4, Jagillonska Street, PL-41200 Sosnowiec, Poland): Study of lipophilicity and application of selected topological indexes in QSAR analysis of nicotinic acid derivatives. Part I. J. Planar Chromatogr. 17, 275-279 (2004). HPTLC of nicotinic acid and selected derivatives (methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate, benzyl nicotinate, nicotinamide, N-methyl nicotinamide) on RP-18 with methanol - water in different volume proportions after chamber saturation for 30 min. Detection under UV light at 254 nm. Investigation of the lipophilicity by TLC and use of the data for quantitative structure-activity relationships.

Pharmaceutical research, HPTLC, qualitative identification, quantitative structure-activity relationships 32a

95 079 T. K. RAVI, Prabhathi KITANIA, M. GANDHIMATHI, P. RAVIMATHI*, Satheesh KUMAR N. (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, 395, Sarojini Naidu Road, Coimbatore 641 044, India): HPTLC method for the estimation of mirtazapine from tablet formulation. IPC 56th 2004, Abstract No. GP-17. HPTLC of mirtazapine in tablet dosage form on silica gel with chloroform - methanol 1:9. The Rf value was 0.50 - 0.52, the linearity range was 0.3 - 1.5 mg/spot. Quantitative determination by scanning at 295 nm. The method was validated for accuracy, precision, linearity, specificity, LOD, and LOQ.

Pharmaceutical research, quality control, comparison of methods, postchromatographic derivatization, quantitative analysis, densitometry, HPTLC, mirtazapine 32a

95 105 M. SAJEWICZ et al., see section 38

95 091 R. T. SANE, S. N. MENON, M. MOTE*, S. INAMDAR, A. MENEZES (*TDM laboratories, Plot No. 194, Scheme No. 15, Road No. 15, Sion (E), Koliwada, Mumbai-22, India): High-performance thin-layer chromatographic determination of aceclofenac in the bulk drug and in pharmaceutical preparations. J. Planar Chromatogr. 17, 238-240 (2004). HPTLC of aceclofenac and mosapride citrate (as internal standard) on silica gel in a twin-trough chamber equilibrated with the mobile phase with toluene - methanol - ethyl acetate - glacial acetic acid 550:250:200:1. Quantitative determination by densitometry at 284 nm.

Quality control, densitometry, HPTLC, quantitative analysis, aceclofenac 32a

95 085 Sapna SHRIKUMAR, A. SAIT, A. JITENDRA*, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore-614044, India): An HPTLC method for the standardization of Curculigo Orchioides and its formulations for antioxidant activity using gallic acid as standard. IPC 56th 2004, Abstract No. G-18. Curculigo Orchioides (Amary llidaceae) is used in various ayurvedic formulations. The rhizomes contain about 5.78 % total phenolis, gallic acid being the major component of the alcoholic extracts. HPTLC of gallic acid on silica gel with toluene - ethyl acetate - glacial acetic acid 25:15:1. The Rf value of gallic acid was 0.19, the linearity range of 150-750 ng/spot. Rhizomes were found to contain 2.54 % gallic acid, formulations contained 5.13 % gallic acid, recovery was 99.5 %.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, HPTLC, Curculigo Orchioides, gallic acid 32a

95 086 Sapna SHRIKUMAR, A. SAIT, Manju GOPI*, A. SUGANTHI, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore 641 044, India): HPTLC method for the standardization of Aphanamixis Polystachya for its antioxidant activity using gallic acid as standard. 56th IPC 2004, Abstract No. GP-36. HPTLC for the standardization of gallic acid in alcoholic extracts of Aphanamixis polystachya (Meliceae) on silica gel with toluene - ethyl acetate - formic acid - methanol 15:15:4:1. Rf value of gallic acid was 0.45, linearity was 15 - 75 mg/mL. Formulations were found to contain 9.56 % of gallic acid. Gallic acid is the main phenolic compound and can be used for standardization of the crude drug.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, Aphanamixis polystachya, gallic acid 32a

95 087 Sapna SHRIKUMAR*, S. CICY, M. SUKUMAR, T. K. RAVI (*Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore 641 044, India): HPTLC method for the estimation and quantification of gallic acid in some ayurvedic formulations of triphala. IPC 56th 2004, Abstract No. GP-26. HPTLC of triphala, an ayurvedic formulation containing about 3.60 % of total phenolics. Separation of alcoholic triphala extracts on silica gel with n-hexane - ethyl acetate 2:1. Rf value of the main spot gallic acid was 0.04 in triphala and its formulation. The method was found to be very specific for gallic acid having a linearity range of 0.2 - 1.6 mg/mL. Several formulations analyzed by HPTLC contained 5.2 - 7.6 % of gallic acid. The reported method is suitable for estimation of gallic acid in raw material and formulations.

Pharmaceutical research, quality control, quantitative analysis, densitometry, comparison of methods, postchromatographic derivatization, HPTLC, triphala 32a

95 088 R. SKIBINSKI, Genowefa MISZTAL* (*Department of Medicinal Chemistry, Medical University of Lublin): Determination of fluvoxamine and moclobemide in tablets by densitometric and videodensitometric TLC. J. Planar Chromatogr. 17, 224-228 (2004). TLC of fluvoxamine and moclobemide on silica gel in horizontal chambers with benzene - acetone - ethanol - 25 % ammonia 9:7:2:1. Densitometric detection and quantification were performed at 249 nm and 236 nm, respectively. The range of linearity was 1 - 10 µg per spot; the RSD was less than 2.5 % for densitometry and less than 5.1 % for videodensitometry.

Quality control, densitometry, quantitative analysis, fluvoxamine, moclobemide 32a

95 009 B. SPANGENBERG et al., see section 3f

95 089 G. SUBRAMANIAN, CH. SRIDEVI NAIDU, Gautam MISHRA, Varadaraj BHAT, N. UDUPA* (*College of Pharmacy, Manipal, Karnataka, India): Stability indicating HPTLC determination of oxcarbazepine in tablets. 56th IPC 2004, Abstract No. GP-47. Stability indicating HPTLC determination of oxcarbazepine in tablet dosage form on silica gel with toluene - methanol 4:1. The Rf value of oxcarbazepine was 0.17. Quantitative determination by scanning at 255 nm. The compound was subjected to acid and alkali hydrolysis, oxidation, dry heat, and photo degradation. All degraded products were well resolved from the pure drug. The method was validated for accuracy, precision, linearity, robustness, and recovery.

Pharmaceutical research, quality control, postchromatographic derivatization, comparison of methods, quantitative analysis, densitometry, HPTLC, oxcarbazepine 32a

95 090 A. SUGANTHI VIPIN PRAKASH*, Sapna SHRIKUMAR, K. A. Mirkasim, T. K. RAVI (*College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641 044, India): HPTLC method for the simultaneous estimation of valdecoxib and tizanidine hydrochlo-

ride in tablets. IPC 56th 2004, Abstract No. GP-3. Simultaneous HPTLC determination of valdecoxib and tizanidine in tablets on silica gel with n-butyl acetate - formic acid - chloroform 7:3:2. Quantitative determination by densitometric scanning at 283 nm. The Rf values of valdecoxib and tizanidine were 0.78 and 0.39 respectively. Linearity range was 200 - 1000 ng/spot and 60 - 300 ng/spot respectively. Mean recovery for both of the compounds was 99.57 - 101.28 %. The method was validated for accuracy, precision, linearity, LOD, and LOQ.

Pharmaceutical research, quality control, densitometry, quantitative analysis, comparison of methods, postchromatographic derivatization, HPTLC, valdecoxib, tizanidine 32a

95 093 S. TAMBE, S. KALE, P. SHAH, S. CHHAJED* (*M.G.V's Pharmacy College, Panchavati, Nasik, India): HPTLC analysis of beta-carotene in oral solid dosage forms. IPC 56th 2004, Abstract No. CP-32. A stability indicating HPTLC method has been developed for the analysis of solid dosage forms containing beta-carotene. HPTLC of beta-carotene on silica gel with petrol ether (40-60 °C) - methanol - toluene 4:8:1. Rf value of beta-carotene was 0.65-0.70. Quantification by densitometric evaluation at 460 nm. The method was validated for accuracy, precision, linearity, and stability, and can be adopted for routine analysis of beta-carotene in formulations.

Pharmaceutical research, quality control, densitometry, comparison of methods, postchromatographic derivatization, quantitative analysis, beta-carotene 32a

95 094 S. TAMBE, S. KALE, S. KULKARNI*, S. CHHAJED (*M.G.V's Pharmacy College, Panchavati, Nasik, India): HPTLC analysis of ondansetron in oral solid dosage forms. IPC 56th 2004, Abstract No. GP-15. Stability indicating HPTLC determination of ondansetron in solid oral dosage forms on silica gel with chloroform - methanol 4:1. Quantitative determination by scanning at 310 nm. The Rf value was 0.62 - 0.64, linearity was 40 - 120 ng. The average recovery was 100.01 %. The method was found suitable for routine analysis of formulations containing ondansetron.

Pharmaceutical research, quality control, densitometry, quantitative analysis, comparison of methods, postchromatographic derivatization, HPTLC, ondansetron 32a

95 034 J. D. VELICKOVIC et al., see section 23e

95 056 J. CHEN (Chen Jiatang)*, J. LIU (Liu Junyi), J. SU (Su Juan) (*Nanjing Tongrentang Pharm. Co., Ltd., Nanjing 210012, China): (Study of the quality standard for compound Yiqi granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 167-169 (2005). TLC on silica gel with 1) ethyl acetate - methyl ethyl ketone - formic acid - water 10:1:1:1; 2) chloroform - methanol 20:1; 3) chloroform - formic acid - water 13:7:2. Detection 1) under UV 254 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating at 105 ºC. Identification by fingerprint technique. Quantification of astragaloside IV by densitometry at 530 nm. Validation of the method by investigation of linearity (1.12 µg - 5.60 µg, r = 0.999); precision (RSD = 1.63 %, n= 5 within plate and RSD = 2.3 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 3.12 %); standard addition recovery (97.69 %, RSD = 2.10 %, n = 5). The results for three real life samples are given.

Pharmaceutical research, herbal, quality control, traditional medicine, qualitative identification, quantitative analysis, densitometry, astragaloside IV 32c

95 057 ZH. CHEN (Chen Zhongyi)*, T. YAO (Yao Tongwei), Y. PENG (Peng Yunzhen), ZH. ZHANG (Zhang Zhijian (*Dep. Pharm. Sci., Zhejiang Univ., Hangzhou, Zhejiang 310006, China): (Assay and related impurity detection for magnesium fructose - diiphosphate) (Chinese). J. Chinese Pharm. Anal. 25 (1), 86-90 (2005). TLC of fructose, fructose-6-phosphate and related impurities on silica gel - carboxy methyl cellulose (CMC) -Na phase with n-butanol - acetone - glacial acetic acid - ammonia - water 35:15:20:3:27. Detection by spraying with 1 % sodium periodate solution followed by spraying with a solution of benzidine - ethanol - acetone - hydrochloric acid

- water 0.8 g:80 mL:30 mL:1.5 mL:70 mL. Identification by fingerprint technique. Quantification by comparison with standards. The detection limits were investigated. In addition, the content of magnesium fructose diphosphate was determined by diphenylamine colorimetric method, and the related impurities are determined with the phosphomolybdic acid colorimetric method.

Pharmaceutical research, herbal, quality control, qualitative identification, quantitative analysis, magnesium fructose, diphosphate, diphenylamine colorimetric method 32c

95 058 J. CUI (Cui Jiucheng)*, X. SONG (Song Xiaomei), Y. CAI (Cai Yan) (*Shanxi Coll. TCM, Xianyang, Shanxi, 712083, China): (Analysis of the processing principle of Fructus Schisandrae Sphenantherae by steaming with wine) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 176-178 (2005). HPTLC on silica gel with 1) cyclohexane; 2) cyclohexane - ethyl acetate 9:1. Detection 1) by spraying with 5 % phosphomolybdic acid in ethanol; 2) by spraying with vanillin - conc. H2SO4 solution. Identification of volatile oil by fingerprint technique. Determination of total lignan content by spectrophotometry. Analysis of the processing principle by comparison of the contents of the volatile oil and lignans in the extracts obtained by using different processing procedures, and discussing of the optimal processing procedures.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, volatile oil 32c

95 062 J. GAO (Gao Jiarong)*, J. ZHANG (Zhang Junru) (*No.1 Affiliated Hosp., Anhui Coll. TCM, Anhui, Hefei 230031, China): (Study of the quality standard for Qieyou Tangjiang extract) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 94-96 (2005). HPTLC on silica gel with 1) n-butanol - glacial acid - water 19:5:5; 2) petroleum ether (30-60 ºC) - formic acetate - formic acid 15:5:1; 3) chloroform - ethyl acetate - methanol - formic acid 200:25:50:1. Detection 1) by spraying with 0.5 % ninhydrin in ethanol; 2) under UV 365 nm; 3) by spraying with vanillin - H2SO4 solution and heating. Identification by fingerprint technique. Quantification of emodin by densitometry at 440 nm. Validation of the method by investigation of linearity (0.1 µg - 0.5 µg, r = 0.998); precision (RSD = 3.8 %, n = 15 within plate and RSD = 3.2 %, n = 5 plate to plate); reproducibility of five time assay towards the same sample (RSD = 4.4 %); standard addition recovery (99.5 %, RSD = 2.2 %, n = 5). The results for some real life samples are given.

Pharmaceutical research, traditional medicine, quality control, densitometry, HPTLC, quantitative analysis, qualitative identification, emodin 32c

95 063 CH. GUO (Guo Changqiang)*, J. LIU (Liu Jinxing), M. ZHANG (Zhang Min), Y. LI (Li Yan), CH. ZHOU (Zhou Chuanguo) (*Shandong Acad. TCM, Jinan, Shangdong 250014, China): (Study of the quality standard for Yijing Bushen granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), 716-719 (2004). TLC on silica gel with 1) ethyl acetate - chloroform - formic acid 3:2:1; 2) chloroform - methanol 7:2; 3) petroleum ether (60-90 ºC) - ethyl acetate 7:3. Detection 1) under UV 365 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating at 105 ºC for 5 min; 3) by spraying with vanillin - H2SO4 solution. Identification by fingerprint technique. Quantification of icarrin by HPLC.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, icarrin 32c

95 064 J. GUO (Guo Jingqiang)*, R. NIU (Niu Ruijie), G. HUANG (Huang Guifen) (*Tianjin municip. Inst. Drug Cont., Tianjin 300070, China): (Determination of astragaloside in Zilongjin tablets by thin-layer chromatography) (Chinese). J. Chinese Trad. and Herb. Drugs (Zhongcaoyao), 36 (2), 222-224 (2005). TLC on silica gel with chloroform - methanol - water 70:35:4. Detection by spraying with 10 % H2SO4 in ethanol and heating at 105 °C for 5 min. Identification by fingerprint technique. Quantification by densitometry at 530 nm. Validation of the method by investigation of linearity (0.458 µg - 2.748 µg, r = 0.998); precision (RSD = 2.48 %, n = 5 within plate and RSD = 4.69 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 2.41 %); and standard addition recovery (96.11 %, RSD = 1.91 %, n = 5). The results for

real life samples are given.

Pharmaceutical research, traditional medicine, quality control, herbal, doping, quantitative analysis, qualitative identification, densitometry, astragaloside 32c

95 065 W. GUO (Guo Wenping), X. BAI (Bai Xiaoshi)*, L. LI (Li Laixiu) (*Sanmenxia People's Hosp., Sanmenxia, Henan 472000, China): (Preparation of Tianma Toufengling capsules and study of its quality standard) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 246-248 (2005). TLC of extracts prepared by different processing technology on silica gel with 1) petroleum ether (60 - 90 ºC) - chloroform - methanol 10:3:2; 2) chloroform - methanol 5:1; 3) benzene - glacial acetic acid 4:1. Detection 1) under UV 254 nm; 2) by spraying with 1 % vanillin - H2SO4 solution and heating at 105 ºC. Identification by fingerprint technique. Quantification of gastrodine by HPLC. Discussion of using the procedures for monitoring the preparation process and the quality control of the medicine products.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, gastrodine 32c

95 067 SH. HU (Hu Shuangfeng) (Ningbo Municip. Inst. Drug Cont., Ningbo, Zhejiang 315040, China): (Differentiation and identification of Xanthium sibiricum Patr. seed and the phoney, Xanthium Spinosam L. seed) (Chinese). Chinese J. Hosp. Pharm. (Zhongguo Yiyuan yaoxue Zazhi) 25 (2), 185-187 (2005). HPTLC on silica gel with n-butanol - glacial acetic acid - water 4:1:5. Detection by exposing to ammonia vapors. Identification by fingerprint technique combined with morphological differentiation and UV spectra comparison.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, differentiation and identification 32c

95 072 CH. LI (Li Chuncheng), X. YANG (Yang Xinghao)*, J. CUI (Cui Jinghao, Y. WANG (Wang Yanfei), J. ZHU (Zhu Jia) (*Pharm. R & D Centre, Nanjing Normal Univ., Nanjing 210097, China): (Separation and purification of the active fraction of Sinisan powder with macroporous resins) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 84-87 (2005). TLC screening of Sinisan powder extracts, purified with macroporous resins, on silica gel with 1) n-butanol - ammonia - ethanol 7:3:1; 2) chloroform - methanol 7:1. Detection 1) by spraying with 5 % AlCl3 in ethanol 2) by spraying with 5 % vanillin - H2SO4 solution. Identification by fingerprint technique. Screening of purification conditions by evaluation of the content of the active principle, and yield of the purified products. Type HP20 macroporous resin has been concluded to be the optimum for active fraction of the recipe in purification efficiency.

Herbal, pharmaceutical research, traditional medicine, quality control, qualitative identification, separation and purification 32c

95 073 C. LI (Li Cunman)*, L. LI (Li Lanfang), Q. ZHANG (Zhang Qinzeng) (*Hebei Provin. Acad. TCM, Shijiazhuang, Hebei 050021, China): (Study of the quality standard for complex Xiaojingtong capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (8), 631-634 (2004). TLC on silica gel with 1) n-hexane - ethyl acetate 9:1; 2) chloroform - diethyl ether 1:1; 3) n-butanol - ethyl acetate - water 4:1:5. Detection by 1) exposing to iodine vapors; 2) spraying with 10 % H2SO4 in ethanol and heating at 105 ºC. Identification by fingerprint technique. Quantification of astragaloside by densitometry at 530 nm. Validation of the procedure by investigation of linearity (1.1 - 5.5 µg per spot), precision (RSD = 1.82 %, n = 5 within plate and 1.99 %, n = 5 plate to plate), repeatability by standard addition recovery (100.1 %, RSD = 1.59, n = 6), etc. The results are given for a group of real samples.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, astragaloside 32c

95 074 F. LI (Li Fengqin) (Puyang Municip. Inst. Drug Cont., Puyang, Henan 457000, China): (Identi-

fication of the main components and the dosage optimization in Shujin Qiefeng capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), app.17-19 (2004). HPTLC on silica gel with 1) toluene - acetone - ethanol - ammonia 20:25:3:2; 2) n-hexane - ethyl acetate - glacial acetic acid 15:5:1; 3) n-hexane - ethyl acetate - ammonia 20:20:1. Detection 1) by spraying with 5 % potassium iodobismuthate solution; 2) by spraying with 1 % potassium permanganate in diluted sulfuric acid followed by heating at 120 ºC, and under UV 360 nm. Identification by fingerprint technique. Determination of the content of aconitine by comparison with standard.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, aconitine 32c

95 076 Q. MENG (Meng Qing)*, H. LIANG (Liang Hanming), G. CHEN (Chen Gengfu), X. GUO (Guo Xiaoling), Y. FENG (Feng Yifan) (*Guangdong Coll. Pharm. Guangzhou 510224, China): (Study of the quality standard for Tongfeng Huadyting tincture) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 158-161 (2005). HPTLC on silica gel with 1) cyclo-hexane - ethyl acetate - methanol 4:5:1; 2) n-hexane - ethyl acetate 3:1; 3) n-hexane - ethyl acetate 9:1; 4) cyclohexane - ethyl acetate - diethylamine 45:20:3. Detection 1) under UV 365 nm; 2) by spraying with 10 % H2SO4 in ethanol and heating; 3) by spraying with diluted potassium iodobismuthate solution followed by spraying with sodium nitrite solution in ethanol. Identification by fingerprint technique. Semi-quantitative determination of aconitine by comparison with the standard. Quantification of strychnine by HPLC. The results for some real life samples are given.

Pharmaceutical research, traditional medicine, quality control, HPTLC, densitometry, quantitative analysis, qualitative identification, aconitine, strychnine 32c

95 077 Q. PENG (Peng Qian)*, H. ZHAO (Zhao Hua), GUO ZHANG (Zhang Guozhu) (*Hanzhong municip. Inst. Drug Cont., Hanzhong, Shanxi 723000, China): (Pharmacognostic identification of Saruma henryi and differentiation of Asarum siebodii) (Chinese). J. Chinese Trad. and Herb. Drugs (Zhongcaoyao), 36 (2), 277-280 (2005). HPTLC on silica gel with toluene - ethyl acetate - water - formic acid 20:10:1:1. Detection under UV 365 nm. Identification of volatile oil by fingerprint technique, combined with microscopy and a chemical method. Quantification of aristolochic acid A by HPLC.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, aristolochic acid A 32c

95 084 L. SHEN (Shen Linni)*, H. YU (Yu Haihong), Y. ZHENG (Zheng Yan) (*Zhejiang Deqing County TCM Hosp., Deqing, Zhejiang 313200, China): (Determination of chlorogenic acid in Liyin tablets by thin-layer chromatography) (Chinese). Chinese J. Hosp. Pharm. (Zhongguo Yiyuan yaoxue Zazhi) 25 (1), 90-92 (2005). TLC on silica gel with chloroform - ethyl acetate - formic acid 2:2:1. Detection under UV light. Identification by fingerprint technique. Quantification by densitometry at 325 nm. Validation of the method by investigation of linearity (0.52 µg - 4.68 µg, r = 0.9992); precision (RSD = 2.20 %, n = 5); reproducibility of five time assay towards the same sample (RSD = 3.10 %); standard addition recovery (99.0 %, RSD = 0.26 %, n = 5). The results for three real life samples are given.

Pharmaceutical research, traditional medicine, quality control, herbal, doping, quantitative analysis, qualitative identification, densitometry, chlorogenic acid 32c

95 095 J. TANG (Tang Jingwen) (Shanghai Shuangji Pharm. Co., Ltd., Shanghai 201319, China): (Study of the quality control for Kangmoling capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 150-154 (2005). TLC on silica gel with 1) chloroform - methanol 15:2; 2) n-hexane - ethyl acetate 9:1; 3) toluene - ethyl acetate - acetone - methanol 50:25:25:3. Detection 1) by spraying with 10 % phosphomolybdic acid and heating; 2) under UV 365 nm; 3) by exposing to acetic anhydride vapors and heating at 140 - 160 ºC and under UV 365 nm. Identification by fingerprint technique. Quantification of flavone glycoside by HPLC. The results for three real life samples are given.

Pharmaceutical research, traditional medicine, quality control, densitometry, quantitative analysis, qualitative identification, flavone glycoside 32c

95 096 X. WANG (Wang Xiaoling) (Luoyang Municip. Inst. Drug Cont., Luoyang, Henan 471003, China): (Identification of the medicinal herb rhubarb and its preparations by thin-layer chromatography) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), app.19-20 (2004). HPTLC on silica gel with petroleum ether (30 - 60 ºC) - formic acetate - formic acid 15:5:1, at 11 ºC and humidity of 40 %. Detection by exposing to ammonia vapors. Identification by fingerprint technique and comparison with the standards.

Pharmaceutical research, traditional medicine, quality control, qualitative identification, HPTLC, emodin 32c

95 097 M. XIN (Xin Meiyu) (Guangdong Wannianqing Pharm. Co., Ltd., Shantou, Guangdong 515031, China): (Study of the quality standard for Buxie Danggui extract) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (2), 225-227 (2004). TLC on silica gel with 1) petroleum ether (30 - 60 ºC) - ethyl acetate 9:1; 2) benzene - glacial acetic acid 4:1. Detection 1) by spraying with 1 % vanillin solution and heating at 105 ºC for 10 min; 2) under UV 365 nm. Identification by fingerprint technique. Quantification of ferulic acid by densitometry at 325 nm. Validation of the method by investigation of linearity (0.16 µg - 1.6 µg, r = 0.9992); precision (RSD = 1.8 %, n= 5 within plate and RSD = 2.3 % plate to plate); reproducibility of five time assay towards the same sample (RSD = 0.5 %); and standard addition recovery (97.17 %, RSD = 0.9 %, n = 5). The results for three real life samples are given.

Pharmaceutical research, traditional medicine, quality control, herbal, doping, quantitative analysis, qualitative identification, densitometry, ferulic acid 32c

95 098 J. ZHANG (Zhang Junping)*, X. HUANG (Huang Xiaolan), H. LE (Le Haiping) (*Nanchang Municip. Inst. Drug Cont., Nanchang, Jiangxi 330003, China): (Study of the quality control of Kangbingdu oral liquid) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App.12-14 (2004). TLC on silica gel with 1) n-butanol - glacial - water 19:5:5; 2) chloroform - methanol - glacial acetic acid - 17:2:1; 3) two fold development with benzene - acetone 9:1. Detection 1) by spraying with ninhydrin solution and heating at 105 ºC; 2) by spraying with 5 % vanillin solution and heating; 3) by spraying with a solution of 8 % vanillin in ethanol - H2SO4, and heating at 105 ºC. Identification by fingerprint technique. Quantification of phyllyrin by densitometry at 280 nm. The quantitative procedure is validated by investigating its linearity (1 - 5 µg/spot, r = 0.9998); precision (RSD = 0.36 % n = 5); repeatability (RSD = 2.92 % n = 5) and standard addition recovery (99.6 %, RSD = 2.4 %, n = 5), etc. The determination results are given for a group of real life samples.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, densitometry, phyllyrin 32c

95 099 Y. ZHANG (Zhang Yujie)*, H. HUANG (Huang Haixin), H. Tian (Tian Hong) (*Nanyang Municip. Inst. Drug Cont., Nanyang, Henan 473061, China): (Study of the quality standard for Pingxiao capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App.14-16 (2004). TLC on silica gel with 1) toluene - ethyl acetate - formic acid 25:20:4; 2) ethyl acetate - methanol - water 100:17:13. Detection 1) by spraying with diazotized para-nitroaniline solution; 2) by spraying with 5 % AlCl3 in ethanol and under UV 365 nm. Identification by fingerprint technique. Quantification of hesperidin by HPLC.

Pharmaceutical research, traditional medicine, quality control, herbal, quantitative analysis, qualitative identification, hesperidin 32c

95 100 ZH. ZHAO (Zhao Zhi Qiang) (Shanghai Leiyunshang Pharm. Co., Ltd., Shanghai 201517, China): (Study of the quality standard for Funing granules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (9), App. 3-5 (2004). TLC on silica gel with 1) n-hexane - ethyl acetate 9:1;

2) chloroform - methanol 5:2; 3) n-hexane - ethyl acetate 3:1. Detection 1) under UV 365 nm; 2) by spraying with 10 % H_2SO_4 in ethanol and heating at 110 ºC; 3) by spraying with 5 % $FeCl_3$ in ethanol. Identification by fingerprint technique. Quantification of icariine by HPLC.

Pharmaceutical research, traditional medicine, quality control, herbal, doping, quantitative analysis, qualitative identification, icariine 32c

95 101 L. ZHOU (Zhou Lingying)*, X. CAO (Cao Xiaolan), X. BAI (Bai Xiaochun) (*Sichuan Enwei Inst. TCM, Chengdu, Sichuan 610041, China): (Study of the quality standard for Jieeryin effervescent tablets) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 27 (1), 34-37 (2005). TLC on silica gel with 1) benzene - acetone - ethyl acetate - ammonia water 10:15:20:1; 2) ethyl acetate - butanone - formic acid - water 5:3:1:1; 3) n-butanol - glacial acetic acid - water 7:1:2. Detection 1) by spraying with potassium iodobismuthate solution; 2) by spraying with 2 % $FeCl_3$ in ethanol; 3) by spraying with vanillin - H_2SO_4 solution and heating; 4) under UV light. Identification by fingerprint technique. Quantification of geniposide by HPLC. The results for ten real life samples are given.

Pharmaceutical research, herbal, quality control, traditional medicine, qualitative identification, quantitative analysis, densitometry, geniposide 32c

95 052 S. BASAR, Angelika KOCH* (*Frohme-Apotheke, Frohmestrasse 14, 22457 Hamburg, Germany): Test of the stability of olibanum resins and extracts. J. Planar Chromatogr. 17, 479-482 (2004). HPTLC of beta-boswellic acid (BA), acetyl-beta-BA, keto-BA, and acetyl-keto-BA and ethanolic extracts of olibanum resin on silica gel with toluene - ethyl acetate - formic acid - heptane 80:20:3:10 in a twin trough chamber without chamber saturation. Quantitative determination by reflectance measurement at 245 and 285 nm. Also two dimensional development with the same mobile phase in the second direction.

Quality control, densitometry, HPTLC, quantitative analysis 32e

95 054 Anne BLATTER*, E. REICH (*CAMAG Laboratory, Sonnenmattstr. 11, CH-4132 Muttenz, Switzerland): High performance thin-layer chromatographic analysis of aristolochic acid in Chinese drugs. J. Planar Chromatogr. 17, 355-359 (2004). HPTLC of aristolochic acid A, B, and C and numerous plant extracts on silica gel in a saturated twin-trough chamber using the upper phase of the mixture toluene - ethyl acetate - water - formic acid 20:10:1:1. Quantitative determination by fluorescence measurement at 366 nm after derivatization with tin(II) chloride reagent. The working range and linearity, LOD and LOQ (based on the calibration plot), and precision (n = 6), were validated with methods described by K. Ferenczi-Fodor et al., J. AOAC Int. 84 (2001) 1265-1276. The stability of the analyte during chromatography was established by two dimensional chromatography. The new method enables visual detection of the acids with certainty at very low levels (400 pg absolute of aristolochic acid A) in plant material and can therefore be used for screening Chinese drugs to ensure their safety on the basis of absence of aristolochic acid.

Quality control, toxicology, HPTLC, quantitative analysis, densitometry, aristolochic acid 32e

95 012 G. HORVÁTH et al., see section 4e

95 075 Q. MA (Ma Quanming)*, SH. LI (Li Shengyou) (*Qinghai Provin. People's Hosp., Xining, Qinghai 810007, China): (Preparation and quality control of Complex Zhike capsules) (Chinese). Chinese J. Trad. Pat. Med. (Zhongchengyao) 26 (8), Append. 9-11 (2004). TLC on silica gel previously immersed in 0.5 % NaOH solution, developed with 1) ethyl acetate - methanol - water 100:17:13; and 2) with the upper phase of toluene - ethyl acetate - formic acid - water 20:10:1:1. Detection by spraying with $AlCl_3$ solution and under UV 365 nm. Identification by fingerprint technique and comparison with the standard. Quantification of scutellarin by HPLC.

Traditional medicine, quality control, pharmaceutical research, herbal, quantitative analysis, qualitative identification, scutellarin 32e

95 019 Z. MALES et al., see section 8a

95 092 R. T. SANE*, S. N. MENON, S. SHAILAJAN, K. K. JARIPATKE (*Ramnarain Ruia College, Matunga, Mumbai-19, India): High-performance thin-layer chromatographic analysis of Asteracantha longifolia Nees. for determination of pharmacokinetics. J. Planar Chromatogr. 17, 483-485 (2004). HPTLC of plant and plasma extracts on prewashed silica gel with toluene - ethyl acetate - methanol 30:3:1 in a twin-trough chamber previously equilibrated with the mobile phase. Quantitative determination by fluorescence/reflectance measurement at 366 nm.

Pharmaceutical research, traditional medicine, HPTLC 32e

95 048 E. A. ABOURASHED* (*Department of Pharmacognosy, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh Saudi Arabia 11451): Validation and application of an HPTLC method for the determination of parthenolide in feverfew herbal products. J. Planar Chromatogr. 17, 375-378 (2004). HPTLC of parthenolide and extracts of feverfew capsules on silica gel with ethyl acetate - n-hexane 3:2 in glass chambers presaturated for 30 min. Detection by dipping in p-anisaldehyde reagent and heating at 105 °C for 5 min, followed by immediate densitometric scanning at 543 nm. The method is precise with CV < 5%; calibration recovery of 101.12 +/- 4.11 % and overall accuracy of 101.14 +/- 4.47 %. The levels of parthenolide in the products analyzed ranged from 0.03 to 0.24 %.

Herbal, quality control, traditional medicine, HPTLC, densitometry, quantitative analysis, feverfew, parthenolide 32g

95 001 J. QU et al., see section 1

33. Inorganic substances

95 102 R. M. BAOSIC, D. M. MILOJKOVIC-OPSENICA, Z. L. TESIC* (*Faculty of Chemistry, University of Belgrade, Studentski trg 16, P. O. Box 158, 11001 Belgrade, Serbia and Montenegro): The effect of the electronegativity of donor atoms in coordinated beta-diketonato ligands on the chromatographic behavior of metal complexes. J. Planar Chromatogr. 17, 250-254 (2004). TLC of three series of beta - diketonato complexes of the type [M(acac)3-n(phacphac)n], [M(acac)3-n(phacphSac)n], and [M(acac)3-n(phSacphSac)n] (where M represents cobalt(III), chromium(III), or ruthenium(III), acac is the 2,4-pentanedionato ion, phacphac is the 1,3-diphenyl-1,3-propanedionato ion, phacphSac is 3-mercapto-1,3-diphenyl-prop-2-en-1-one, phSacphSac is the 3-mercapto-1,3-diphenyl-prop-2-en-1-thion ion, and n = 0-3) on silica gel with five mono-component (chloroform, toluene, dichloromethane, xylene, tetrahydronaphthalene) and five two-component eluents (n-amyl acetate - carbon tetrachloride 1:1, n-butyl acetate - carbon tetrachloride 2:3, chloroform - carbon tetrachloride 1:1 and 3:7, and dichloromethane - carbon tetrachloride 4:1. Separations were performed in a horizontal chamber after equilibration for 30 min. After development the colored spots were readily visible.

Qualitative identification 33a, 2c

95 103 Iva REZIC*, L. BOKIC, A. J. M. HORVAT (*Laboratory of Analytical Chemistry, Department of Textile Chemistry and Material Testing, Faculty of Textile Technology, University of Zagreb, Pierottieva 6, 10000 Zagreb, Croatia): TLC separation and identification of heavy metals present in cotton material. J. Planar Chromatogr. 17, 305-308 (2004). TLC of manganese(II), chromium(III), nickel(II), cobalt(II), iron(III), and zinc(II) on cellulose with acetonitrile - hydrochloric acid - water 73:15:12 as optimum ternary mobile phase. Detection by spraying with 0.1 g quercetin in 100 mL 2-propanol and 10 g dimethylglyoxime in 100 mL ethanol and exposition to

ammonia vapor. Recording of the colored spots under white light by means of a highly sensitive CCD color video camera.

Environmental, densitometry, qualitative identification, quantitative analysis 33a

35. Other technical products and complex mixtures

95 104 L. WILLIAMS*, R. SJOVIK, M. L. FALCK-PEDERSEN (*SINTEF Applied Chemistry, P. O. Box 124 Blindern, N-0314 Oslo, Norway): ChemScreen: Planar synthesis, separation and screening of antioxidants. J. Planar Chromatogr. 17, 244-249 (2004). TLC of thirty compounds (coumarin derivatives) including by-products on silica gel with ethyl acetate - hexane 2:3 or, for more polar synthesized compounds, with methanol - dichloromethane 7:93 in a saturated chamber. Detection under UV light at 254 nm, and by derivatization if necessary, e. g. by iodine reagent. Quantitative determination by densitometry at 292 - 363 nm. Direct screening of the reaction mixture on the plate for antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test radical.

Environmental, densitometry, quantitative analysis, ChemScreen 35b, 4e

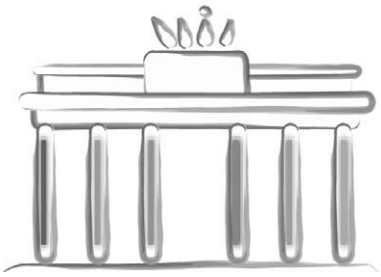
37. Environmental analysis

95 016 W. WEBER et al., see section 4e

38. Chiral separation

95 105 M. SAJEWICZ, R. PIETKA, Teresa KOWALSKA* (*Institute of Chemistry, Silesian University, 9 Szkolna Street, 40-006 Katowice, Poland): Chiral separation of S-(+)- and R-(-)-ibuprofen by thin-layer chromatography. An improved analytical procedure. J. Planar Chromatogr. 17, 173-176 (2004). TLC of R,S-(+-)-ibuprofen and S-(+)-ibuprofen on silica gel prewashed with methanol - water 9:1 and impregnated with L-arginine by conventional dipping for 2 s in a 0.03 mol/L solution of the compound in methanol. One-dimensional development with acetonitrile - methanol - water 5:1:1 plus several drops of acetic acid to adjust the pH to 4.8, and two-dimensional chromatography with the same solvent mixture in two directions. Quantitation by densitometry at 210 nm.

Quality control, densitometry, quantitative analysis, ibuprofen 38, 32a



INTERNATIONAL SYMPOSIUM FOR PLANAR CHROMATOGRAPHY/ INSTRUMENTAL HPTLC

Berlin (Germany), 9–11 October 2006

We are enthusiastic to learn how many analysts are now confronted with situations where Instrumental High-Performance Thin-Layer Chromatography is a suitable solution to their problems and is favored over better known and more widely used analytical methods. At the same time it is rather difficult nowadays to find a place for this technique in the minds of opinion leaders, even if the need exists in analytical laboratories.

This has arisen through inadequate information and training. How to select the method; how to use planar chromatography when it is described as a method of choice; avoidance of usual mistakes; which other samples may be well covered by this technique, etc.

To address these issues an exchange of knowledge is foremost, from which sprang our motivation to hold again an international event with the Interlaken series spirit, last held in 1997.

The first issue, which was held in Lyon (France) in 2003, held its promises with 116 participants from 17 countries, 2 workshops, 15 lectures, 26 posters, and a 4 manufacturers' session. After this success we are happy to welcome you to Berlin from the 9th to the 11th October 2006. Berlin, the capital of Germany, is dynamic, cosmopolitan and creative, allowing for every kind of lifestyle. East meets West in the metropolis at the heart of a changing Europe. Germany's largest city is a city of opportunities just waiting to be seized in all areas, like entertainment, recreation, economy, science and academic life. This appears to us as the best choice for this 2nd issue.

With the firm idea to look straight forward, the program expects to provide exactly what you need. The main symposium, featuring lectures and posters, will provide a real overview of the most recent advances in all the application fields where TLC has a place. Training and the manufacturers' sessions will be held in conjunction with the main symposium but functioning as a separate program. The training program is strongly focused to provide all your needs in a short time. This time a special workshop will be targeted on validation. The best experts will be there to guide you through each step and explain what you need to know, independent of your level of expertise.

Such an event wouldn't be possible without support from the manufacturers. As in all other high technology fields, they are at the forefront of innovation and technical improvement. Their collaboration with world leaders in the field from the early days of TLC has ensured development of Instrumental HPTLC to a modern analytical technique.

We are looking forward to share our knowledge, ideas and enthusiasm!

Call for papers

The scientific program will feature invited keynote speakers, selected submitted lectures and poster presentations. Contributions are invited from all areas of planar chromatography, especially from colleagues working in the pharmaceutical, food, environmental and medical fields.
Papers on theory, method development, validation, instrumental methods, hyphenated techniques, and quantitative applications in all areas of chemistry are most welcome.

Colleagues wishing to participate in the scientific program should submit a brief abstract to
Prof. Dr. Lothar W. Kroh, Institute for Food Chemistry, Technical University Berlin, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany or to
committee@hptlc.com
by 15th June 2006 stating whether they wish to present an oral presentation or a poster.

Abstract should be no more than 250 words and 2 figures/tables in a MS Word file (in Arial, font size 12, justified). The abstract should indicate the title, the author's names (with the presenting author underlined), affiliation (with e-mail address) and a brief description of the work to be presented. All submissions will be reviewed by the scientific organizing committee and authors will be informed of their decision by 31st July 2006.

All accepted contributions will be published in the symposium proceedings. Papers presented at the symposium will be also published after review, in a special issue of an analytical journal. Complete manuscripts must be delivered to the publishers' representative at the time of the symposium. Further details on the format for manuscripts will be obtained from the editorial office of the journal.

Prächromatographische in situ-Derivatisierung von Glyphosat und AMPA

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▲ *Dr. Holger Hegewald*

Dr. Hegewald* ist Leiter eines Analytiklabors in Évora, Portugal. Er beschäftigt sich mit Analysen von Pestiziden sowie von pflanzlichen Hormonen im Rahmen von Forschungsvorhaben mit der Universität Évora. Ausserdem werden Analysen von PAK in Wasser, Aflatoxin M1 in Milch, sowie von Anthocyanen und organische Säuren in Wein durchgeführt. Dabei wird ausschliesslich die Planar-Chromatographie benutzt.

Einleitung

Im Gegensatz zum vorhergehenden Beitrag, der eine in vitro-Derivatisierung nutzt, wird hier eine Methode zur prächromatographischen in situ-Derivatisierung vorgestellt.

Diese Art der Derivatisierung direkt auf der Startzone der Platte (in situ) ist nur in der Planar-Chromatographie möglich. Vorteile sind eine automatisierte, reproduzierbare Umsetzung mit minimalem Reagenzienverbrauch sowie die Vermeidung chlorhaltiger Lösungsmittel. Die in situ Derivatisierung dauert nicht viel länger als eine normale Probenauftragung.

Probenvorbereitung

Wird für diese Arbeit nicht dargestellt. Möglich ist z.B. die Festphasenextraktion an Ionenaustauschmaterialien.

Standardlösungen

Je 0,5 ng/µL Glyphosat und AMPA gelöst in Wasser-Methanol 3:2

Schicht

HPTLC-Platte Kieselgel 60 F_{254} (Merck), 20×10 cm.

Probenauftragung und Derivatisierung

Proben- und Standardlösungen flächenförmig auftragen, Auftragefläche 7×3 mm, Abstand zwischen den Bahnen 10 mm. Danach Übersprühen mit je 3 µL 75 mM Borat-NaOH-Puffer pH 10,5 und je 3 µL 9-Fluorenylmethylchlorformiat (FMOCCl, 2 mg/mL Acetonitril). Reaktionszonen mit einer Glasplatte abdecken und 10 min bei Zimmertemperatur inkubieren. Vor der Chromatographie 5 min mit dem Fön trocknen. Bei Bedarf könnte eine Fokussierung mit Methanol bis zur Auftragefläche-Oberkante die Peakschärfe verbessern (hier nicht durchgeführt).

Chromatographie

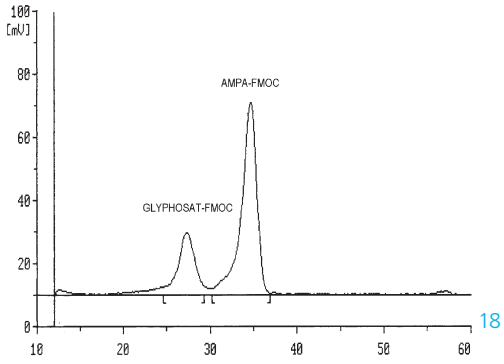
In der Doppeltrogkammer ohne Sättigung in n-Butanol-Essigsäure-Wasser 5:1:1, Laufstrecke vom unteren Plattenrand 70 mm. Nach Trocknung tauchen oder besprühen mit Paraffin-Toluen 1:1 - dadurch wird die Fluoreszenz ca. 10-fach erhöht.

Densitometrische Auswertung

TLC-Scanner mit CATS-Software, Fluoreszenzmessung mit der Quecksilberlampe (erlaubt eine empfindlichere Detektion) bei UV 265/Sekundärfilter M 360 nm, lineare Kalibration über die Peakhöhe

Ergebnisse und Diskussion

Die Bestimmungsgrenzen von Glyphosat-FMOC und AMPA-FMOC liegen bei 0,5 und 0,25 ng absolut pro Substanzzone. Die Kalibration ist zwischen 0,25 und 35 ng linear. Das erlaubt die sichere Quantifizierung von Glyphosat und AMPA in einem Wasservolumen von etwa 15 mL.



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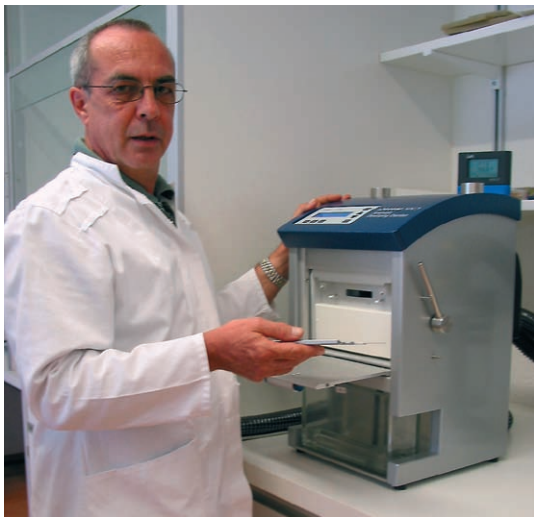
▲ *Densitogramm einer Standardbahn mit je 5 ng Glyphosat-FMOC und AMPA-FMOC*

Weitere Informationen sind beim Autor auf Anfrage erhältlich.

* Dr. Holger Hegewald, Lacrome Lda, Rua César Batista 6 D, P-7000-715 Évora, Portugal, lacrome@clix.pt

Qualität und Reproduzierbarkeit der Kammersättigung in der neuen Automatischen Entwicklungskammer ADC 2



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▲ *Daniel Handloser bei der Durchführung der ADC 2-Tests im CAMAG-Labor*

Die Qualität und Reproduzierbarkeit der Kammersättigung in der ADC 2 im Vergleich zur herkömmlichen Doppeltrogkammer wurde im CAMAG-Labor am Beispiel der Trennung eines Sulfonamidgemisches und den Fingerprints eines Rhabarberwurzelextrakts aufgezeigt.

Einleitung

Im CBS 94 (letzte Umschlagsseite) wurde die neue Automatische Entwicklungskammer ADC 2 bereits vorgestellt. Die ADC 2 erlaubt die vollautomatische Entwicklung von DC/HPTLC-Platten, und so erfolgt der wichtigste Schritt der Chromatographie, die Entwicklung, reproduzierbar und unabhängig von Umwelteinflüssen. Dies ist besonders wichtig, wenn das Fliessmittel leichtflüchtige Bestandteile enthält und die Methode sensibel auf Änderungen der mobilen Phase sowie Gasphase reagiert. Eine ausführliche Beschreibung der Vorgänge während der Chromatogramm-Entwicklung und die Bedeutung der Gasphase wurden bereits dargestellt in Parameter der Planar-Chromatographie, Chromatogramm-Entwicklung – Kammerform und Kammersättigung, Teil 1 (CBS 87).

Die Reproduzierbarkeit der chromatographischen Trennung ist nicht nur für die Quantifizierung unabdingbar, sondern auch ein wichtiges Element bei der Routine-Identifizierung der komplexen Fingerprints von Pflanzenextrakten (Botanicals). Alle entscheidenden Parameter können in der ADC 2 programmiert und automatisch überwacht werden. Neben Umwelteinflüssen werden manuelle Unregelmässigkeiten ausgeschlossen, während der Benutzer sich gleichzeitig weiteren Aufgaben zuwenden kann. Bestehende Methoden können weitgehend übernommen werden, da für die Entwicklung in der ADC 2 eine herkömmliche 20×10 cm Doppeltrogkammer eingesetzt wird.

Trennung von Sulfonamiden

Die Reproduzierbarkeit wurde anhand eines hinsichtlich der Gasphase sensiblen Modell-Systems ermittelt. Die Trennung der 5 Sulfonamide mit dem Fliessmittel Dichlorethan – Methanol – 2-Propanol – 25% Ammoniak 25:5:5:1 zeigt in Abhängigkeit von der Homogenität und Sättigung der Gasphase Schwankungen der R_F-Werte, und die Fliessmittelfront verläuft wellenförmig. In der Praxis wird eine gute Trennung der Sulfonamide jedoch mit einem einfacheren Fliessmittel erreicht. Unter ungesättigten und gesättigten Bedingungen wurden je fünf Platten in einer herkömmlichen Doppeltrogkammer entwickelt und mit fünf in der ADC 2 entwickelten Platten verglichen.

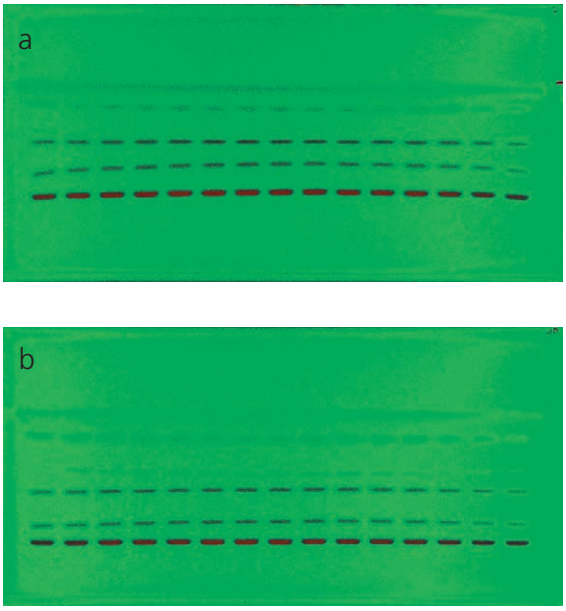
Chromatographische Bedingungen

- Standardlösung: Sulfamethoxazol (160 ng/µL), Sulfamerazin (50 ng/µL), Sulfamethazin (50 ng/µL), Trimethoprim (32 ng/µL) und Dimetridazol (100 ng/µL) wurden in Chloroform – Methanol 1:1 gelöst.
- Schicht: HPTLC-Platten Kieselgel 60 F_{254} (Merck), 20×10 cm
- Probenauftragung bandförmig mit DC-Probenautomat 4, 15 Bahnen, Auftragevolumen 5 µL, Bandlänge 8 mm, unterer Randabstand 8 mm, seitlicher Randabstand 15 mm, Bahnabstand 12 mm
- Chromatographie in der Doppeltrogkammer 20×10 cm bzw. ADC 2 bei unterschiedlicher Kammersättigung mit Dichlorethan – Methanol – 2-Propanol – 25% Ammoniak 25:5:5:1
- Densitometrische Auswertung mit TLC-Scanner 3 und winCATS-Software, Absorptionsmessung bei UV 254 nm

Ergebnisse und Diskussion

Generell kann der Einfluss der Kammersättigung am Verhalten der R_F-Werte und der Fliessmittelfront abgelesen werden. Unter gesättigten Bedingungen, wenn die Schicht mit Fliessmittelmolekülen beladen ist und man daher die Laufstrecke anhand der virtuellen Front misst, liegen die R_F-Werte tiefer als nach Entwicklung in der ungesättigten Kammer. In der ADC 2 werden bei gleicher Sättigungszeit tiefere R_F-Werte als in der Doppeltrogkammer beobachtet, demnach ist die ADC 2 dichter und garantiert bessere Sättigungsbedingungen.

Zunächst wurde im teilweise gesättigten System chromatographiert (10 mL Fliessmittel nur in einem Trog, 10 min Wartezeit, kein Filterpapier). Obwohl die Bedingungen in der Doppeltrogkammer und der ADC 2 vergleichbar sind, weisen die nachfolgenden Chromatogramme Unterschiede auf. Zum Einstellen der Platte muss die Doppeltrogkammer geöffnet werden. Dadurch wird die partielle Sättigung auf nicht reproduzierbare Weise gestört und das Chromatogramm zeigt in der Mitte höhere R_F-Werte (wellenförmig). In der ADC 2 stellt sich in der gleichen Zeit eine höhere Sättigung ein (etwas tiefere R_F-Werte), und das Chromatogramm ist gleichmässiger.

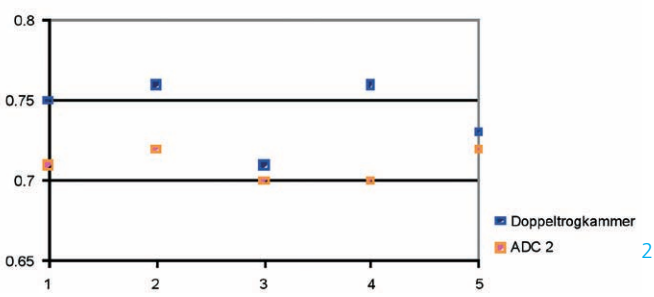


20

▲ *Trennung eines Sulfonamidgemisches unter teilweise gesättigten Bedingungen in der a) Doppeltrogkammer und b) ADC 2, Aufnahme unter UV 254 nm.*

In einem zweiten Experiment wurde die Kammersättigung erhöht, indem die Kammer vor der Entwicklung statt partiell 10 min nun 20 min mit Fliessmittel gesättigt wird, in der herkömmlichen Doppeltrogkammer wird für dieses Experiment ein Filterpapier in einen der zwei Tröge gestellt und mit 10 mL Fliessmittel benetzt. Auch der zweite Trog wird mit 10 mL Fliessmittel beschickt und die Kammer geschlossen. Nach 20 min wird der Deckel abgehoben und die Platte in die Kammer gestellt. Diese manuelle Operation stört jedoch die vorher aufgebaute Kammersättigung, auch wenn sie sehr sorgfältig ausgeführt wird. Dies zeigt sich beim Vergleich der Resultate von Platte zu Platte: Die Homogenität der Chromatographie innerhalb einer Platte verbessert sich dadurch weiter, aber die pro Platte gemittelten R_F-Werte schwanken erheblich.

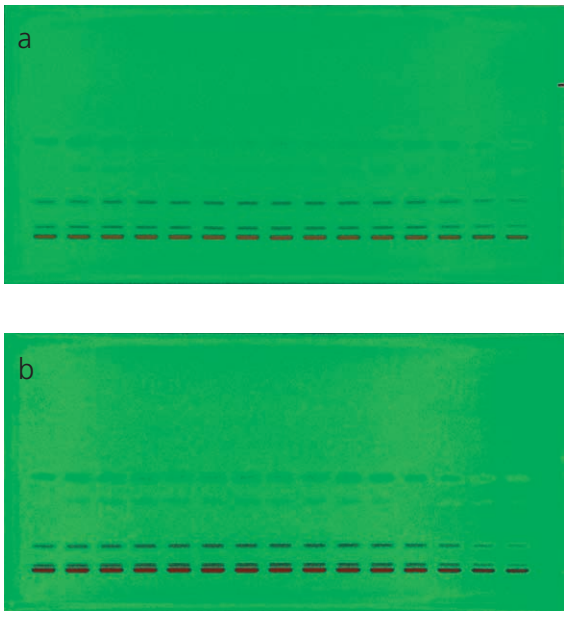
Die Entwicklung in der ADC 2 bei gleichem Vorgehen ist wieder reproduzierbarer als in der Doppeltrogkammer. Dies ist in nachfolgender Abbildung am Beispiel der R_F-Werte von Dimetridazol dargestellt. Da in der ADC 2 ein dickeres Filterpapier verwendet und dieses mit 25 mL statt 10 mL Fliessmittel benetzt werden muss, ist die erzielbare Kammersättigung etwas höher, was zu vergleichsweise niedrigeren R_F-Werten führt.



21

▲ *Über die Platte gemittelte R_F-Werte (n=15) von Dimetridazol auf 5 Platten (gesättigt 20 min mit Filterpapier) ohne Vorkonditionierung der Schicht. Die relative Standardabweichung der R_F-Mittelwerte (n= 5) ist ± 2.9 % in der Doppeltrogkammer bzw. ± 1.3 % in der ADC 2.*

Ein noch stabileres chromatographisches System wird erhalten, wenn die Platte in der gesättigten Kammer vor der Entwicklung 10 min der Gasphase ausgesetzt, d.h. vorkonditioniert, wird. Die RF-Werte werden dadurch weiter abgesenkt, und gleichzeitig erhöht sich die Reproduzierbarkeit der Chromatographie.

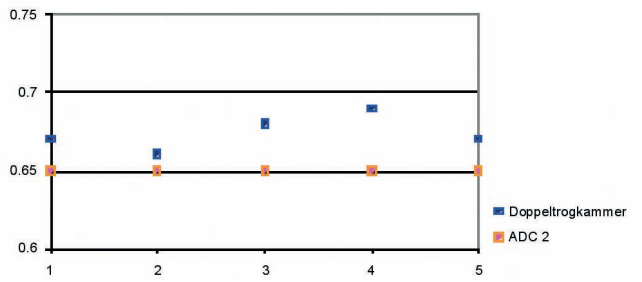


22

▲ *Trennung eines Sulfonamidgemisches in der gesättigten Kammer (20 min mit Filterpapier) mit 10 min Vorkonditionierung der Schicht; a) Doppeltrogkammer und b) ADC 2; Aufnahme unter UV 254 nm.*

In der Doppeltrogkammer wird in einem Trog ein Filterpapier mit 10 mL Fliessmittel benetzt. In den noch leeren zweiten Trog wird die Platte gestellt und während 10 min vorkonditioniert. Danach muss die Kammer kurz geöffnet werden, um in den leeren Trog 10 mL Fliessmittel geben zu können. Durch das einseitige Einfüllen des Fliessmittels verläuft die Chromatographie zunächst ungleichmässig, d.h. an der Einfüllseite etwas früher, ausserdem wird die aufgebaute Gasphase beim Öffnen des Deckels stark gestört.

In der ADC 2 fallen diese manuellen Schritte weg. Beide Tröge der Kammer werden gleichzeitig mit Fliessmittel befüllt. Während des Vorkonditionierens hängt die Platte im Gasraum. Sie wird nach der vorgegebenen Zeit (10 min) in das Fliessmittel gesenkt. Bei jeder Entwicklung wird so ein sehr gut reproduzierbares gesättigtes System erreicht. Die verbesserte Reproduzierbarkeit der RF-Werte zeigt nachfolgende Abbildung am Beispiel von Dimetridazol.



23

▲ *Über die Platte gemittelte R_F-Werte (n=15) von Dimetridazol auf 5 Platten; (gesättigt 20 min mit Filterpapier) plus 10 min Vorkonditionieren der Schicht. Die relative Standardabweichung der R_F-Mittelwerte (n= 5) ist jetzt ± 1.6 % in der Doppeltrogkammer bzw. 0 % in der ADC 2.*

Fingerprints eines Rhabarberwurzelextrakts

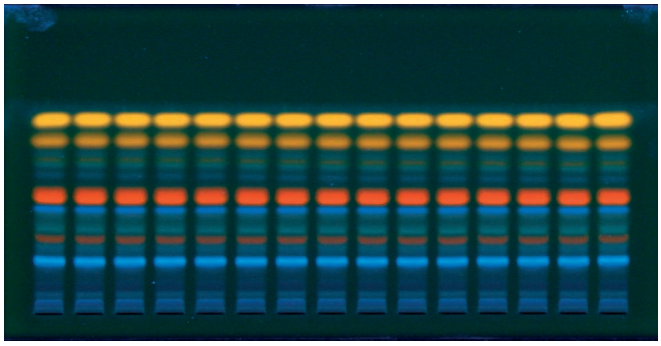
Die am Beispiel der Sulfonamid-Trennung dargestellten Einflüsse der Kammersättigung auf die Stabilität und Reproduzierbarkeit des chromatographischen Systems werden anhand eines Rhabarberwurzel-Extrakts bestätigt. Durch die reproduzierbare Entwicklung in der ADC 2 ist die Homogenität der R_F-Werte im resultierenden Fingerprint kein Zufallsergebnis.

Chromatographische Bedingungen

- Probenvorbereitung: 500 mg Rhabarberwurzel wurden 15 min bei 60 °C im Ultraschallbad mit 5 mL Methanol extrahiert, danach zentrifugiert.
- Schicht: HPTLC-Platte Kieselgel 60 F_{254} (Merck), 20×10 cm
- Probenauftragung bandförmig mit DC-Probenautomat 4, 15 Bahnen, Auftragevolumen 5 µL, Bandlänge 8 mm, unterer Randabstand 8 mm,

seitlicher Randabstand 15 mm, Bahnabstand 12 mm.

- Chromatographie in der ADC 2 mit 20 min Kammersättigung mit Methanol – Dichlormethan 1:4.



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▲ *Fingerprint eines Rhabarberwurzel-Extraktes, Aufnahme unter UV 366 nm.*

Ausblick

Neben der verbesserten Reproduzierbarkeit der R_F-Werte ermöglicht die ADC 2 auch das kontrollierte Einstellen der Schichtaktivität und gewährleistet damit die Reproduzierbarkeit der Trennung bei unterschiedlicher relativer Luftfeuchte im Labor. Die Anwendungen der ADC 2 sind vielfältig – **wir würden uns freuen, von Ihren eigenen praktischen Erfahrungen mit dem System zu hören!**

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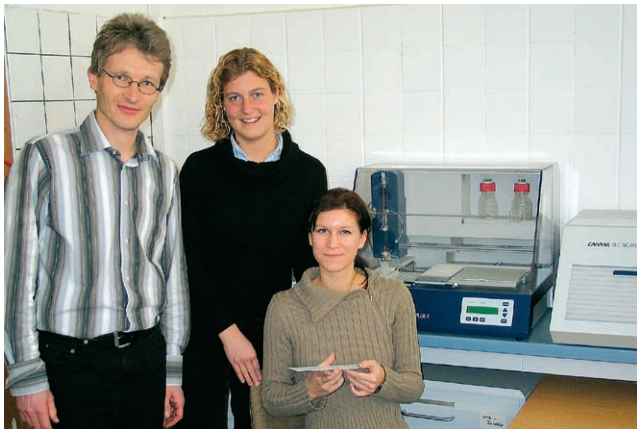
ADC 2 – Reproduzierbarkeit, Sicherheit und Komfort

Die Automatische Chromatogramm-Entwicklungskammer ADC 2 ist universell einsetzbar und liefert unübertroffen reproduzierbare Ergebnisse. Sie ist so konzipiert, dass alle in der konventionellen Entwicklung notwendigen manuellen Operationen automatisch ablaufen. Dabei wird, und das ist eine der Stärken der ADC 2, für den Entwicklungsvorgang eine herkömmliche CAMAG Doppeltrogkammer 20×10 cm eingesetzt. Somit können beim Einsatz dieser Kammer die bereits bestehenden Analysenvorschriften weitgehend übernommen werden.

Das bei manueller Entwicklung nötige Öffnen der Entwicklungskammer sowie alle personen- bzw. umweltabhängigen Einflussfaktoren werden in der ADC 2 beseitigt bzw. kontrolliert. Sobald die für das Chromatogramm vorgesehene Frontposition erreicht ist, wird die Platte automatisch aus der Kammer gehoben und unter strömungsoptimierten Bedingungen zügig und effektiv getrocknet.

Quantifizierung von in vitro Lipolyseprodukten mittels HPTLC



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▲ *Von links nach rechts: Prof. Dr. Karsten Mäder, Andrea Rübe, Sandra Klein*

Die Arbeitsgruppe von Professor Mäder*, Institut für Pharmazeutische Technologie und Biopharmazie, Martin-Luther-Universität Halle, beschäftigt sich unter anderem mit der Herstellung von bioabbaubaren Nanokapseln und Nanosphären auf Lipidträgerbasis. Mit diesen Arzneiformen kann eine erhöhte Resorption schwerlöslicher Arzneistoffe unter Verminderung der Variabilität erzielt werden. Beim Mechanismus der Resorptionserhöhung spielt die Verdauung des Arzneiträgersystems eine wichtige Rolle.

Einleitung

Zur Optimierung der Wirkstoffcarrier werden Testverfahren benötigt, die die Prozesse des Abbaus der Arzneiformen in vitro simulieren. Der künstliche Verdau mittels Lipasen ist hierbei ein häufig genutztes Verfahren. Dabei werden die Arzneiformen mit einem Gemisch aus Pancreatin, als Quelle für Lipase und Colipase, und Gallenextrakt inkubiert. Nach vollendetem Verdau werden die einzelnen Lipidbestandteile extrahiert, auf HPTLC-Platten aufgetragen, chromatographiert und anschliessend quantitativ detektiert. Die Planar-Chromatographie stellt ein geeignetes Verfahren zur Auswertung der in vitro assays von Verdauungsprozessen dar. Man kann im Gegensatz zur häufig verwendeten pH-stat Methode, bei der die entstehenden Fettsäuren titrimetrisch erfasst werden, den Verdau genauer charakterisieren, da jede einzelne Lipidklasse quantifizierbar ist. Damit trägt die HPTLC zum besseren Verständnis des Abbauverhaltens von kolloidalen Lipidträgersystemen und somit zur Optimierung dieser Arzneiformen bei.

Probenvorbereitung

Die Proben werden zunächst mit 1N HCl angesäuert, um die Dissoziation der Fettsäuren zurückzudrängen, dann mittels Ultraturrax homogenisiert. Anschliessend werden die Lipide mit Chloroform extrahiert.

Stationäre Phase

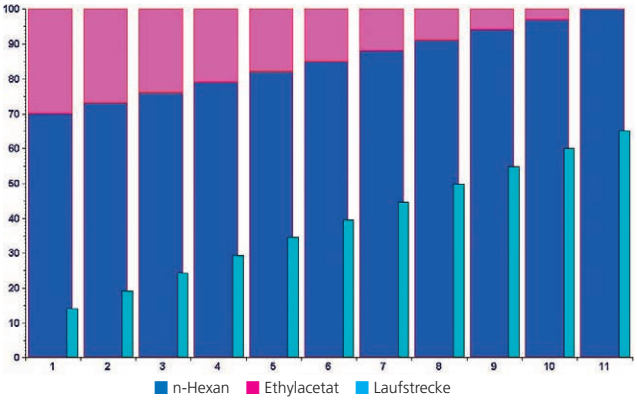
HPTLC-Platten Kieselgel 60 F_{254} (Merck) 20×10 cm

Probenauftragung

Bandförmig mit DC-Probenautomat 4, 18 Bahnen, Auftragevolumen variabel, Bandlänge 8 mm, Bahnabstand 10 mm, unterer Randabstand 8 mm, seitlicher Randabstand mind. 15 mm

Chromatographie

Im AMD 2-System mit einem 11-Stufen-Gradienten auf der Basis von Ethylacetat. Zwischen den einzelnen Entwicklungsschritten wird 90 s getrocknet und anschliessend die Schicht mit 4 M Essigsäure konditioniert. Die Laufstrecke beträgt max. 65 mm, die Gradientendauer insgesamt 110 min.



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▲ *AMD2-Gradient zur Trennung von Lipolyseprodukten*

Postchromatographische Derivatisierung

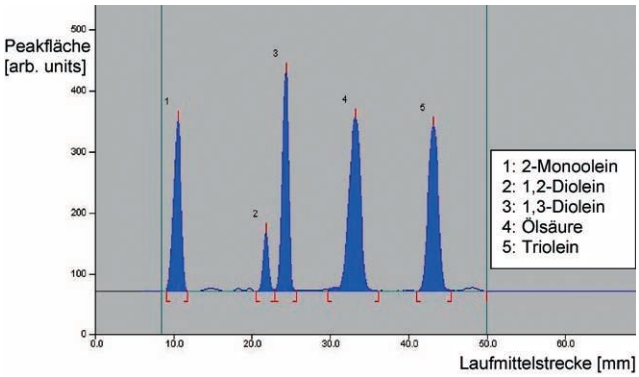
Mit Hilfe der Chromatogramm-Tauchvorrichtung wird die Platte 20 s in eine wässrige Kupfersulfatlösung (10% CuSO4, 8% H3PO4 und 5% Methanol) getaucht und anschliessend 30 min bei 150 °C erhitzt. Langkettige Lipide färben sich braun.

Densitometrische Auswertung

Mit TLC-Scanner 3 und winCATS Software, Absorptionsmessung bei 675 nm, Auswertung über die Peakfläche mittels Kalibration nach Hill-Kinetik

Ergebnisse und Diskussion

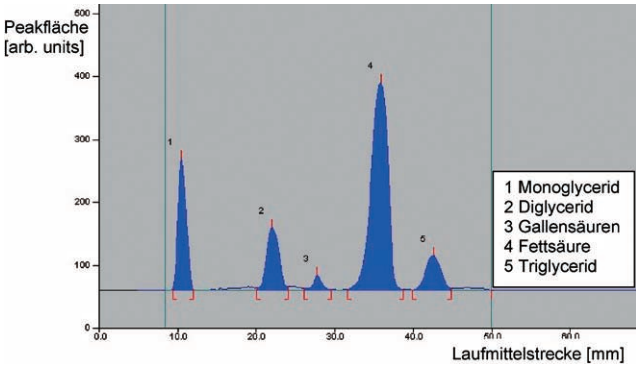
Nachfolgendes Densitogramm zeigt die AMD2-Trennung einer Lipid-Standardmischung.



28

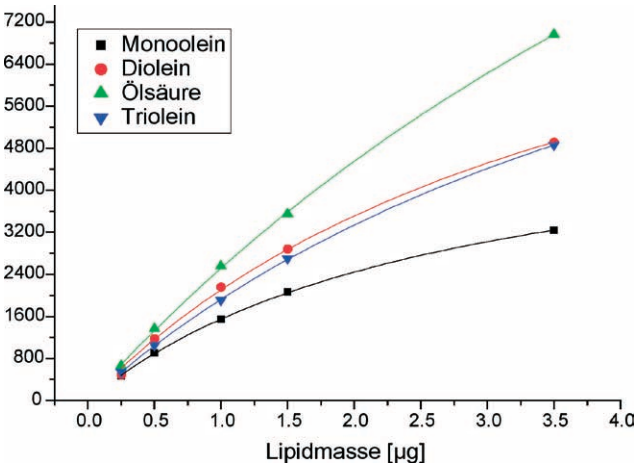
▲ *Densitogramm der Lipid-Standardmischung*

Im Densitogramm der extrahierten Lipolyseprodukte sind die 4 Spezies des Standardgemisches wieder auffindbar. Des Weiteren tritt ein zusätzlicher Peak (3) auf, der durch Gallensäuren bedingt wird. Die mit Hilfe der Kalibrierkurven berechneten Lipidkonzentrationen geben Auskunft über das Abbauverhalten des Lipidträgersystems.



29

▲ *Densitogramm nach künstlichem Verdau extrahierter Lipolyseprodukte*



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▲ *Kalibrierkurven des Standardgemisches (Hill-Kinetik), Korrelationskoeffizienten für Monoolein 0.996, Diolein 0.996, Ölsäure 0.998 und Triolein 0.999*

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

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