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In diesem Heft
Planar-Chromatographie zur Bestimmung von Schadstoffen in Spielzeug und weitere interessante Anwendungen

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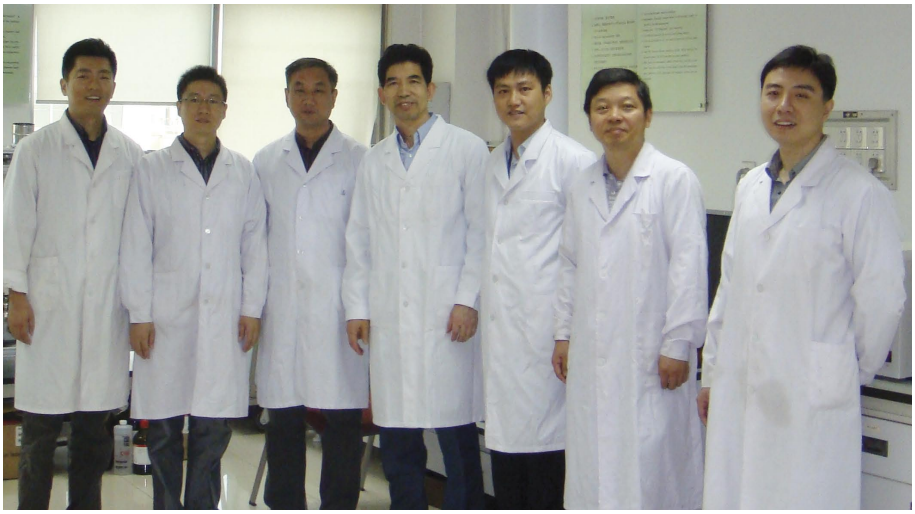
Simultane Bestimmung der Pestizide Temephos und Fenitrothion in Grünem Tee

Kurzfassung aus einem Beitrag von W. Fan, Y. Yue *et al.* in Journal of Planar Chromatography 24 (2011) 1, 53–56, mit freundlicher Genehmigung von Akadémiai Kiadó



Von links: Dr. Xi Yao, Dr. Jing Wang, Prof. Feng Tang, Prof. Yongde Yue, Dr. Ping Lu, Dr. Xuefang Guo, Dr. Jia Sun

Die nachfolgend beschriebene HPTLC-Methode wurde von der Forschungsgruppe von Prof. Yue* am International Center for Bamboo and Rattan, Beijing, China, entwickelt und validiert. Sie bietet entscheidende Vorteile gegenüber den bisher bekannten GC- und HPLC-Methoden.

Einleitung

Phosphororganische Pestizide wie Temephos und Fenitrothion werden häufig in Teeplantagen in Asien zur Steigerung der Produktionsausbeute eingesetzt. Sie sind jedoch potenziell gesundheitsgefährdend. Infolge der komplexen Matrix sind die Analysenverfahren für derartige Pestizide oft kompliziert und zeitraubend.

Die hier beschriebene HPTLC-Methode bietet eine verbesserte Identifizierung und Quantifizierung und ist somit eine wertvolle Ergänzung zu den GC- und HPLC-Analysenverfahren. Die Vorteile sind: Einfachheit der Methode, keine Störung durch die mobile Phase, alle Komponenten bleiben auf der Platte gespeichert und können nach Belieben mit geänderten Parametern untersucht werden und, nicht zuletzt, Kostenersparnis.

Probenvorbereitung

Teeblätter werden bei Raumtemperatur durch mechanisches Schütteln mit Ethylacetat extrahiert. Die Extrakte werden mittels Festphasenextraktion an einer Tandemsäule adsorbiert, dann mit Dichlormethan eluiert. Aliquots der Eluate werden nach Bedarf mit Standardlösung aufgestockt.

Standardlösungen

Die Stammlösungen mit je 1 mg/mL Temephos bzw. Fenitrothion in Methanol werden mit dem gleichen Lösungsmittel auf 10 und 1 µg/mL verdünnt.

Schicht

HPTLC-Platten Kieselgel 60 F_{254} (Merck) 20 x 10 cm, vorgewaschen durch Entwicklung mit Methanol, getrocknet durch 30 min Erhitzen auf 110 °C.

Probenauftragen

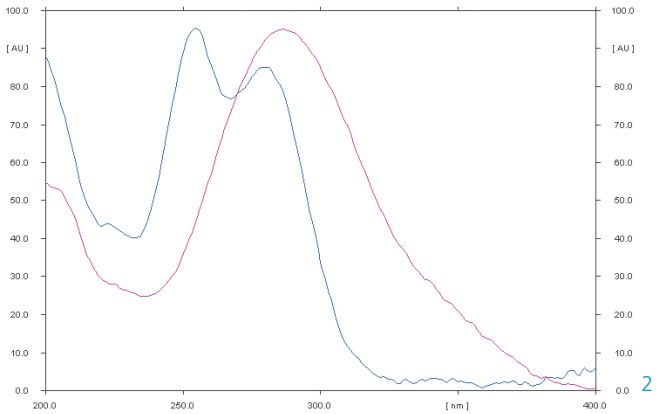
Bandförmig mit Linomat 5, Bandlänge 5 mm, 10 mm vom unteren Plattenrand, 15 mm Seitenabstand, Bahnabstand 10 mm.

Chromatographie

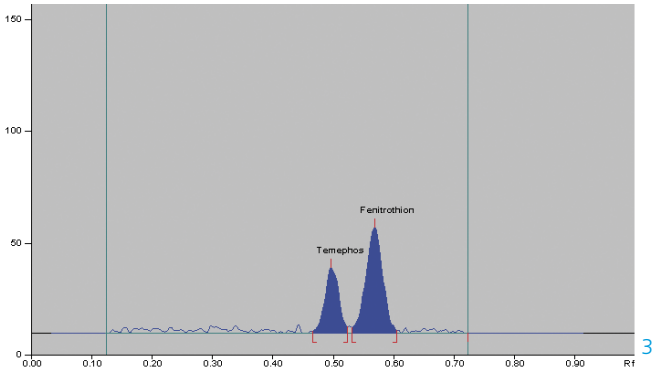
In der Doppeltrogkammer 20 x 10 cm mit Aceton – Hexan 3:7, Lauftrecke 70 mm.

Densitometrische Auswertung

TLC-Scanner 3 mit winCATS Software; Spektrenaufnahme 200–400 nm; quantitative Auswertung durch Absorptionsmessung bei 290 nm, Spaltgrösse 4.00 x 0.30 mm, Messgeschwindigkeit 20 mm/s; Quantifizierung über Peakfläche mit linearer Regression.



2 *UV-Spektren 200-400 nm von Temephos (blau) und Fenitrothion (rot)*



3 *Densitogramm, Absorptionsmessung bei 290 nm*

Ergebnisse und Diskussion

Korrelationskoeffizienten und Detektionsgrenzen

Pestizid	hR_F	Korrelations-koeffizient	Detektionsgrenze [ng/band]
Temephos	55	0.9981	20
Fenitrothion	69	0.9994	10

Wiederfindungsraten und *RSD* von Temephos und Fenitrothion in Tee

Pestizid	Aufstockung [mg/kg]	Mittl. Wiederfindung [%]	*RSD* [%]
Temephos	0.2	107	14.7
	0.4	92.6	9.9
	4.0	83.3	11.7
Fenitrothion	0.2	78.7	20.2
	0.4	93.1	4.4
	4.0	86.8	10.0

Fazit

Die HPTLC-Methode ist geeignet für die simultane Bestimmung von Temephos- und Fenitrothion-Rückständen in grünem Tee. Verglichen mit bisher beschriebenen GC- und HPLC-Rückstandsanalysenverfahren von Organophosphor-Pestiziden bietet diese Methode Vorteile hinsichtlich Einfachheit, Schnelligkeit und Kostenersparnis.

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich.

Kontakt: Prof. Dr. Yongde Yue*, International Center for Bamboo and Rattan, No.8, Futong East Street, Wangjing area, Chaoyang district, 100102 Beijing, China, yuey@cbr.ac.cn

Eine Kopie des Artikels in JPC kann von CAMAG angefordert werden.

Analyse von Insulin-Proben mittels HPTLC–MS

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5

Von links: M. Schulz, S. Minarik, B. Schubach und I. Fahr

Die Möglichkeit, Planar-Chromatographie auf unkomplizierte Weise mit Massenspektrometrie zu koppeln, hat auch innerhalb der Firma Merck neue Anwendungen gefunden. Der vorliegende Beitrag zur Analytik von Insulin ist ein Beispiel. Er entstand in Zusammenarbeit des DC-Forschungslabors mit dem Analytiklabor für Massenspektrometrie.

Einleitung

Wie schon in früheren Arbeiten gezeigt, eignet sich die Planar-Chromatographie sehr gut für die Peptid-Analytik. Peptide aus dem tryptischen Verdau von Proteinen können mit HPTLC aufgetrennt und mit MS ausgewertet werden [1–4].

Auch für die Analytik der intakten Proteine ist die Planar-Chromatographie geeignet. Mögliche Anwendungen im Bereich der Insulin-Analytik sind prozessbegleitende Analysen während der Insulin-Herstellung, Nebenkomponenten-Analyse bzw. Reinheitsprüfung oder Identitätsprüfung. In dieser Arbeit wird eine Methode zur Unterscheidbarkeit von Insulin-Proben verschiedener Spezies mit HPTLC-MS beschrieben. ProteoChrom® Schichten mit einer Schichtdicke von 100 µm und ihrem optimierten Binder-System ermöglichen hohe Empfindlichkeiten und gute Wasserstabilität.

Es wird gezeigt, dass Human-, Schweine- und Rinder-Insulin mittels HPTLC-MS analysiert und eindeutig identifiziert werden können. Diese werden direkt von der Platte mit dem TLC-MS Interface eluiert und mit dem Massenspektrometer identifiziert.

Schicht

ProteoChrom® HPTLC Kieselgel Si 60 F_{254s}, (Merck) 20 × 10 cm

Probenvorbereitung

Human Insulin recombinant (PAN Biotech), Insulin porcine pancreas (Sigma-Aldrich) and Insulin bovine pancreas (Sigma-Aldrich) gelöst in 0,1 % wässriger Trifluoressigsäure (1 mg/mL)

Probenauftragung

Bandförmig mit dem DC-Probenautomat 4, Bandlänge 5 mm, Bahnabstand 10 mm, Abstand vom unteren Plattenrand 10 mm, Auftragevolumen 5 µL

Chromatographie

In der Flachbodenkammer 20 × 10 cm mit 2-Butanol – Pyridin – Ammoniak (25%) – Wasser 39:34:10:26 bis zu einer Laufstrecke von 5 cm (Laufzeit 50 min)

Postchromatographische Derivatisierung

Mit dem DC-Sprühgerät wird die Platte mit Fluorescamin-Reagenz (0,02 % Fluorescamin in Aceton) besprüht und 10 min bei Raumtemperatur getrocknet. Die Platte wird nur zur Hälfte besprüht und die andere Hälfte für die MS verwendet.

Dokumentation

Mit dem DigiStore 2-System unter UV 366 nm

Massenspektrometrie

Die Banden werden mit dem TLC-MS Interface eluiert und mit dem Thermo LTQ XL Orbitrap Massenspektrometer analysiert. Ionenquelle: Electrospray-Ionisierung (positiver Modus). Eluent: Wasser (Merck LiChrosolv) – Acetonitril (Merck hypergrade für LC-MS) 50:50

Ergebnisse und Diskussion

Die Chromatogramm-Zonen von Human-, Schweine- und Rinder-Insulin wurden auf der linken Plattenhälfte mit Fluorescamin angefärbt (hR_F 50). Weitere Banden wurden im Rahmen dieser Arbeit nicht untersucht. Auf der rechten Plattenhälfte – mit den gleichen Proben – wurden die Positionen markiert, mit Wasser – Acetonitril 50:50 eluiert und dem Massenspektrometer zugeführt.

Das Insulin-Molekül besteht aus zwei Aminosäureketten, die über Sulfid-Brücken am Cystein miteinander verbunden sind. Human-Insulin hat eine Molmasse von 5807 Da. Die Struktur des Schweine-Insulins ist der des Human-Insulins sehr ähnlich, nur die Stelle B30 in der Aminosäuresequenz ist Alanin anstelle von Threonin. Die Struktur des Rinder-Insulins unterscheidet sich von der Aminosäuresequenz des menschlichen Insulins an zwei Stellen der A-Kette und einer Stelle der B-Kette. A8 ist Alanin statt Threonin, A10 ist Valin statt Isoleucin und B30 ist Alanin statt Threonin. Daraus ergeben sich Molmassen von 5778 Da für Schweine-Insulin und 5733 für Rinder-Insulin.

Die unterschiedlichen Signale der 3–6-fach geladenen Ionen können eindeutig den Molmassen der verschiedenen Insulin-Proben zugeordnet werden. Die Insulinbanden auf der Platte zeigen weniger Verunreinigungen als die Orginalproben. Daher sind die Signale, die nicht den Hauptkomponenten zugeordnet werden können, bei der Messung von der Platte deutlich reduziert.

Massenspektrometrie-Ergebnisse

Human-Insulin	Schweine-Insulin	Rinder-Insulin
968,7816 $[M+6H]^{6+}$	963,78 $[M+6H]^{6+}$	956,4421 $[M+6H]^{6+}$
1162,5367 $[M+5H]^{5+}$	1156,3347 $[M+5H]^{5+}$	1147,5288 $[M+5H]^{5+}$
1452,6680 $[M+4H]^{4+}$	1445,1658 $[M+4H]^{4+}$	1434,1584 $[M+4H]^{4+}$
1936,5513 $[M+3H]^{3+}$	1926,5491 $[M+3H]^{3+}$	1912,2065 $[M+3H]^{3+}$
Molmasse 5807 g/mol	Molmasse 5778 g/mol	Molmasse 5733 g/mol

Die Primärstruktur von Insulin ist intakt, und die Massengenauigkeit ist < 1,5 ppm. Das Potenzial der Methode für Nebenkomponentenanalyse beziehungsweise Reinheitsprüfungen wird anhand dieser Ergebnisse deutlich. Sowohl die Anfärbung mit Fluorescamin als auch die Massenspektren zeigen eine Abtrennung von Nebenkomponenten, die mit dieser Methode weiter charakterisiert werden können.

[1] J.F. Emory et al. Eur J Mass Spectrom 16 (2010) 21

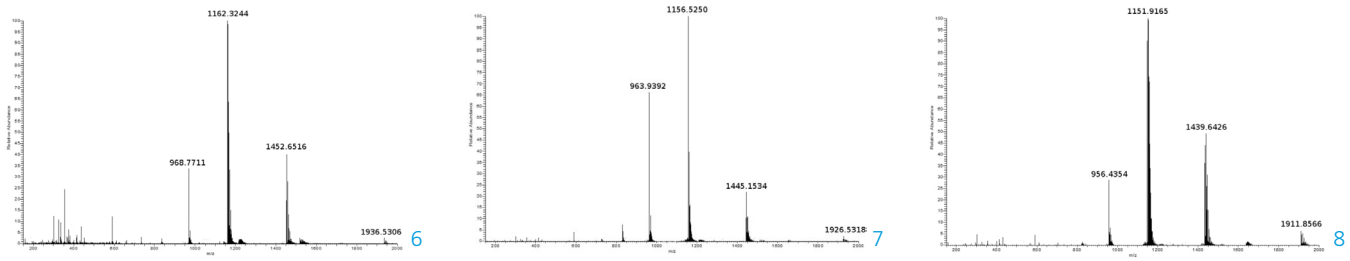
[2] M. Schulz et al. CBS 106 (2011) 5

[3] M. Walworth et al. Anal Chem 83 (2011) 591

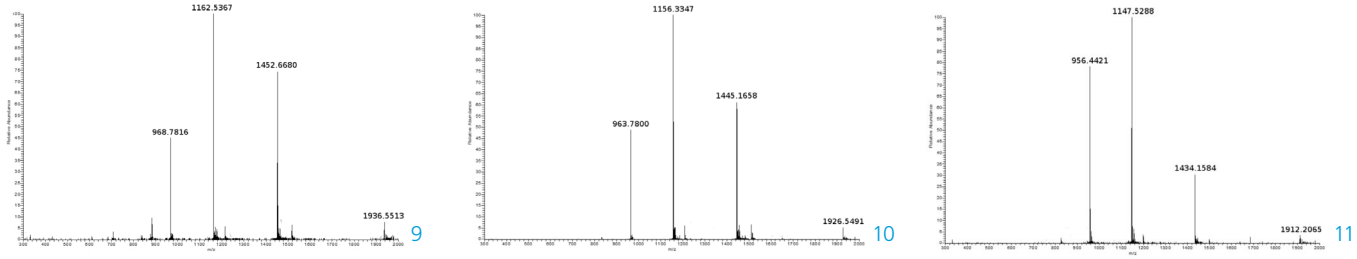
[4] M. Walworth et al. Rapid Commun Mass Spectrom 26 (2012) 37

Weitere Informationen sind von den Autoren auf Anfrage erhältlich.

Kontakt: Michael Schulz, Merck KGaA, MM-LER-CP, Frankfurter Str. 250, 64293 Darmstadt, michael.schulz@merckgroup.com



Referenzspektrum von Human-Insulin (links), Schweine-Insulin (Mitte), Rinder-Insulin (rechts)



Human-Insulin (links), Schweine-Insulin (Mitte), Rinder-Insulin (rechts) gemessen von der ProteoChrom® HPTLC Si60 F_{254s} Platte

Simultane Bestimmung von Pioglitazon, Metformin und Glimepirid in Pharmaka

Rajendra Kakde und Dipak Kale*

Kurzfassung einer Veröffentlichung in Journal of Planar Chromatography 24 (2011) 331-336 mit freundlicher Genehmigung von Akadémiai Kiadó. Dr. Kakde *et al* erhielten für diese Arbeit den Dr. P. D. Sethi Award 2011 for Best Research Paper in Pharmaceutical Analysis.

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Forschungsgruppe von Prof. Rajendra Kakde at Deptt. of Pharmaceutical Sciences, RTM Nagpur University, Maharashtra, India.

Die hier beschriebene unkomplizierte HPTLC-Methode ist geeignet für die zuverlässige und präzise Bestimmung von Pioglitazon, Methformin und Glimepirid sowie deren Zersetzungsprodukte in Rohprodukten und pharmazeutischen Antidiabetes Präparationen. Die Methode wurde validiert hinsichtlich Präzision, Genauigkeit, Spezifität und Robustheit.

Einleitung

Pioglitazon (PIO) und Metformin (MET) dienen zur Behandlung von Diabetes Typ II [1], Glimepirid (GLM) ist ein orales Antidiabetikum und gehört zur Gruppe der Sulfonyl-Harnstoffe.

Metformin erhöht die Insulinempfindlichkeit der Leber und erhöht die Aufnahmefähigkeit des Gewebes für Glukose [2]. GLM hat Langzeitwirkung bei der physiologischen Insulinsekretion. Patienten, die an Diabetes Typ II leiden, benötigen eine Behandlung mit mehr als nur einem antihyperglykämischen Wirkstoff für ihre optimale Einstellung [2, 3].

Standard Lösungen

75 mg, 500 mg und 25 mg der reinen Standards von PIO, MET und GLM wurden eingewogen in jeweils drei verschiedene 50 mL Messkolben und mit Methanol aufgefüllt. Das ergab Lösungen von 1.5 mg/mL, 10 mg/mL und 0.5 mg/mL PIO, MET bzw. GLM.

Probenvorbereitung

Zwanzig *Ziglim plus-2* Tabletten wurden fein gepulvert. Eine Menge entsprechend 7.5 mg PIO wurde in einen 50 mL Messkolben eingewogen, 25 mL Methanol zugegeben, der Kolben 30 min im Ultraschallbad behandelt, und nach Abkühlung auf Raumtemperatur zur Marke aufgefüllt. Die Lösung wurde durch ein Whatman Typ I Filter filtriert und danach direkt zum Probenauftragen verwendet.

Schicht

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

Probenauftragen

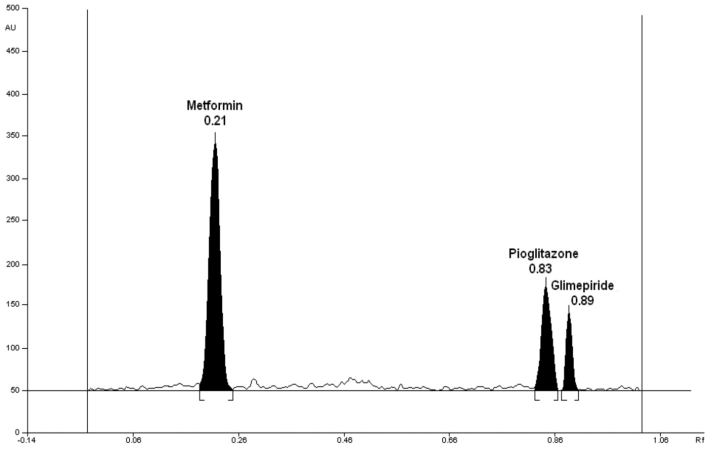
Bandförmig mit DC-Probenautomat (ATS 4), 8 Bahnen, Bandlänge 6 mm, Bahnabstand 10 mm, Abstand vom Plattenrand 10 mm; jeweils 8 µL Proben und Standards

Chromatographie

Doppeltrogkammer 10 x 10 cm, nach 10 min Kammersättigung mit Acetonitril-Methanol-Propanol-Ammoniumacetat-Lösung 7:2:1:1, Laufstrecke 80 mm vom unteren Plattenrand; 5 min Lufttrocknung

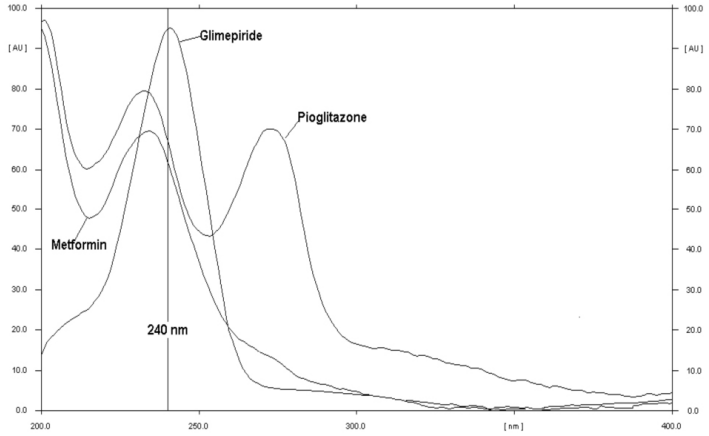
Densitometrie

TLC Scanner 3 mit winCATS Software, Absorptionsmessung bei 240 nm, Spaltdimensionen 3.00 x 0.45 mm, Messgeschwindigkeit 20 mm/s



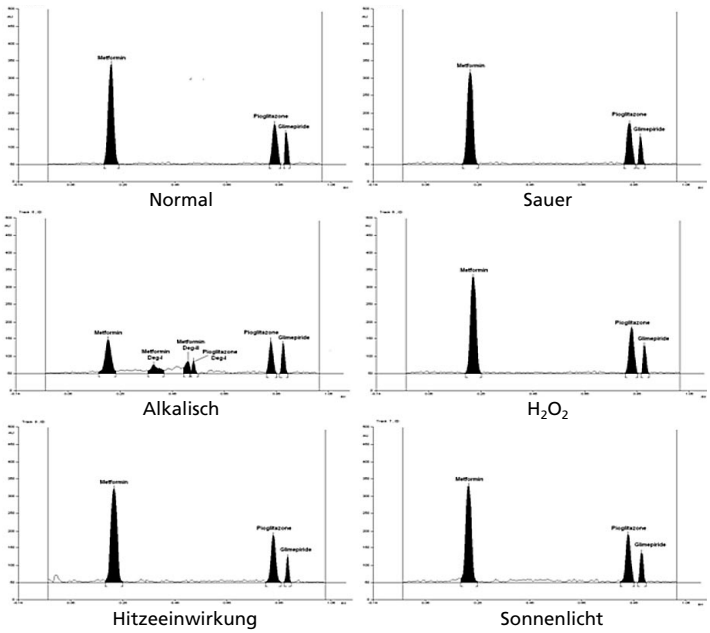
13

Trennung von Pioglitazon, Metformin und Glimepirid in einem Handelspräparat



14

Spektren der pharmazeutischen Wirkstoffe in Ziglim plus-2 Tabletten



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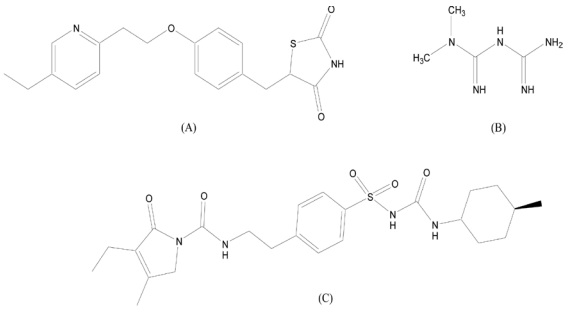
HPTLC Densitogramme von Pioglitazon, Metformin und Glimepirid; die im Rahmen der Stabilitätsstudie durchgeführten Tests ergaben nur im basischen pH-Bereich Zersetzungserscheinungen.

Ergebnisse und Diskussion

Das typische Densitogramm (Abb. 13) zeigt die gute Auftrennung von Metformin (hR_F 21), Pioglitazon (83) und Glimepirid (89). Die Methode wurde validiert hinsichtlich Präzision, Genauigkeit, Spezifität, Linearität und Arbeitsbereich, Detektionsgrenze (LOD), Bestimmungsgrenze (LOQ), sowie Robustheit gemäss ICH-Richtlinien.

Die Kalibrierfunktion erwies sich als linear in den Konzentrationsbereichen 0.3–1.2, 10–40 bzw. 0.04–0.16 µg/Bande bei Auswertung über Peakfläche mit Korrelationskoeffizienten von 0.995, 0.996 bzw. 0.998 für PIO, MET und GLM. Die Methode ist unkompliziert, schnell, genau und kostengünstig. Sie ist somit geeignet für die Quantifizierung, z.B. in der Qualitätskontrolle von Handelspräparaten.



Strukturformeln von Pioglitazon (A), Metformin (B) und Glimepirid (C)

[1] P.B. Musholt, *et al.*, J. Diabetes. Sci. Technol. 3 (2009) 1442

[2] K. Aljabri, *et al.*, Am. J. Med. 116 (2004) 230

[3] E. Draeger, Hoechst AG, Diabetes Res. Clin Pract. 28 (1995) 139

Weitere Informationen sind von den Autoren auf Anfrage erhältlich.

*Dr. Rajendra B. Kakde, University Dept. of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Campus, Nagpur 440033, MS, India, drkakde@yahoo.com

Bereich Produktion unter neuer Leitung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Walter Rahm (58) übernahm im April 2011 die **Leitung des Bereichs Produktion** von Herrn Jürg Bär, der nach 40 Jahren engagierter und erfolgreicher Tätigkeit für CAMAG in den wohlverdienten Ruhestand trat. Herr Rahm kam 1990 zu CAMAG, um das Qualitätsmanagementsystem gemäss ISO Norm 9000 aufzubauen. Er verliess dann CAMAG 1994, kehrte jedoch 2003 zu uns zurück und übernahm die Leitung der Abteilung Einkauf. Er gehört somit zu den nicht wenigen Mitarbeitern, die zum zweiten Mal bei CAMAG sind, was ihre Verbundenheit mit dem Unternehmen erkennen lässt.


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Frau Lilian Budmiger (52) **leitet die Abteilung AVOR**. Sie ist bei CAMAG seit 1995, arbeitete zuerst in der Vormontage, übernahm dann Aufgaben in der Baugruppenprüfung und der Arbeitsvorbereitung, bis sie Ende 2009 deren Leitung übernahm. Zu ihren Aufgaben gehören Produktionsplanung und Disposition gemäss den Vorgaben der Geschäftsleitung, Erstellen und Überwachen von Bestellvorschlägen und Fertigungsaufträgen sowie die Pflege der Produktionsdokumente und der EDV-Daten.


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Andreas Muheim (55), **Leiter der Montageabteilung**, seit 1984 bei CAMAG, übernahm 1996 die Leitung dieser Abteilung. Seine Hauptaufgabe ist es, sämtliche Montageaufträge gemäss Prioritätenvorgabe der Geschäftsleitung umzusetzen und zu überwachen. Um die Übernahme neuer Produkte zu optimieren, wird Herr Muheim bei Entwicklungsprojekten frühzeitig beigezogen, um die Belange der Fertigung einzubringen. Schwerpunkte dabei sind »produktionsfreundliche« Konstruktion bei der Gerätestruktur, Aufteilung der Baugruppen, Berücksichtigung vorhandener Werkzeuge, Sicherstellen der Montageanweisungen usw. Dadurch wird sicher gestellt, dass die Geräte bereits von Anfang an in einer hohen »Produktionsreife« hergestellt werden können.


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Rolf Lützelschwab (55) **ist verantwortlich für die Endprüfung**. Er ist bei CAMAG seit 1987 und arbeitete mehrere Jahre in der Montageabteilung. Seit 2004 ist er zuständig für die Endprüfung sämtlicher CAMAG Geräte, wobei seine langjährige Erfahrung in der Montage von grossem Nutzen ist. Er ist Stellvertreter von Herrn Muheim als Montageleiter.

Zielsetzung unserer Produktion ist die Herstellung von fehlerfreien, qualitativ hochwertigen Geräten und die Sicherstellung ihrer Verfügbarkeit. Einzelteile und Baugruppen beziehen wir von ausgewählten Lieferanten, die schon bei der Konstruktion beigezogen werden. Um die Erfüllung der hohen Anforderungen an die Endprodukte zu gewährleiten, erfolgt die Montage komplexer Baugruppen sowie die Endmontage aller Geräte im Hause. Wir produzieren in Losgrössen von 25 oder 50 Geräten auf Lager, nicht auf Kundenbestellung.

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Liebe Freunde

Dieses Heft ist ein bunter Strauss von Publikationen, die die vielseitigen Einsatzmöglichkeiten der HPTLC belegen. Ein Beispiel ist die Analytik von polyaromatischen Kohlenwasserstoffen in Spielzeug. Weitere Beispiele sind im Bereich der Rückstandsanalytik von chinesischem Grüntee und der pharmazeutischen Analytik in Indien.

Das TLC-MS-Interface ist inzwischen ein steter Begleiter der CBS-Ausgaben geworden. In diesem Heft wird es erstmalig und überzeugend für die Insulin-Analytik eingesetzt. Inzwischen ist es ein unentbehrliches Instrument für HPTLC-Anwender geworden, aber auch bei anderen Analytikern gewinnt die Planar-Chromatographie durch die Verbindung mit der Massenspektrometrie an Interesse.

Die Vorzüge der Matrixtoleranz und simultanen Derivatisierung ermöglichen einen charakteristischen Fingerprint und eine eindeutige Zuordnung von Biopolymeren. Diese Verjüngungskur bekommt der Analytik von Dickungsmitteln, Hydrokolloiden und Gelen sehr gut!

Zum Abschluss noch ein Wort in eigener Sache: Durch meine Übernahme des Lehrstuhls für Lebensmittelwissenschaften an der Justus-Liebig-Universität Giessen ist die Fertigstellung dieses Hefts ein wenig in Verzug geraten. Die positive Seite der Medaille, es gibt damit ein zusätzliches HPTLC-Kompetenzzentrum in Deutschland!

Herzlichst

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

This issue contains a real bouquet of applications representing today's multifacetted use of HPTLC. One example is the analysis of polyaromatic hydrocarbons (PAHs) in toys. Other applications of topical interest are the residue analysis of pesticides in Green Tea and a pharmaceutical analysis from India.

Meanwhile hyphenating planar chromatography and mass spectrometry with the TLC-MS Interface continues to be a repetitive subject in the latest CBS issues. This time it is the application for insulin analysis in different species. This interface has become an almost indispensable tool for the HPTLC user, plus it makes planar chromatography more and more attractive for analysts.

The benefits of tolerance for high matrix loads combined with simultaneous derivatization allow for a fast fingerprint and an unambiguous assignment of biopolymers. This appears to be a real rejuvenating cure for the analysis of tricky samples, *i.e.* thickening agents, hydrocolloids and gels.

My taking over the Chair of Food Science at the Justus Liebig University of Giessen resulted in a somewhat delayed release of this CBS issue. On the other hand, the benefit is the establishment of one more Center of HPTLC Competence in Germany!

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG

MARCH
2012

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THE CBS CLASSIFICATION SYSTEM

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

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

1. Reviews and books

108 001 E. REICH*, Valeria WIDMER (*CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Switzerland; eike.reich@camag.com): Plant analysis 2008 - planar chromatography. Planta Med. 75, 711-718 (2009). For many decades, planar chromatography has been used for the analysis of plants, in particular today in its most advanced form of HPTLC. The technique is *e. g.* used for the identification of medicinal plants and dietary supplements, and for the detection of adulteration and quantitative determination of marker substances. Reliable qualitative and quantitative results can be achieved based on suitable instrumentation and adequate methodological concepts. The manageability of the entire planar chromatographic process has improved. Integration of biological detection systems as well as hyphenation to mass spectroscopy has widened the applicability of planar chromatography as an important analytical technique. The introduction is followed by explanation of HPTLC, use of HPTLC in plant analysis, limitations, applications (identification, detection of adulteration and quantitation), and instrumentation (chromatogram development, documentation, detection and evaluation).

traditional medicine, herbal, quality control, review, HPTLC, densitometry, quantitative analysis, qualitative identification 1a, 32e

108 002 J. SHERMA (Department of Chemistry, Lafayette College, Easton, Pennsylvania 18042-1782, USA, shermaj@lafayette.edu): Review of advances in thin-layer chromatography of pesticides: 2008-2010. Journal of Environmental Science and Health, Part B 46, 557-568 (2011). Review on techniques and applications of TLC and HPTLC for separation, detection, qualitative and quantitative determination and preparative isolation of pesticides. Covered are sample preparation techniques, stationary phases, sample application, mobile phases, development methods using different chambers, detection under UV or by derivatization with various reagents, identification based on hR_F values or by online HPTLC-MS, quantification by scanning densitometry or videodensitometry, preparative layer chromatography and thin-layer radiochromatography. Various applications are described. In the review period especially forensic analyses of human and animal samples for pesticides were numerous. The identification and quantification of components from plant extracts with pesticide activity is also reviewed and it is expected that this area will be especially active in the future given the large amount of ongoing worldwide research on phytochemical compounds.

environmental, HPTLC, review, densitometry, quantitative analysis, qualitative identification, comparison of methods 1, 29

108 003 J. SHERMA (Lafayette College, Department of Chemistry, Easton, PA 18042, USA; shermaj@lafayette.edu): Review of HPTLC in drug analysis: 1996-2009. J. AOAC Int. 93, 754-764 (2010). The review describes analytical methods for drug substances, formulations, and clinical samples analyzed and validated by HPTLC during the period 1996-2009. Procedures, materials, and instrumentation for the different steps in the chromatographic procedure and validation of results are given; application to bulk drugs, formulations, stability studies, biological samples (*e.g.*, urine and plasma), and hydrophobicity studies; and prospects for the future use of HPTLC for drug analysis are described. The sections cover the experimental procedures (sample preparation, stationary phases, mobile phases, application of standards and samples, chromatogram development, detection, documentation of chromatograms, densitometric quantitative analysis), determination of hydrophobicity, confirmation of zone identity, method validation, chiral separations, micropreparative layer chromatography, applications of HPTLC-densitometry, and future prospects for HPTLC in drug analysis. 155 references are reviewed.

quality control, pharmaceutical research, clinical chemistry research, HPTLC,

quantitative analysis, qualitative identification, densitometry, review 1

108 004 Agata SKORUPA, A. GIERAK* (*Institute of Chemistry, Jan Kochanowski University of Humanities and Science, Swietokrzyska St. 15G, 25406 Kielce, Poland; Andrzej.Gierak@ujk.edu.pl): Detection and visualization methods used in thin-layer chromatography. J. Planar Chromatogr. 24, 274-280 (2011). Presentation of new reagents and visualization methods which enable the detection of particular substances. The best of them provide high selectivity and low detection limits and lead to a reliable analysis of the developed chromatogram. Three groups of chemical reactions are used for detection: oxidation, reduction, and complexation, as well as precipitation of colored precipitates. Oxidation and reduction are most widely used due to their selectivity, which allows detection and identification of the separated substances. Like this the target substance can be selectively visualized, as for example antioxidants in complex natural matrices. Innumerable chemical, physical, and biological methods of visualization are known. Required are new, more efficient reagents fo a selective visualization of a defined part of a substance or, in some cases, for a specific determination of a specific substance. The article describes the detection of food ingredients, medicines and pharmaceuticals, organic compounds not present in food, and inorganic compounds.

pharmaceutical research, review, HPTLC, densitometry, postchromatographic derivatization, quantitative analysis 1, 3e

2. Fundamentals, theory and general

108 005 A.P. BOICHENKO*, I.V. MAKHNO, A.Y. RENKEVICH, L.P. LOGINOVA (*Department of Analytical Biochemistry, University Centre for Pharmacy, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands; boichenko@univer.kharkov.ua; oleksandr.boychenko@gmail.com): The mobile phase motion in ascending micellar thin-layer chromatography with normal-phase plates. J. Planar Chromatogr. 24, 463-469 (2011). Investigation of the surface tension and viscosity of micellar mobile phases based on the cationic surfactant cetylpyridinium chloride and additives of alcohols (ethanol, 1-propanol, 1-butanol, 1-pentanol). The effect of mobile phase properties on the motion of mobile phase in ascending TLC was investigated. The applicability of a quadratic relationship between the time of mobile phase motion and the distance from the solvent entry position and front of mobile phase was validated for micellar TLC.

2d

108 006 D. DABIC, Maja NATIC*, Z. DZAMBASKI, R. MARKOVIC, D. MILOJKOVIC, O. TESIC (*Faculty of Chemistry, University of Belgrade, P. O. Box 51, 11158 Belgrade, Serbia, mmandic@chem.bg.ac.rs): Quantitative structure-retention relationship of new N-substituted 2-alkylidene-4-oxothiazolidines. J. Sep. Sci. 34, 2397-2404 (2011). HPTLC of 23 newly synthesized N-substituted 2-alkylidene-4-oxothiazolidines on RP-18 with 1) methanol - water, 2) acetonitrile - water, and 3) tetrahydrofurane - water. A quantitative structure-retention relationship study allowed to understand the chromatographic behavior of similar compounds.

pharmaceutical research, preparative TLC, HPTLC, quantitative structure-retention relationship 2c

108 007 G. OROS, C. CSERHATI* (*Research Institute of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O.Box 17, H-1525 Budapest, Hungary, tevi@chemres.hu): Reversed phase thin-layer chromatographic behavior of some acylanilide fungicides. J. Liq. Chromatogr. Relat. Technol. 32, 1317-1330 (2009). New study of relationship

between structure and chromatographic behaviour of acylanilide derivatives. TLC of acylanilide derivatives on TLC aluminum foil with acetone - water and methanol - water mixtures. The hR_F values related to the molecular lipophilicity and to the specific hydrophobic surface area of the analytes were calculated.

pharmaceutical research, qualitative identification, quantitative structure-retention relationship 2c

108 008 A. PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellon´ska Street, 41-200 Sosnowiec, Poland, apyka@sum.edu.pl): Use of selected topological indexes for evaluation of lipophilicity of steroid compounds investigated by RP-HPTLC. J. Liq. Chromatogr. Relat. Technol. 32, 3056-3065 (2009). HPTLC of steroid compounds (androsterone, epi-androsterone, dehydroepi-androsterone, testosterone, stigmasterol, beta-sitosterol, estradiol, hydrocortisone, and cholesterol) on RP-18 with methanol - water and acetonitrile - water in different volume compositions. Detection by spraying with sulfuric acid - methanol 1:9, followed by heating at 120 ºC for 15 min. The chromatographic parameters of lipophilicity of the studied steroids were determined.

pharmaceutical research, qualitative identification, quantitative structure activity relationships 2c, 13

108 009 Grazyna ZYDEK*, Elzbieta BRZEZINSKA (*Department of Analytical Chemistry, Medical University of Lodz, Ul. Muszynskiego 1, 90-151 Lodz, Poland): Normal and reversed phase thin layer chromatography data in quantitative structure-activity relationship study of compounds with affinity for serotonin (5-HT) receptors. J. of Chromatogr. B 879, 1764-1772 (2011). Quantitative structure-activity relationship (QSAR) analysis of 20 drugs with affinity for serotonin (5-HT) receptors by calculation of a set of physicochemical parameters and TLC data. TLC on silica gel and RP-2 impregnated with solutions of aspartic acid, serine, phenylalanine, tryptophan, tyrosine, asparagine, threonine and their mixtures, with two mobile phases - the systems were chosen as models of drug-5-HT-receptor interaction. The relationships between chromatographic data, molecular descriptors and biological activity data were found by regression analysis. The resulting correlations for the compounds with serotoninergic activity represent their interaction with the proposed biochromatographic models. The presented regression models based on biochromatographic studies can be an efficient tool in the QSAR analysis for initial prediction of compounds activity direction within 5-HT receptors.

pharmaceutical research, HPTLC, TLC, biochromatography, multiple regression analysis, quantitative structure-activity relationships 2

3. General techniques

108 010 F. DING (Ding Fengyan), D. WANG (Wang Dongyuan)*, S. SONG (Song Shixia), S. XU (Xu Shuying) (*Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016, China; wdylxsy@hotmail.com): Preliminary investigation of sintered plates bonded or end-capped with phenyl for planar electrochromatography. J. Planar Chromatogr. 24, 10-15 (2011). TLC of Sudan II, Sudan III, p-methoxyazobenzene, p-aminoazobenzene on silica gel reversed-phase sintered glass plates with numerous mobile phases. RP-phenyl sintered plates, *i. e.* sintered plates bonded with phenyl, were unsuitable for planar electrochromatography if the mobile phase contained no buffer salts, irrespective of whether or not the plates were end-capped. RP-18 sintered plates end-capped with an appropriate amount of phenyl were suitable for PEC and showed a similar separation ability but a higher electroosmotic flow than RP-18 sintered

plates end-capped with an appropriate amount of methyl.

electrochromatography, RP-phenyl sintered phases 3b

108 011 B. KANNAN*, M.A. MARIN, K. SHRESTHA, D.A. HIGGINS, Maryanne COLLINSON (*Dep. of Chem., Virginia Commonwealth Univ., Richmond, VA 23284-2006, USA): Continuous stationary phase gradients for planar chromatographic media. J. Chromatogr. A 1218 (52), 9406-9413 (2011). Description of a simple, elegant method for the formation of a continuous stationary phase gradient for use in chromatographic separations, at the example of TLC. Gradient stationary phases were formed on activated HPTLC plates using a newly developed methodology termed „controlled rate infusion“. Reaction of the SiOH groups on the activated HPTLC plates with 3-aminopropyltriethoxysilane in a time dependent fashion by using a programmable syringe pump to control the rate of 3-aminopropyltriethoxysilane infusion into the deposition reservoir. The profile of the gradient was controlled by the infusion rate and visualized by the concentration-dependent color reaction of amino groups and ninhydrin. The advantages of such gradients were shown by optimizing the retention and separation of various components in different mixtures of 1) four weak acids and bases and (2) three widely used over-the-counter drugs. The separation was better on gradient stationary phases than on NP-TLC phases or amino phases. The retention and separation can be controlled by strategically modifying the steepness of the gradient.

HPTLC 3d

108 012 L. KOMSTA*, L. CIESLA, Anna BOGUCKA-KOCKA, Aleksandra JÓZEFCZYK, J. KRYSZEN, Monika WAKSMUNDZKA-HAJNOS (*Dep. of Med. Chem., Med. Univ. of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland): The start-to-end chemometric image processing of 2D thin-layer videoscans. J. of Chromatogr. A 1218 (19), 2820-2825 (2011). A unified procedure for image preprocessing of 2D TLC videoscans saved as JPG files is proposed for further supervised or unsupervised chemometric analysis. The procedure was based on open source software and included denoising using a median filter, baseline removal with the rollerball algorithm and nonlinear warping using spline functions. The application of the proposed procedure enabled filtration of random differences between images, such as changes in the intensity of the background as well as differences in the location of the zones. After the preprocessing only the zone intensity had an influence on the statistical analysis by principal component analysis (PCA) or other techniques. The proposed technique was successfully applied for the determination of the differences between three Carex species based on the 2D videoscans of the extracts.

pharmaceutical research, quality control, traditional medicine, herbal, quantitative analysis, comparison of methods, qualitative identification 3f

108 013 A. MOHAMMAD*, A. MOHEMAN (*Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh Muslim University, Aligarh-202 002, India; alimohammad08@gmail.com): A new spray reagent for selective detection and quantification of dichlorvos in bluish tinged maize grains by TLC-spectrophotometry. J. Planar Chromatogr. 24, 113-115 (2011). TLC of dichlorvos on silica gel with cyclohexane - acetone - methanol 16:6:1. Detection by spraying with 2 % sodium hydroxide solution and subsequent spraying with 2 % thiobarbituric solution followed by heating at 90 °C for 10 min. The hR_F of dichlorvos was 50. The limit of detection was approximately 18 µg/zone. Spectrophometric analysis of dichlorvos was performed by measuring the absorbance of the sample solution. The linearity was in the range of 50-350 µg/mL dichlorvos. On alkaline hydrolysis dichlorvos forms dimethylphosphoric acid and dichloroacetaldehyde; the latter reacts with 2-thiobarbituric acid to give a sharp pink

spot. The reagent is selective for dichlorvos, and does not react with other organophosphorus, organochlorine, carbamate, and synthetic pyrethroid insecticides.

toxicology, food analysis, qualitative identification 3e, 29a

108 014 Claudia OELLIG, W. SCHWACK* (*nst. of Food Chem., Univ. of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany): Planar solid phase extraction - A new clean-up concept in multi-residue analysis of pesticides by liquid chromatography - mass spectrometry. J. of Chromatogr. A 1218 (37), 6540-6547 (2011). New approach and application of highly automated planar chromatographic tools for powerful clean-up, called high-throughput planar solid phase extraction (HTpSPE), which is indispensable for preventing matrix effects in multi-residue analysis of pesticides in food by liquid and gas chromatography coupled to mass spectrometry, employing TLC to completely separate pesticides from matrix compounds and to focus them into a sharp zone, followed by extraction of the target zone by the TLC-MS interface, thus resulting in extracts nearly free of interference and free of matrix effects, as shown for seven chemically representative pesticides in four different matrices (apples, cucumbers, red grapes, tomatoes), and completion of clean-up of one sample in a manner of minutes. Regarding the clean-up step, quantification by LC-MS with mean recovery (against solvent standards) of 90-104% and relative standard deviations of 0.3-4.1% ($n = 5$) for two spiking levels of 0.1 and 0.5 mg/kg.

quality control, herbal, pharmaceutical research, food analysis, environmental, HPTLC, qualitative identification, quantitative analysis, preparative TLC 3a, 29

108 015 Claudia OELLIG*, W. SCHWACK (*Institute of Food Chemistry, University of Hohenheim, 70599 Stuttgart, Germany, claudia.oellig@uni-hohenheim.de): Planar solid phase extraction - a new clean-up concept in residue analysis of pesticides. CBS 107, 9-10 (2011). Extraction of pesticides from fruit and vegetable samples by QuEChERs method. TLC of acetamiprid, azoxystrobin, chlorpyriofos, fenarimol, mepanipyrim, penconazole and pirimicarb on amino phase aluminum foil (prewashed with acetonitrile) with acetonitrile over a migration distance of 75 mm in the first direction. After drying development in the backwards direction over 45 mm with acetone. Evaluation under UV 254 nm, UV 366 nm, white light and under UV 366 nm after immersion in primuline solution. Extraction of the target zone by TLC-MS interface with acetonitrile – 10 mM ammonium formate 1:1. Average recoveries of the seven pesticides were 90-104 % with *%RSD* of 0.3-4.1 % ($n = 5$). This new high-throughput planar solid phase extraction method for multi-residue analysis of pesticides in food allows a rapid and efficient clean-up at low costs and low solvent consumption.

food analysis, quality control, qualitative identification, quantitative analysis 3a, 29

108 016 K.V. SEDNEV*, V.G. BEREZKIN (*Belarussian State Agricultural Academy, ul. Michurina 5, Gorki, Mogilev Oblast, 213407 Belarus; sednevkv@mail.ru): Thin-layer chromatography of polar and ionic compounds using active gas flow over the siliga gel adsorbent layer. J. Planar Chromatogr. 24, 181-187 (2011). Study of the TLC separation of polar and ionic substances in carbon dioxide and ammonia atmospheres on silica gel plates with reduced moisture content by single and multiple development with countercurrent drying. The possibility of simple gradient separation by passage of polar vapor was shown. TLC of a six-dye and a two-dye sample on silica gel with toluene. The technique and a special apparatus is described. The active gas flow over the adsorbent layer has several advantages for the separation of polar ionic substances: activation of the adsorbent, stabilization of the mobile phase pH and ionic strength, saturation of the adsorbent with mobile phase, evaporation of the mobile phase and creating gradients is possible by simple

passage of gases in one or other direction over the adsorbent layer.

qualitative identification 3d

108 004 Agata SKORUPA et al., see section 1

108 017 B. SPANGENBERG (University of Applied Sciences Offenburg, Badstrasse 24, 77652 Offenburg, Germany; spangenberg@hs-offenburg.de): A new way of using chemiluminescence in thin-layer chromatography. J. Planar Chromatogr. 24, 357-359 (2011). TLC of benzo[a]pyrene on RP-18, RP-8, and RP-2 with acetone - ethyl acetate 7:3 in an unsaturated twin-trough chamber. Detection by dipping for 1 s into a solution of 250 mg bis(2,4-dinitrophenyl)oxalate in 40 mL of *n*-butyl acetate and 5 mL of hydrogen peroxide (upper phase). For videodensitometric evaluation the sheets were measured for 2 min and monitored over a period of 2 h. The reaction energy is transferred to the fluorescent compound (benzo[a]pyrene) which emits light while relaxing from its excited state. The advantage of this chemiluminescence reaction performed on a TLC plate is the improvement of the detection limit by extending the measuring time. The detection limit is 50 pg benzo[a]pyrene which is identical to that achievable in chemiluminescence HPLC. The calibration curve for benzo[a]pyrene was linear in the range of 50-8000 pg. The new procedure overcomes the problem of uneven illumination with CCD cameras.

comparison of methods, quantitative analysis, qualitative identification 3e

108 018 A. SUNEETHA*, B. SYAMASUNDAR (*Dept. Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur (AP), India): Development and validation of HPTLC method for the estimation of almotriptan malate in tablet dosage form. Ind. J. Pharma. Sci. 72(5), 629-632 (2010). TLC of almotriptan malate on silica gel aluminum foil with butanol - acetic acid - water 3:1:1. Quantitative determination by densitometry in absorbance mode at 300 nm. The calibration curve was linear over the range of 100-700 ng/band for almotriptan malate. The method was successfully applied to the analysis of drug in a pharmaceutical form.

pharmaceutical research, quality control, densitometry, quantitative analysis 3e

4. Special techniques

108 019 Elena CHERNETSOVA*, A. REVELSKY, Gertrud MORLOCK (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany, chern_es@mail.ru): Some new features of direct analysis in real time mass spectrometry utilizing the desorption at an angle option. Rapid Commun. Mass Spectrom. 25, 2275-2282 (2011). The authors explored the possibility of the desorption at an angle scanning analysis of surfaces from direct analysis in real time mass spectrometry (DART-MS), including the coupling of planar chromatography with DART-MS. A method for the visualization of the impact region of the DART gas stream was developed, as well as the DART-MS detectability of liquids was increased, improving the capabilities of DART-MS in trace analysis.

pharmaceutical research, HPTLC, quantitative analysis, DART-MS 4e

108 020 M. EIBISCH, S. ZELLMER, R. GEBHARDT, R. SUB, B. FUCHS, J. SCHILLER* (*University of Leipzig, Faculty of Medicine, Institute of Medical Physics and Biophysics, Härtelstrasse 16-18, 04107 Leipzig, Germany, juergen.schiller@medizin.uni-leipzig.de): Phosphatidylcholine dimers can be easily misinterpreted as cardiolipins in complex lipid mixtures: a matrix-assisted laser desorption/ionization time-of-flight mass spectrometric study of lipids from hepatocytes.

Rapid Commun. Mass Spectrom. 25, 2619-2626 (2011). HPTLC of cardiolipins in hepatocyte sample on silica gel with chloroform - ethanol - water - triethanolamine 30:35:7:35. Qualitative determination by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Caution is required if cardiolipin is analyzed directly from the total lipid extract because phospholipid dimers may be interpreted as cardiolipins.

pharmaceutical research, HPTLC, qualitative identification 4e

108 021 H. KIM, M. OH, J. HONG, Y. JANG* (*Kyung Hee East-West Pharmaceutical Research Institute, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea, ypjang@khu.ac.kr): Quantitative analysis of major dibenzocyclooctane lignans in Schisandrae Fructus by online TLC-DART-MS. Phytochem. Anal. 22, 258-262 (2011). TLC of gomisin A (1), gomisin N (2) and schisandrin (3) in the fruits of Schisandrae chinensis on silica gel with toluene - ethyl acetate - formic acid 14:6:1. Quantitative determination by direct analysis in real time mass spectrometry (DART-MS). Linearity of (1) - (3) was between 0.5 and 5 nmole. The limits of detection and quantification were 60-200 pmole for (1), and 58-192 pmole for (2) and (3). Recovery (by standard addition) for (1) - (3) was between 104.0 and 120.2 %. TLC-DART-MS method provides faster and more specific quantification compared with the conventional densitometric and HPLC-UV methods.

herbal, quality control, preparative TLC, comparison of methods, quantitative analysis, TLC-DART-MS 4e

5. Hydrocarbons and halogen derivatives

108 022 Irena MALINOWSKA*, M. STUDZINSKI, H. MALINOWSKI (*Plac Marii Curie-Sklodowskiej 3/216, 20-031 Lublin, Poland, irena.malinowska@poczta.umcs.lublin.pl): Some aspects of TLC in homogenous magnetic fields. J. Sep. Sci. 34, 2397-2404 (2011). TLC of polyaromatic hydrocarbons (PAH) to examine the influence of magnetic field on the retention of compounds on silica gel, with monocomponent mobile phases containing *n*-hexane, *n*-heptane, *n*-octane, benzene, and toluene and as binary phases, mixtures of *n*-hexane - benzene 9:1, 7:3, and 1:1. The magnetic field influences the retention and separation efficiency of investigated chromatographic systems.

pharmaceutical research, HPTLC, qualitative identification, magnetochromatography 5b

6. Alcohols

108 023 H.A. AHMED*, N.F. YOUSSEF (*National Organization for Drug Control and Research (NODCAR), P. O. Box 29, 12553 Cairo, Egypt; hanan_egypt1@yahoo.com): Validated HPTLC method of salmeterol xinafoate determination in inhaled pharmaceutical product and spiked human urine. J. Planar Chromatogr. 24, 423-427 (2011). HPTLC of salmeterol xinafoate (as salmeterol base SAL and xinafoic acid XIN) on silica gel with ethyl acetate - methanol - 33 % ammonia 16:3:1 with chamber saturation for 1 h. Detection under UV light at 254 nm. Quantitative determination by densitometry at 300 nm (SAL) and at 250 nm (XIN). The hR_F value was 48 for SAL and 36 for XIN. Linearity was betwen 1-6 µg/zone for SAL and 0.5-4 µg/zone for XIN. The recovery (by standard addition) was 100.6 % for SAL and 99.8 % for XIN. The intra-day and inter-day precision, as *%RSD*, was 0.7 and 1.1 % for SAL, and 0.9 and 1.0 % for XIN. The limit of detection and of quantification was 0.4 and 1.2 µg/zone for SAL and 0.1 and 0.3 µg/zone for XIN.

quality control, HPTLC, densitometry, quantitative analysis 6

108 024 Barbara MARCINIEC*, M. OGRODOWCZYK, A.KWIECIEN (*Poznan University of Medical Sciences, Department of Pharmaceutical Chemistry, Grunwaldzka 6, 60-780 Poznan, Poland;

bmarcin@ump.edu.pl): Effect of radiation sterilization on alprenolol in the solid state studied by high-performance thin-layer chromatography. J. AOAC Int. 93, 792-797 (2010). HPTLC of alprenolol on silica gel with methanol - 25 % ammonia 99:1 in a chamber saturated for 10 min. Quantitative determination by absorbance measurement at 270 nm. The linearity range was between 1.03 and 20.6 µg/zone. The *%RSD* of precision was 3.9 %. The recovery of alprenolol was at 80 % level 100.1 % with a *%RSD* of 2.2 %, at 100 % level 99.9 % with a *%RSD* of 3.9 %, and at 120 % level 104.4 % with a *%RSD* of 2.9 %. The LOD was 520 ng/zone and LOQ 1550 ng/zone.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 6

108 025 B. RAMESH, P. NARAYANA, A. REDDY, P. DEVI* (*Analytical Chemistry Div, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, (A.P.), India): Spectrodensitometric evaluation and determination of fluconazole and its impurities in pharmaceutical formulation by high-performance thin-layer chromatography. Journal of Pharmacy Research 4(5), 1401-1404 (2011). HPTLC of fluconazole and its structurally related impurities, on silica gel (prewashed with methanol) with *n*-butanol - water - acetic acid 8:2:1 with chamber saturation for 20 min. The hR_F value of fluconazole was 67 and of the two separated impurities 49 (impurity B) and 79 (impurity E). Quantitative determination by densitometry in absorbance mode at 254 nm. The method was linear in the range of 1-6 µg/band for fluconazole and 0.5-2.5 µg/band for both impurities.

pharmaceutical research, HPTLC 6

108 026 B. RAMESH, P. NARAYANA, A. REDDY, P. DEVI* (*Analytical Chemistry Division, Indian Institute of Chemial Technology, Tarnaka, Hyderabad-500007, India; sitadevi@iict.res.in): Stability-indicating HPTLC method for analysis of venlafaxine hydrochloride, and use of the method to study degradation kinetics. J. Planar Chromatogr. 24, 160-165 (2011). HPTLC of venlafaxine hydrochloride in bulk and formulations on silica gel with butanol - acetic acid - water 3:1:1. Quantitative determination by densitometry in absorbance mode at 284 nm. The hR_F value was 58. Linearity was between 100 and 600 ng/zone. The limit of detection and quantification was 39 and 131 ng/zone, respectively. The repeatability and the intermediate precision (*%RSD*, $n = 6$), were between 0.3-0.7 % and 0.6-0.9 %, respectively. The recovery was between 99.1 and 101.7 %.

quality control, pharmaceutical research, HPTLC, densitometry, quantitative analysis 6

108 027 D.H. SHEWIYO, E. KAALE, P. G. RISHA, H. B. SILLO, B. DEJAEGHER, J. SMEYERS-VERBEKE, Y. VANDER HEYDEN* (*Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium; yvanvdh@vub.ac.be): Development and validation of a normal-phase HPTLC-densitometric method for the quantitative analysis of fluconazole in tablets. J. Planar Chromatogr. 24, 529-533 (2011). HPTLC of fluconazole (2-(2-difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)-2-propanol) on silica gel with ethyl acetate - methanol - ammonia - diaminoethane 170:20:10:1. Quantitative determination by densitometry at 216 nm. The hR_F value was 40. The *%RSD* for repeatability and intermediate precision were 1.6 and 3.1 %, respectively. The mean recovery was 99.1 %.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 6

7. Phenols

108 028 H. FARAJI, M. SABER-TEHRANI, A, MIRZAIE, S. WAQIF-HUSAIN* (*Department of Chemistry, Faculty of Science, Science and Research Branch, Islamic Azad University, P. O. Box 14515-775, Poonak-Hesarak, Tehran, Iran; syedwaqifhusain@yahoo.com): Application of liquid-

liquid microextraction - high-performance thin-layer chromatography for preconcentration and determination of phenolic compounds in aqueous samples. J. Planar Chromatogr. 24, 214-217 (2011). HPTLC of six phenolic compounds (phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol) in water samples extracted using microliter volumes of 1-undecanolon, on RP-18 with methanol - water 2:1 in a twin-trough chamber. Detection by spraying with a mixture of iron(III) chloride and potassium ferricyanide. Quantitative determination by densitometry in absorbance mode at 254 and 725 nm. The calibration curves were linear in the range of 0.025-4.0 and 0.1-20.0 mg/band. The limits of detection and quantification were between 8-35 and 25-98 ng/band, respectively. The relative standard deviation for repeatability was between 2.6 % and 6.4 %. Recovery (by standard addition) was between 89.2-101.7 %.

environmental, quality control, HPTLC, densitometry, quantitative analysis 7, 37c

108 029 A. MISHRA, V.S. GOWDRA, K. ARUMUGAM*, S. S. HUSSEN, K. BHAT, N. UDUPA (*Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal-576 104, Karnataka, India; millinkarthik@gmail.com): Stability-indicating HPTLC method for analysis of tolterodine in the bulk drug. J. Planar Chromatogr. 24, 150-153 (2011). HPTLC of tolterodine in the bulk drug on silica gel with toluene - methanol 1:1 + 1 drop aqueous ammonia in a saturated twin-trough chamber lined with filter paper; optimum saturation time was 30 min. The hR_F value was 40. Quantitative determination by densitometry in absorbance mode at 284 nm. Linearity was between 200 and 1800 ng/band (r^2 >0.990). The limits of detection and quantification were 100 and 200 ng/band, respectively. The repeatability and intermediate precision (*%RSD*, $n = 6$), was between 0.7-0.9 % and 0.6-1.8 %, respectively. Mean recovery was 100.9 %.

quality control, pharmaceutical research, HPTLC, densitometry, quantitative analysis 7

8. Substances containing heterocyclic oxygen

108 030 Elena CHERNETSOVA, Gertrud MORLOCK* (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany, gerda.morlock@uni-hohenheim.de): Fast quantification of 5-hydroxymethylfurfural in honey. CBS 107, 13-15 (2011). HPTLC of 5-hydroxymethylfurfural (HMF) in honey on silica gel, prewashed with methanol - water 6:1, with ethyl acetate. Quantitative determination by densitometry in absorbance mode at 290 nm. Optional detection by immersion in p-aminobenzoic acid reagent followed by heating at 110 °C for 5-10 min. The hR_F value of HMF was 80. The calibration function was polynomial in the range of 0.8-80 ng/band whilst Michaelis Menten 2 regression was suitable for higher concentrations. The LOD of HMF in honey samples was 0.75 mg/kg (12 µL applied) and the LOQ 2.4 mg/kg. The method complies with the requirement of max. 15 mg/kg of HMF in honey. The results with this method were compared with those obtained by the spectrophotometric Winkler method and by HPLC-UV and mean differences were minor (3.3 % or 0.9 mg/kg). Complementary confirmation by HPTLC-MS online coupling. HMF zones identified under UV were eluted and analyzed by ESI-MS in full-scan and SIM mode.

quality control, food analysis, comparison of methods, densitometry, HPTLC, quantitative analysis 8b

108 031 F. SOPONAR, A.C. MOT, C. SARBU* (*Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos, 400028, Cluj Napoca, Romania; csarbu@chem.ubbcluj.ro): High-performance thin-layer chromatography and three-dimensional image analysis for the determination of rutin in pharmaceutical preparations. J. AOAC Int. 93, 804-810 (2010). HPTLC

of rutin on amino phase with ethyl acetate - formic acid - methanol - water 100:9:11:17 at room temperature. Detection by spraying with natural products reagent. Linearity was between 0.95 and 4.78 µg/zone. LOD and LOQ were 330 and 630 ng/zone, respectively. The *%RSD* for six replicates at three concentration levels was less than 3 %, while the recovery was between 97.3-103.3 %. For the three-dimensional image analysis of chromatograms images were taken from each plate using a HP flatbed scanner at an optical resolution of 300 dpi in normal mode. The pictures were stored in TIFF file format. Evaluation was performed using Melanie 7.0 software. Following automatic detection of the zones the program computed the 3D image of a selected region from the HPTLC plate. The volume of the resulting cone-shaped zone was used as numerical data to construct the calibration curve.

pharmaceutical research, quality control, HPTLC, quantitative analysis 8a

10. Carbohydrates

108 032 A. MOHAMMAD*, S. LAEEQ (*Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering & Technology, Aligarh Muslim University, Aligarh, India; alimohammad08@gmail.com): Identification of coexisting pentose, hexose, and disaccharides with preliminary separation through hydrophilic interaction on silica HPTLC plate using aqueous sodium deoxycholate-acetonitrile mobile phase system. J. Planar Chromatogr. 24, 491-496 (2011). HPTLC of pentose, hexose and disaccharides in pharmaceutical formulations on silica with aqueous micellar bile salt, sodium deoxycholate in acetonitrile 1:5 with chamber saturation. This mobile phase provided the best separation of the 22 phases tested. Detection by spraying with ethanolic orcinol solution.

pharmaceutical research, quality control, HPTLC, qualitative identification 10a

108 033 Gertrud MORLOCK*, G. SABIR (*Institute of Food Chemistry, University of Hohenheim, Garbenstrasse 28, 70599, Stuttgart, Germany, gerda.morlock@uni-hohenheim.de): Comparison of two orthogonal liquid chromatographic methods for quantitation of sugars in food. J. Liq. Chromatogr. Relat. Technol. 34, 902-919 (2011). HPTLC of seven sugars (D-glucose, D-galactose, D-mannose, beta-D-fructose, alpha-D-fructose, sucrose, maltose, lactose) in food samples on silica gel with *n*-butanol - isopropanol - acetic acid - boric acid solution (200 mg boric acid in 10 mL water) 6:14:1:3. Detection by dipping into either aniline diphenylamine *o*-phosphoric acid reagent or p-aminobenzoic acid reagent. Quantitative determination by absorbance measurement at 370 nm. The HPTLC method was more sensitive by a factor of 8 for detection of sugars when compared to HPLC; only fructose showed a slightly better LOQ (difference by factor of 3). LOQ was better than 63 ng for HPTLC and about 500 ng for HPLC. Method comparison showed a good correlation and only a mean difference between both methods of 1.5 % sugar content for many food samples analyzed. HPTLC is a fully compliant method for determination of sugars in food. Application in the bioanalytical field was shown as well.

food analysis, quality control, comparison of methods, quantitative analysis, HPTLC, densitometry 10a

108 034 K. TAKÁCS, A. SZABÓ, I. WINKLER, B. ERDÉLYI* (*Fermentia Ltd, Berlini út 47-49, 1045 Budapest, Hungary; biofil@chello.hu; info@fermentia.hu): TLC method for monitoring the formation and degradation of bacterial exo-polysaccharides. J. Planar Chromatogr. 24, 211-213 (2011). HPTLC of exo-polysaccharides from bacterial fermentation and a series of mono-, oligo-, and polysaccharide reference solutions on silica gel with chloroform - toluene - 35 % formic acid - methanol 10:2:2:7 in a twin-trough chamber saturated for 1 h. Detection of starch-type poly-

saccharides by placing the plate into a twin-trough chamber saturated with iodine vapor. After drying determination by densitometry in absorbance mode at 400 nm. The plates were then immersed for 1 min in a solution of 10 % concentrated sulfuric acid in *n*-propanol - toluene 1:1, followed by heating at 115 °C for 10 min. The zones were evaluated visually and densitometrically at 400 nm.

quality control, pharmaceutical research, HPTLC, densitometry, quantitative analysis, qualitative identification, fermentation 10b

108 035 Catherine TESSINI*, M. VEGA, N. MUELLER, L. BUSTAMANTE, D. VON BAER, A. BERG, Claudia MARDONES (*Departamento de Análisis Instrumental, Facultad de Farmacia, Universidad de Concepción, Casilla 237, Correo 3, Concepción, Chile): High-performance thin-layer chromatography determination of cellobiosan and levoglucosan in bio-oil obtained by fast pyrolysis of sawdust. J. of Chromatogr. A 1218 (24), 3811-3815 (2011). HPTLC of sugars in bio-oil fractions on silica gel with acetonitrile - water 4:1, or mixtures of butanol and formic acid, followed by detection with the aniline - diphenylamine - *o*-phosphoric acid reagent. The method allowed for the separation of the anhydrosugars levoglucosan and cellobiosan, as well as glucose, arabinose, xylose and cellobiose without the need of pre-treatment and pre-derivatization of samples. Volatile compounds present in bio-oil did not interfere with sugar analysis, and the detrimental effect of the complex bio-oil matrix on columns and detector lifetime is avoided by using disposable HPTLC plates. It was found that the concentrations of levoglucosan and cellobiosan in bio-oil samples obtained from *Pinus radiata* sawdust were ranged between 1.3-2.3 % and 1.0-2.0 % respectively, while a higher levoglucosan concentration was in a bio-oil sample obtained from native wood.

HPTLC, quantitative analysis, qualitative identification 10

11. Organic acids and lipids

108 036 M. ANAND, Purvi GANDHI, Nancy PANDITA, S. GANDHI, P. DESHPANDE* (*Dept. of Pharmaceutical Chemistry, School of Pharmacy & Technology Management, SVKMs NMIMS University, Vile-Parle (w) Mumbai, India): Validated high-performance thin-layer chromatographic method for estimation of olopatadine hydrochloride as bulk drug and in ophthalmic solutions. International Journal of ChemTech Research 2(3), 1372-1375 (2010). TLC of olopatadine hydrochloride on silica gel aluminum foil with methanol - water - glacial acetic acid 40:10:1 with chamber saturation for 20 min. The hR_F value was 37. Quantitative determination by densitometry in absorbance mode at 247 nm. The method was linear in the range of 200-1200 ng/band. The average recovery was 100.5 %.

pharmaceutical research, quality control, densitometry, quantitative analysis 11a

108 037 J.L. COUNIHAN, K.E. HUEGLIN, C.R. WAGNER, S. P. GADOMSKI, P.A. ZANI, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton, PA, USA 18042; friedb@lafayette.edu): The effect of a diapause on neutral lipids in the pitcher-plant mosquito Wyeomyia smithii as determined by HPTLC-densitometry. J. Planar Chromatogr. 24, 206-210 (2011). HPTLC of free sterols, free fatty acids, triacylglycerols, methyl esters, and steryl esters in larvae samples on silica gel with concentration zone, with petroleum ether - diethyl ether - glacial acetic acid 80:20:1 in a twin-trough chamber saturated for 20 min at 21 +/- 1 °C and a relative humidity of 25 %. Detection by spraying with 5 % ethanolic phosphomolybdic acid solution. Quantitative determination by densitometry at 610 nm.

HPTLC, densitometry, quantitative analysis, biological research 11c

108 038 J. COUNIHAN, P. ZANI, B. FRIED, J. SHERMA* (*Department of Biology, Lafayette College, Easton, PA 18042, USA, friedb@lafayette.edu): Characterization and quantification of the polar lipids in the lizard Uta stansburiana by HPTLC-densitometry. J. Liq. Chromatogr. Relat. Technol. 32, 1289-1298 (2009). HPTLC of phosphatidylcholine (1), phosphatidylethanolamine (2), sphingomyelin (3), sulfatides (4), and cerebrosides (5) in tissue samples of the lizard Uta stansburiana on silica gel with chloroform - methanol - water 65:25:4. Detection by spraying with 10 % cupric sulfate in 8 % phosphoric acid, followed by heating at 140 ºC for 30 min. Quantitative determination by absorbance measurement at 370 nm. The hR_F values of (1)-(5) were 40, 56, 39, 51 and 71, respectively.

pharmaceutical research, HPTLC, densitometry, quantitative analysis 11c

108 039 Fatma HELMY*, A. MORRIS (*Department of Biological Sciences, Delaware State University, 1200 N. DuPont Highway, Dover, DE 19901, USA; fhelmy@desu.edu): A comparative study of the lipid composition of the brain of chicken and rat during myelination. A chromatographic and densitometric analysis. J. Planar Chromatogr. 24, 325-330 (2011). TLC on silica gel with A) 1-propanol - ethyl acetate - chloroform - methanol - water 50:50:50:21:18 for phospholipids, B) chloroform - methanol 9:2 for glycolipids (phospholipids remain at the origin), and C) chloroform - methanol - water 280:99:11 for the resolution of two ethanolamin plasmalogens (PE1, PE2). TLC on aluminum oxide with chloroform - methanol - water 65:30:4 for choline lipids (*e. g.*, phosphatidyl choline and sphingomyelin). 2D TLC on aluminum oxide after a hydrolysis step (1 % HCl) with mobile phase A in the first direction and hexane - diethyl ether 4:1 in the second direction for alkenyl lipids, followed by reaction with Schiff leucofuchsin reagent. Different detection methods were used: thionine reagent, Biebrich scarlet reagents (for cholin phopholipids), and the periodic acid Schiff reaction (for detection of glycolipids). Quantitative determination by densitometry at 600 nm (thionine), or 560 nm (leucofuchsin), or 520 nm (Biebrich).

densitometry, quantitative analysis 11e

108 040 K. KHANDAGLE, S. GANDHI, P. DESHPANDE, A. KALE, P. DESHMUKH (*Dept. of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Pune, India): High-performance thin-layer chromatographic determination of cefixime and ofloxacin in combined tablet dosage form. J. Chem. Pharm Res. 2(5), 92-96 (2010). TLC of ofloxacin and cefixime on silica gel with methanol - ethyl acetate - 25 % ammonia 7:7:3. The hR_F value of ofloxacin was 61 and of cefixime 78. The method was linear in the range of 50-500 ng/band. The recovery was in the range of 99.3-102.2 %. Quantitative determination by densitometry in absorbance mode at 295 nm.

pharmaceutical research, quality control, densitometry, quantitative analysis 11a

108 052 I. NAGUIB et al., see section 17

108 041 Hasumati RAJ*, Sadhana RAJPUT, J. DAVE, C. PATEL (*Shri Sarvajanik Pharmacy College, Near Arvind baug, Mehsana-384001, Gujarat, India): Development and validation of two chromatographic stability-indicating methods for determination of rosuvastatin in pure form and pharmaceutical preparation. International Journal of ChemTech Research 1(3), 677-689 (2009). HPTLC of rosuvastatin in raw material and tablet dosage formulation on silica gel with ethyl acetate - toluene - acetonitrile - formic acid 60:35:5:2. The hR_F value was 85. Quantitative evaluation by absorbance measurement at 243 nm. The method was found to be linear in the range of 318-3816 ng/band. The mean recovery was 99.7 %. The sample was subjected to different stress

conditions and the degradation products were well separated from the main drug.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 11a

108 042 A. RAJASEKARAN, R. ARIVUKKARASU, D. ARCHANA* (*Dept. of Pharmaceutical Analysis, KMCH College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore, India): HPTLC method for estimation of gallic acid and rutin in Haritaki - an ayurvedic formulation. International Journal of PharmTech Research 3(2), 986-999 (2011). TLC of methanolic extracts of Haritaki and gallic acid and rutin as markers on silica gel with toluene - ethyl acetate - formic acid 3:6:1 for gallic acid and chloroform - ethyl acetate - methanol - formic acid 7:10:1:2 for rutin. Quantitative determination by densitometry in absorbance mode at 280 nm for gallic acid and 254 nm for rutin. The method was linear in the range of 100-500 ng/band for gallic acid and 1000-5000 ng/band for rutin. The recovery was 99.1 % for gallic acid and 97.9 % for rutin. The LOD and LOQ of gallic acid was 71 and 213 ng/zone and of rutin 63 and 189 ng/zone.

traditional medicine, herbal, densitometry, quantitative analysis 11a,8b

108 043 Janhavi RAO*, Kamini SETHY, Savita TADAV (*Dept. of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Erandwane Pune, Maharashatra, India): Validated HPTLC method for simultaneous quantitation of cefixime and ofloxacin in bulk drug and in pharmaceutical formulation. Pharmacie Globale (IJCP) 4(05), 1-4 (2011). TLC of cefixime and ofloxacin in combined tablet dosage formulation on silica gel with *n*-butanol - 25% ammonia - water - DMSO 8:3:1:2. The hR_F value of cefixime was 55 and of ofloxacin 65. Quantitative evaluation by absorbance measurement at 297 nm. The method was linear in the range of 30-180 ng/band for both drugs.

pharmaceutical research, quality control, clinical chemistry research, densitometry, quantitative analysis 11a

108 044 A. SEEMUNGAL, A. PETRÓCZI, D.P. NAUGHTON* (*School of Life Sciences, Kingston University, Kingston upon Thames, Surrey KT1 2EE, UK; D.Naughton@kingston.ac.uk): Application of thin-layer chromatography to rank the efficacies of five antioxidants in red wine. J. Planar Chromatogr. 24, 320-324 (2011). TLC of a red wine sample and gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and quercetin as standards on silica gel with toluene - ethyl actate - formic acid 6:5:1 with chamber saturation for 1 h. Detection by treatment with 1 % methanolic diphenylborinic acid 2-aminoethyl ester (natural products reagent) followed by 5 % ethanolic polyethylene glycol. Evaluation under UV light at 254 and 366 nm. Also spraying with 0.04 % methanolic DPPH radical reagent. The hR_F value was 65, 60, 56, 47, and 7 for p-coumaric acid, quercetin, caffeic acid, gallic acid, and chlorogenic acid, respectively.

food analysis, qualitative identification 11a

13. Steroids

108 045 H. LI (Li Huimin) (Huizhou Municipal Inst. for Drug Cont., Guangdong, Huizhou 516003, China): (Quick screening of 10 glucocorticoids in traditional Chinese formulations and some health-care foods by thin-layer chromatography) (Chinese). J. of China Pharm. 19 (23), 24-25 (2010). TLC of the extracts of traditional Chinese formulations and some health-care foods on silica gel with dichloromethane - acetone 4:1. Detection under UV 254 nm. This TLC method was better than the previously used one with the mobile phase dichloromethane - diethyl ether - methanol - water 385:60:15:2 and detection reagent 2 % tetrazolium solution - 2N NaOH - methanol 3:10:5.

Identification of dexamethasone, prednisone, prednisolone acetate, prednisone acetate, cortisone acetate, hydrocortisone, betamethasone, hydrocortisone acetate, dexamethasone acetate, fluocinolone acetonide acetate by comparison with the standards. The improved procedure proved to be simple, fast, robust and suitable for quick screening of 10 glucocorticoids illegally added to some traditional Chinese formulations and health-care foods.

pharmaceutical research, quality control, traditional medicine, food analysis, qualitative identification 13

108 046 A. PYKA (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellon´ska Street, 41-200 Sosnowiec, Poland, apyka@sum.edu.pl): Spectrodensitometry application to analytical identification of estradiol, hydrocortisone, testosterone and cholesterol on diol plates. J. Liq. Chromatogr. Relat. Technol. 32, 1084-1095 (2009). HPTLC of estradiol (1), hydrocortisone (2), testosterone (3), and cholesterol (4) on diol phase with chloroform. Detection by dipping the plate into a solution of sulphuric acid - methanol 1:19 for 15 s, followed by heating at 120 ºC for 10 min. Quantitative determination by absorbance measurement at 200 nm for (1) and (4) and 248 nm for (2) and (3). The hR_F values of (1)-(4) were 18, 2, 28 and 46, respectively.

quantitative analysis, HPTLC 13

108 008 A. PYKA, see section 2

14. Steroid glycosides, saponins and other terpenoid glycosides

108 047 D. KHERA, K. KOHLI*, N. PARMAR (*Faculty of Pharmacy, Department of Pharmaceutics, Hamdard University, Hamdard Nagar, New Delhi 110062, India, kanchan.kohli@hotmail.com): Development and validation of stability-indicating HPTLC method for determination of glycyrrhizic acid in bulk drug and pharmaceutical formulations. J. Liq. Chromatogr. Relat. Technol. 34, 1502-1517 (2011). HPTLC of glycyrrhizic acid in bulk drug and pharmaceutical formulations on silica gel with butanol - glacial acetic acid - water 7:1:2. Quantitative determination by absorbance measurement at 254 nm. The hR_F of glycyrrhizin was 24. Linearity was 200-1000 ng/zone. Limits of detection and quantification were found to be 80 and 200 ng/band. The intermediate/inter-day/intra-day precision was below 0.6 % (n=3). Recoveries (by standard addition) were 98.1-99.5 %.

pharmaceutical research, traditional medicine, HPTLC, densitometry, quantitative analysis 14

17. Amines, amides and related nitrogen compounds

108 048 M. ANAND, A. FONSECA, S. GANDHI, P. DESHPANDE* (* Dept. of Pharmaceutical Analysis, A.I.S.S.M.S. College of Pharmacy, Kennedy Rd., Near R.T.O.,Pune,(M.S.),India): Development and validation of high-performance thin-layer chromatographic method for estimation of brimonidine tartrate as bulk drug and in ophthalmic solutions. International Journal of PharmTech Research 2(3), 1376-1379 (2010). TLC of brimonidine tartrate on silica gel with methanol – 25 % ammonia 40:1 with chamber saturation for 20 min. The hR_F value was 52. Quantitative determination by densitometry in absorbance mode at 250 nm. The method was linear in the range of 200-1200 ng/band. The intra-day and inter-day precision, as *%RSD*, was in the range of 0.7-1.8 % and 0.9-1.8 %, respectively. The mean recovery (by standard addition) was 99.7 %.

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

108 049 D. JUN, P. STODULKA, M. HRABINOVA, M. PROHANKA, B. DOLEZAL, K. KUCA* (*Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Trebesska

1575, Hradec Kralove 500 01, Czech Republic; kucakam@pmfhk.cz): TLC analysis of twelve different salts of oxime HI-6 - reactivator of nerve agent inhibited AChE. J. Planar Chromatogr. 24, 105-107 (2011). TLC of twelve bisquaternary acetylcholinesterase reactivator HI-6-salts (sulfate, chloride, acetate, bromide, phosphate, mesylate, tartrate, iodide, malonate, salicylate, maleinate, and tosylate) on cellulose with acetone - acetic acid - water - toluene 5:2:2:1, butanol - acetic acid - water - toluene 1:1:1:1, isopropanol - acetic acid - water - toluene 5:2:2:1, and isopropanol - formic acid - water - toluene 6:2:2:1 in a twin-trough chamber. Detection under UV light at 254 nm and by spraying with Dragendorff's reagent.

quality control, toxicology, pharmaceutical research, qualitative identification 17a

108 050 D. KALE*, R. KAKDE (*Dept. of Pharmaceutical Sciences, R.T.M. Nagpur University, Amravati Rd., Nagpur, India): HPTLC estimation of nateglinide in bulk drug and tablet dosage form. Asian Journal of Chemistry 23(10), 4351-4354 (2011). HPTLC of nateglinide in tablet dosage form on silica gel with *n*-hexane - methanol - isopropanol 75:15:10. The hR_F value was 56. Quantitative evaluation by absorbance measurement at 210 nm. The method was linear in the range of 300-1000 ng/band. The average recovery was 98.5 %.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 17a

108 051 D.M. KHATRI, P.J. MEHTA* (*Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, Sarkhej-Gandhinagar Highway, Ahmadabad 382482, Gujarat, India; drpritimehta@nirmauni.ac.in): Stability-indicating HPTLC method for determination of milnacipran hydrochloride in pharmaceutical forrmulations. J. Planar Chromatogr. 24, 412-418 (2011). HPTLC of milnacipran hydrochloride on silica gel, prewashed with methanol, with chloroform - methanol - ammonia 64:25:2 in a twin-trough chamber saturated for 20 min. Detection under UV light at 245 and 366 nm. Quantitative determination by absorbance measurement at 220 nm. The hR_F value of milnacipran was 45. Linearity was between 500 and 6000 ng/zone. The method precision, intra-day precision, inter-day precision, and different analyst precision (n = 6 each, *%RSD*), was 1.2, 1.9, 1.6, and 1.9 %, respectively. The mean recovery (n = 3) was 99.2-99.8 % with a *%RSD* between 0.6-1.7 %.

pharmaceutical research, quality control, HPTLC, quantitative analysis, densitometry 17c

108 052 I. NAGUIB, M. ABDELKAWY* (*Analytical Chemistry Dept., Faculty of Pharmacy, Beni-Suef University, 62111, Egypt): Development and validation of stability indicating HPLC and HPTLC methods for determination of sulpiride and mebeverine hydrochloride in combination. European Journal of Medicinal Chemistry 45, 3719-3725 (2010). TLC of sulpiride and mebeverine hydrochloride on silica gel with absolute ethanol - methylene chloride - triethylamine 35:15:1. Quantitative determination by absorbance measurement at 221 nm. The method was linear in the range of 0.4-1.4 µg/band for sulpiride and 0.2-1.6 µg/band for mebeverine hydrochloride. The recovery was 100.4-101.0 %. The hR_F value of sulpiride was 42 and of mebeverine hydrochloride 62. The results obtained by this TLC method were comparable with those by HPLC.

pharmaceutical research, quality control, quantitative analysis, comparison of methods 17c,11a

108 053 J.R. RAO*, M. KUMAR, L. SATHIYANARAYANAN, S. SAVITA, V. YADAV (*Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwana, Pune-411038, India; janhavirao@rediffmail.com): Application of a stability-indicating

HPTLC method for quantitative analysis of sertraline hydrochloride in pharmaceutical dosage forms. J. Planar Chromatogr. 24, 140-144 (2011). HPTLC of sertraline hydrochloride on silica gel, prewashed with methanol, with toluene - ethyl actate - ethanol - ammonia 80:20:95:1 in a twin-trough chamber saturated for 30 min at 25 +/- 2 °C. The hR_F value was 33. Quantitative determination by densitometry in absorbance mode at 273 nm. The linearity was in the range of 2-12 µg (r^2 = 0.9996). The intra-day and inter-day precision for sertralin hydrochloride standard was 0.6, 0.4, 0.3 % and 0.8, 0.4, 0.2 %, respectively; for sertraline hydrochloride in commercial tablets 0.6, 0.2, 0.1 % and 0.6, 0.3, 0.2 %, respectively. The LOD was 670 ng/band and the LOQ 710 ng/band. *[Note of the editor: LOD/LOQ difference arises questions.]*

pharmaceutical research, quality control, HPTLC, quantitative analysis, densitometry 17a

108 054 D. VASTAG, Nada PERISIC-JANJIC*, J. TOMIC, S. PETROVIC (*Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia; nadap@uns.ac.rs): Evaluation of the lipophilicity and prediction of biological activity of some N-cyclohexyl-N-substituted-2-phenylacetamide derivatives using RP-TLC. J. Planar Chromatogr. 24, 435-440 (2011). TLC of nine N-cyclohexyl-N-substituted-2-phenylacetamides on RP-18 with different aqueous eluents; water - acetone, water - acetonitrile, and water - dioxane. The volume fraction of the organic modifier in the aqueous mobile phase comprised 60-80 % (dioxane, acetone) and 70-90 % (acetonitrile) and varied in steps of 5 %. Detection under UV light at 254 nm. The effects on retention behavior of all investigated modifiers were similar. The linear relationship between retention parameters and organic modifier content allowed the extrapolation procedure. The result of the investigation showed that reversed-phase RM0 proved to express the lipophilic nature of an investigated compound as well as its biological activity.

clinical chemistry research, qualitative identification 17c

108 055 A. WIECKOWSKA, M. BAJDA, K. WIECKOWSKI, Barbara MALAWSKA* (*Faculty of Pharmacy, Department of Physicochemical Drug Analysis, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland; mfmalaws@cyf-kr.edu.pl): Chromatographic and computational studies of the physicochemcal properties of cholinesterase inhibitors - alkyl- and arylcarbamate derivatives of N-benzylpiperidine and N-benzylpiperazine. J. Planar Chromatogr. 23, 359-364 (2010). TLC of four series of carbamates of 3-hydroxy- and 4-hydroxyphenyl-acetamides, 4-benzylpiperidine and 1-benzylpiperazine on RP-18 with mixtures of acetonitrile and water containing 45-90 % organic modifier, with chamber saturation for 2 h at ambient temperature. Detection under UV light at 254 nm. The results obtained were compared with theoretical lipophilicity calculated by use of ChemOffice, QikProp, and Pallas software.

pharmaceutical research, quality control, qualitative identification 17c

18. Amino acids and peptides, chemical structure of proteins

108 056 A. CHOMICKI, K. KLOC, T. H. DZIDO* (*Department of Physical Chemistry, Medical University of Lublin, Chozki 4a, 20-093 Lublin, Poland; tadeusz.dzido@umlub.pl): Two-dimensional separation of some amino acids by HPTLC and pressurized planar electrochromatography. J. Planar Chromatogr. 24, 6-9 (2011). Two-dimensional separation of eight amino acids (arginine, phenylalanine, histidine, glutamic acid, leucine, lysine, threonin, and tryptophan) by HPTLC in the first dimension and pressurized planar electrochromatography in the second, orthogonal direction. HPTLC on RP-18, prewashed by dipping into methanol for 1 min, with acetonitrile - buffer solution. Buffer solutions of pH 3.2-7.0 were prepared by mixing 0.1 M citric acid with 0.2 M disodium hydrogen phosphate. Chromatogram development was performed with 10 % aceto-

nitrile in buffer (pH 3.2) in a horizontal chamber after saturation for 15 min. Then the HPTLC plate was prewetted with mobile phase, inserted into the PPEC chamber and separation was performed for 21 min at a polarization voltage of 2.5 kV. Detection by spraying with ninhydrin reagent followed by heating under a hot air stream. Combination of HPTLC and PPEC in a two-dimensional process leads to substantial enhancement of amino acid separation.

HPTLC, qualitative identification 18a

108 057 F.A. MEHTA*, B.G. PATEL, S.S. PANDYA, K.B. AHIR (*Indukaka Ipcowala College of Pharmacy, P. O. Box 53, P. O. Vithal Udyognagar, Beyond GIDC Phase IV, New Vallabh Vidyanagar, Gujarat, 388 121, India; fm999@ymail.com): High-performance thin-layer chromatographic analysis of betaine in alcohol extracts of Achyranthes aspera L. J. Planar Chromatogr. 24, 136-139 (2011). HPTLC of ethanolic extracts of plant material and betaine on silica gel, prewashed with methanol, with methanol - water 9:1 in a twin-trough chamber saturated for 30 min. Detection under UV light at 254 and 366 nm and by spraying with Dragendorff's reagent or with sulfuric acid reagent followed by heating at 120 °C for 10 min (Dragendorff's reagent) or at 110 °C until the intensity of the fluorescent zones reached a maximum (sulfuric acid). Quantitative determination by densitometry at 550 nm. The calibration range was between 4 and 30 µg/band. LOD and LOQ was 1 and 4 µg/band, respectively. The repeatability (n = 6) was 1.7 %. The recovery was between 99.1-101.9 %. The inter-day and intra-day precision (n = 3) was between 1.1- 2.2 % and 1.2-2.2 %, respectively.

traditional medicine, herbal, quality control, HPTLC, quantitative analysis, densitometry 18a

20. Enzymes

108 058 A. ANKLI*, D. HANDLOSER, V. WIDMER, E. REICH, E. CENIVIVA (*CAMAG Laboratory, Sonnenmattstr. 11, 4432 Muttenz, Switzerland, anita.ankli@camag.com): Rapid test for content uniformity of coenzyme Q10 in soft gel capsules by HPTLC. CBS 107, 5-7 (2011). HPTLC of coenzyme Q10 on silica gel (prewashed with methanol) with toluene in a horizontal developing chamber or a twin-trough chamber without chamber saturation. Quantitative determination by densitometry in absorbance mode at 282 nm. The hR_F value of coenzyme Q10 was 20. Linearity was between 20-50 ng/zone. By development in the horizontal chamber from both sides of the plate the analysis of 72 samples (10 samples from 6 different batches and 12 standards) takes only 86 min.

quality control, HPTLC, densitometry, quantitative analysis 20

108 059 Irena VOVK*, Gordana POPOVIC, Breda SIMONOVSKA, A. ALBREHT, Danica AGBABA (*National Inst. of Chem., Lab. for Food Chem., Hajdrihova 19, SI-1000 Ljubljana, Slovenia): Ultra-thin-layer chromatography mass spectrometry and thin-layer chromatography mass spectrometry of single peptides of angiotensin-converting enzyme inhibitors. J. of Chromatogr. A 1218 (20), 3089-3084 (2011). Comparison of the separation of the structurally related angiotensin-converting enzyme (ACE) inhibitors lisinopril, cilazapril, ramipril and quinapril and their corresponding active diacid forms (prilates) by conventional TLC on silica gel with the separation on monolithic ultra-TLC (UTLC) phase. Technical modifications of the commercially available equipment for sample application, development and detection were necessary for the use with UTLC plates. Development in a modified horizontal developing chamber with ethyl acetate - acetone - acetic acid - water 16:4:1:2. Detection by absorbance measurement at 220 nm and after exposure to iodine vapors under daylight, as well as by image analysis. As a result the monolithic layer was more efficient for the separation of structurally similar polar compounds, such as prilates, than conventional silica layers. Confirmation of the identity of the compounds by ESI-MS

after their online extraction from the UTLC and TLC plates.

clinical chemistry research, HPTLC, densitometry, qualitative identification, quantitative analysis, comparison of methods 20

27. Vitamins and various growth regulators

108 060 D.V. DEMCHENKO, O.N. POZHARITSKAYA, A.N. SHIKOV*, V.G. MAKAROV (*Saint Petersburg Institute of Pharmacy, 47/5, Piskarevskiy pr., St. Petersburg, Russia; alexs79@mail.ru): Validated HPTLC method for quantification of Vitamin D3 in fish oil. J. Planar Chromatogr. 24, 487-490 (2011). HPTLC of vitamin D3 in fish oil after saponification and purification on silica gel with chloroform - diethyl ether 9:1 with chamber saturation for 20 min. Quantitative determination at 280 nm. Linearity was between 200 and 1000 ng/band. The LOD and LOQ were 29 and 232 ng/band, respectively. The repeatability (*RSD*) was 4.6 %; the *%RSD* for intermediate precision was 0.9; the *%RSD* for intra-day precision ($n = 6$) was between 2.9-5.9 % and the *%RSD* for inter-day precision ($n = 6$) was between 3.2-6.4 %. Recovery was 97.6 - 104.2 % with a *%RSD* of 3.9 - 4.8 %.

food analysis, quality control, HPTLC, quantitative analysis 27

28. Antibiotics, Mycotoxins

108 061 K. BOBER (Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, 41-200, Sosnowiec, Poland, bober@sum.edu.pl): The visualizing agents for selected quinolones and fluoroquinolones. J. Liq. Chromatogr. Relat. Technol. 32, 3049-3055 (2009). HPTLC of cinoxacin (1), pipemidic acid (2), ofloxacin (3), and pefloxacin (4) on silica gel with buffer solution (pH 5.5) - methanol 4:1 and acetonitrile - water - acetic acid, 3:20:2. Detection by dipping into various visualization agents. The detection limits for (1) to (4) were 0.1, 0.1, 0.5, and 0.75 µg/zone, respectively. Best detection conditions for (1) and (2) were obtained by dipping in Janus blue and immediate evaluation, and for (3) and (4) by dipping in Cresol red followed by heating at 120 ºC for 10 min for (3) and (4).

pharmaceutical research, HPTLC, quantitative analysis 28a

108 062 E.M. GRZELAK, B. MAJER-DZIEDZIC, Irena M. CHOMA* (*University of Maria-Curie-Sklodowska, Department of Chromatographic Methods, Lublin, Poland; Irena.Choma@umcs.lublin.pl): Development of a novel direct bioautography - thin-layer chromatography test: Optimization of growth conditions for Gram-negative bacteria, Escherichia coli. J. AOAC Int. 94, 1567-1572 (2011). TLC of flumequin as standard on silica gel using various incubation times. After incubation, the plates were sprayed with 0.2 % aqueous MTT solution and incubated for 0.5 h at 37 °C. The regression coefficients were 0.9977 and 0.9968, respectively, for intraday and interday curves. The calibration curves showed good linearity in the range of 5-500 ng (0.5-50.0 µg/mL). The established LOD of flumequine was 5 ng/zone (0.5 µg/mL). One drop of triton X-100/10 mL aqueous MTT solution was found to enhance the intensity of the color.

pharmaceutical research, qualitative identification, bioautography 28a

108 063 J. NOWAKOWSKA, P. PIKUL*, P. ROGULSKI (*Medical University of Gdansk, Faculty of Pharmacy, Department of Physical Chemistry, al. Gen. Hallera 107, 80-416 Gdansk, Poland; pikul.piotr@gumed.edu.pl): TLC of aclarubicin and doxycycline with mixed *n*-alcohol mobile phases. J. Planar Chromatogr. 23, 353-358 (2010). TLC of aclarubicin and doxycycline on silica gel, RP-18, cellulose, polyamide 11, and HPTLC on silica gel and RP-18 with a wide range (from 0 to 100 %) of mixtures of *n*-alcohols with DMSO, hexamethyldisiloxane, acetonitrile, and water in chambers saturated with mobile phase at room temperature. Detection by spraying with sul-

furic acid - methanol 1:4, anisaldehyde - methanol - acetic acid - orthophosphoric acid - sulfuric acid 1:100:10:10:5 (for cellulose) and 5 % aluminium chloride in methanol (for polyamide). The effect of mobile and stationary phases on the chromatographic behavior of the compounds was studied.

pharmaceutical research, quality control, qualitative identification, HPTLC 28a

108 064 Eva PETRLÍKOVÁ*, K. WAISSER (*Department of Inorganic and Organic Chemistry, Charles University in Prague, Faculty of Pharmacy in Hradec Králové, Heyrovského 1203, 50005 Hradec Králové, Czech Republic; Eva.Petrlikova@faf.cuni.cz): A TLC study of the lipophilicity of new antimycobacterial active benzoxazine derivatives containing a thioxo group. J. Planar Chromatogr. 24, 196-200 (2011). Experimental RM values of a series of 3-(4-alkylphenyl)-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones and 3-(4-alkylphenyl)-2H-1,3-benzoxazine-2,4(3H)-ones were obtained by a reversed-phase TLC system. The mobile phase was a phosphate buffer with volume fractions of acetone between 80 and 60 %. TLC of 14 compounds on RP-18 with phosphate buffer pH 7.4 and acetone (80, 75, 70, 65, and 60 %). Detection under UV light. The retention constants RM0 were compared with the partition coefficients calculated using different software products.

qualitative identification 28a

108 065 B.K. SINGH*, D.V. PARWATE, S. SRIVASTAVA, S. K.SHUKLA (*Department of Chemistry, RTM Nagpur University Campus, Amravati Road, Nagpur, India-440033; singhbab2001@rediffmail.com): Thin-layer chromatographic selective and stability-indicating method for assay of cefixime in pharmaceuticals. J. Planar Chromatogr. 24, 524-528 (2011). TLC of cefixime on silica gel with toluene - ethyl acetate - formic acid - water 5:29:11:5 with chamber saturation for 30 min at 25 +/- 2 °C. The hR_F value was 54. Quantitative determination by densitometry in absorbance mode at 293 nm. Linearity was between 500 and 1500 ng. The repeatability (*%RSD*) was below 2 %. The limit of detection and the limit of quantification was 9 and 42 ng/zone, respectively. The recovery was between 98.9-100.3 %.

pharmaceutical research, quality control, quantitative analysis, densitometry 28a

108 066 Irena VOVK*, B. SIMONOVSKA (*National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia, and EN-FIST Centre of Excellence, Dunajska cesta 156, SI-1000 Ljubljana, Slovenia; irena.vovk@ki.si): Development and validation of a high-performance thin-layer chromatographic method for determination of ofloxacin residues on pharmaceutical equipment surfaces. J. AOAC Int. 94, 735-742 (2011). HPTLC and TLC of ofloxacin on silica gel with ethanol - conc. ammonia 4:1 in a horizontal chamber. Quantitative determination by fluorescence measurement at 313 nm. Simulated samples at a residue level of 1 mg/m² were prepared by spreading the calculated amount of ofloxacin solution on 1, 5, and 10 dm² stainless steel surfaces. The hR_F of ofloxacin was 56. The mean recovery ($n = 6$) was 88.6-95.3 % with a CV of 3.8-4.9 %. The LOD was 0.6 ng/zone and the LOQ was 2 ng/zone, but it was shown that these can be lowered by immersion of the developed plate into a solution of liquid paraffin - *n*-hexane 1:2 to approximately 0.3 and 0.9 ng/zone. The repeatability (system precision) was 4.2 % for 2 ng, and 3.7 % for 20 ng. The recovery was between 88.6-95.3 %.

pharmaceutical research, quality control, HPTLC, quantitative analysis, densitometry 28a

29. Pesticides and other agrochemicals

108 067 J. BHATIA*, J. D. SHARMA (*Forensic Science Laboratory, Port Blair, Andaman and Nicobar Islands-744101, India; jitesh007bhatia@yahoo.com): Thin-layer chromatographic detection

of carbosulfan by 4-aminoantipyrine reagent. J. Planar Chromatogr. 24, 545-546 (2011). TLC of carbosulfan and other commonly available carbamate pesticides, organochlorine pesticides, organophosphorus pesticides, synthetic pyrethoids and extracts from biological tissues on silica gel with toluene - acetone 9:1 with chamber saturation for 30 min. Detection by spraying with 10 % sodium hydroxide solution followed by 4-aminoantipyrine reagent and then with potassium hexacyanoferrate(III) reagent. The red-colored zone for carbosulfan was observed immediately. Carbaryl, propoxur, and carbofuran form a red-colored zone at the hR_F values of 52, 45, and 48, respectively, whereas the hR_F value of carbosulfan was 94.

toxicology, qualitative identification 29c

108 068 K.K. KULKARNI*, D.B. SHINDE, D.V. MANE, R.B. TOCHE, M.V. GARAD (*Directorate of Forensic Science Laboratory, State of Maharastra, Home Department, Vidyanagari, Kalina, Santacruz, (East), Mumbai-400 098, India; krishnakulkarni96@yahoo.com): New chromogenic spray reagent for detection and identification of carbosulfan. J. Planar Chromatogr. 23, 373-375 (2010). HPTLC of carbosulfan on silica gel with *n*-hexane - acetone 4:1 in a saturated chamber. Detection by spraying with 10 % sodium hydroxide solution followed by potassium ferricyanide reagent. Semi-quantitative analysis after extraction is done against standards. Other carbamate, organophosphorus, organochlorine, and pyrethroid insecticides and constituents of viscera do not interfere. The detection limit of carbosulfan is ca. 500 ng.

toxicology, HPTLC, qualitative identification 29f

108 070 D. LIU*, C. QIAN, Y. WANG (*Dept. of Applied Chemistry, College of Science, China Agriculture University, Beijing 100193, P.R., China): Determination of organophosphorus pesticides in rice by TLC. Asian Journal of Chemistry 23(5), 2011-2013 (2011). Methods are reported for determination of organophosphorus pesticides using TLC-cholinesterase inhibition as well as GC-FID. The powdered rice sample was initially extracted with ethyl acetate. The extract was cleaned up by GPC, eluted with cyclohexane - ethyl acetate 1:1, evaporated, and taken up in acetone. This extract was analysed by TLC on silica gel with ethyl acetate to a developing distance of 10-12 cm. After development the plate was air dried, exposed to bromine vapors, sprayed with enzyme solution and incubated at 37 °C for 30 min and sprayed with the reagent. White spots appeared against bluish-red background. The sample was also analysed by GC and the GC method was very sensitive. However, the TLC method is recommended for preliminary screening of samples.

quality control, comparison of methods, qualitative identification 29b

108 013 A. MOHAMMAD *et al.*, see section 3

108 014 Claudia OELLIG *et al.*, see section 3

108 015 Claudia OELLIG *et al.*, see section 3

108 002 J. SHERMA, see section 1

30. Synthetic and natural dyes

108 071 H. KANDLER, M. BLEISCH, Valeria WIDMER, E. REICH* (*CAMAG Laboratory, Sonnen-

mattstrasse 11, 4132 Muttenz, Switzerland, eike.reich@camag.com): A validated HPTLC method for the determination of illegal dyes in spices and spice mixtures. J. Liq. Chromatogr. Relat. Technol. 32, 1273-1288 (2009). HPTLC of Sudan I (1), II (2), III (3), IV (4), Sudan Red B (5), Sudan Red 7B (6), Sudan Red G (7), Para Red (8), FD&C Orange 2 (9), Butter Yellow (10), Citrus Red 2 (11), Toluidine Red (12), and Disperse Orange 11 (13) in paprika, chili, and curry on RP-18 with acetonitrile - ammonia 25 % 19:1. Quantitative determination by absorbance measurement at absorption maxima of each dye. The hR_F values of compounds (1) - (13) were 61, 54, 48, 29, 18, 11, 69, 63, 56, 48, 39, 18 and 11, respectively. Visual detection limits were 3 ppm for most dyes in either matrix, 5 ppm for Sudan I, 13 ppm for Disperse Orange, and 7 ppm for Butter Yellow. The limits of detection by densitometry were lower by a factor of 2 for all dyes and values of 1-3 ppm were reached except for Disperse Orange with a limit of detction of 7 ppm. Average recoveries ranged from 95.0-110.8 %. The HPTLC method is successfully applied in the routine control of illegal dyes in food by surveillance authorities.

toxicology, food analysis, HPTLC, densitometry, quantitative analysis, qualitative identification 30a

108 072 A.N. SHIKOV, V.I. OSSIPOV, O. MARTISKAINEN, Olga POZHARITSKAYA, Svetlana IVANOVA, V.G. MAKAROV* (*Saint-Petersburg Inst. of Pharmacy, 47/33, Piskarevsky pr., 195067 St.-Petersburg, Russia): The offline combination of thin-layer chromatography and high-performance liquid chromatography with diode array detection and microTOF-Q mass spectrometry for the separation and identification of spinochromes from sea urchin (Strongylocentrotus droebachiensis) shells. J. of Chromatogr. A 1218 (50), 9111-9114 (2011). A short communication on the fractionation, separation and identification of spinochrome pigments from sea urchin (Strongylocentrotus droebachiensis) shells by TLC with off-line HPLC coupled to diode array detection and microTOF-Q mass spectrometry (HPLC-DAD-MS). Two fractions of pigments were obtained and separated by TLC, then eluted with methanol directly into the MS using the TLC-MS Interface. The HPLC-DAD-MS analysis of the fractions indicated the presence of six sea urchin pigments: spinochrome monomers B and D, three spinochrome dimers (anhydroethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin) and its isomer and ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin)), and one pigment that was preliminary identified as a spinochrome dimer with the structural formula $C_{22}H_{16}O_{16}$.

herbal, quality control, HPTLC, qualitative identification, preparative TLC 30b

32. Pharmaceutical and biomedical applications

108 073 H. ADHAMI, T. LINDER, H. KAEHLIG, D. SCHUSTER, M. ZEHL, Liselotte KRENN* (*Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria, liselotte.krenn@univie.ac.at): Catechol alkenyls from *Semecarpus anacardium*: Acetylcholinesterase inhibition and binding mode predictions. J. Ethnopharmacol. 139, 142-148 (2012). HPTLC of 1,2-dihydroxy-3-pentadec-8-enylbenzene (A) and 1,2-dihydroxy-3-pentadeca-8,11-dienylbenzene (B) in the fruits of *Semecarpus anacardium L. f.* (Anacardiaceae) on RP-18 with acetonitrile - water 199:1. Detection by spraying with anisaldehyde - sulfuric acid reagent. The hR_F values of (A) and (B) were 31 and 42, respectively. The method was combined with ESI-MS and NMR for compound identification.

pharmaceutical research, traditional medicine, HPTLC, qualitative identification 32e

108 074 E. AGUIRRE-HERNÁNDEZ, M.E. GONZÁLEZ-TRUJANO, G. PÉREZ-ORTEGA, R.E. LLANOS-ROMERO, Patricia GUEVARA-FEFER* (*Departamento de Ecología y Recursos Natu-

rales, Facultad de Ciencias, Universídad National Autónoma de México, Ciudad Universitaria Coyoacán, 04510 México; patriciaguevara@ciencias.unam.mx): TLC fingerprint profile and antioxidant and anti-inflammatory effects of aqueous extracts from species of Cleyera and Ternstroemia genera. J. Planar Chromatogr. 24, 400-405 (2011). TLC of plant extracts and kaempferol, quercetin, and rutin as standards on silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:27 with chamber saturation for 30 min. Detection by spraying with 1 % methanolic diphenylborinic acid 2-aminoethylester followed by 5 % ethanolic polyethylene glycol 4000. Evaluation under UV 366 nm. Detection of the antioxidant activity with DPPH radical reagent. Videodensitometric analysis of the derivatized TLC plates. The hR_F value was 24 for rutin, 98 for quercetin and 100 for kaempferol. *[Note of the editor: Such high hR_F values should be avoided by reduction of the elution strength of the mobile phase.]*

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

108 075 M. AMIR, M. MUJEEB, A. SABIH, S. AHMAD, A. AHMAD*, W. A. SIDDIQUI (*Plant Tissue Culture Laboratory, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdad, New Delhi 110062, India; aamirx1@gmail.com): Validation of HPTLC method for estimation of diosgenin in callus and rhizome of *Dioscorea deltoidea*. Planta Med. 76, 523 (2010). HPTLC of diosgenin in callus and rhizome of *Dioscorea deltoidea* on silica gel with petroleum ether - isopropanol 12:1. The hR_F value of diosgenin was 76. Quantitative determination by densitometry in absorption mode at 366 nm after spraying with methanolic sulfuric acid. The linear regression analysis data showed good linear relationship with $r = 0.991$ and 0.995 for diosgenin with respect to peak height and peak area, respectively. LOD and LOQ were 17 and 50 ng/zone, respectively.

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

108 076 B. ASGHARI, S.N. EBRAHIMI, F. MIRZAJANI, H.Y. ABOUL-ENEIN* (*Pharmaceutical and Medicinal Chemistry Department, The Pharmaceutical and Drug Industries Research Division, National Research Center, Dokki, Cairo 12311, Egypt; haboulenein@yahoo.com): Development and validation of a simple stability-indicating TLC method for the determination of levamisole in pharmaceutical tablet formulation. J. Planar Chromatogr. 24, 419-422 (2011). TLC of levamisole on silica gel with methanol - toluene - chloroform 14:35:50 in a twin-trough chamber saturated for 15 min at 25 °C. The hR_F value was 30. Quantitative determination by densitometry in absorbance mode at 223 nm. Linearity was between 50 and 2000 ng/zone. The recovery was 88.4-102.6 %. The *%RSD* for intra-day and inter-day precision was 1.9 % and 2.0 %, respectively. The LOD and LOQ was 2 and 7 ng/zone, respectively.

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

108 077 A. BAZYLKO, M. TOMCZYK*, A. FLAZINSKA, A. LEGAS (*Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Bialystok, ul. Mickiewicza 2 a, 15-230 Bialystok, Poland; tomczy@umweb.edu.pl): Chemical fingerprint of Potentilla species by using HPTLC method. J. Planar Chromatogr. 24, 441-444 (2011). HPTLC of plant extracts and 17 polyphenolic compounds (apigenin, apigenin-7-glucoside, ellagic acid, hyperoside, isoquercitrin, kaempferol, kaempferol-3-glucoside, kaempferol-3-glucuronide, luteolin, luteolin-7-glucoside, methyl brevifolincarboxylate, myricetin, quercetin, quercetin-3-glucuronide, rutin, tiliroside, ellagic acid 3,3‘-di-O-methyl ether 4-xylopyranoside) on silica gel (prewashed with methanol) with toluene - ethyl formate - formic acid 7:5:1 in an automatic developing chamber set with a twin-trough chamber at 22 °C and a relative humidity of 48 %. Detection under UV light at 254 and 366 nm,

and at 366 nm after spraying with 1.0 % methanolic diphenylborinic acid 2-aminoethylester.

pharmaceutical research, traditional medicine, herbal, quality control, HPTLC, qualitative identification 32a

108 078 D. CASONI, L. TUHUTIU, C. SARBU* (*Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Cluj Napoca, Romania, csarbu@chem.ubbcluj.ro): Simultaneous determination of parabens in pharmaceutical preparations using high-performance thin-layer chromatography and image analysis. J. Liq. Chromatogr. Relat. Technol. 34, 805-816 (2011). HPTLC of methyl (1), ethyl (2), propyl (3), and butylparaben (4) on RP-18 first with methanol 60 % and in a second development with methanol 30 %. Quantitative determination by absorbance measurement at 254 nm. The hR_F values of parabens (1) - (4) were 57, 47, 37 and 28, respectively. Linearity was between 0.46-2.74 µg/band for (1), 0.50-2.99 µg/band for (2), 0.54-3.24 µg/band for (3) and 0.58-3.49 µg/band for (4). The LOD and LOQ were between 100-370 ng/zone and 200-440 ng/zone, respectively. Relative standard deviation of precision was below 3.5 %. Recovery (by standard addition) was higher than 96.3 % in all cases.

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

108 079 L. CHEN (Chen Li-Yu), Q. CHEN (Chen Qian-Liang), D. XU (Xu Dan), J. HAO (Hao Jian-Guo), M. SCHLÄPPI, Z. XU * (Xu Zi-Qin) (*Institute of Life Science, Northwest University, Taibaibeilu 229, Xi'an, 710069 Shanxi, People's Republic of China, ziqinxu@nwu.edu.cn): Changes of gentiopicroside synthesis during somatic embryogenesis in *Gentiana macrophylla*. Planta Med. 75, 1618-1624 (2009). TLC of gentiopicroside and methanolic plant extracts on silica gel containing 1 % CMC-Na with ethyl acetate - ethanol - water 20:2:1. Detection by spraying with 2 % vanillin-sulfuric acid reagent and by evaluation under UV 254 nm.

traditional medicine, herbal, qualitative identification 32e

108 080 H. CHOUHAN, S. SINGH* (*Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, India, sksingh.phe@itbhu.ac.in): Phytochemical analysis, antioxidant and anti-inflammatory activities of *Phyllanthus simplex*. J. Ethnopharmacol. 137, 1337-1344 (2011). HPTLC of phyllanthin (A) and gallic acid (B) in the whole plant of *Phyllanthus simplex* on silica gel with hexane - ethyl acetate 5:1 for (A) and ethyl acetate - formic acid 44:3 for (B). Detection by spraying with anisaldehyde - sulfuric acid reagent. Quantitative determination by absorbance measurement at 260 and 520 nm. The hR_F values of (A) and (B) were 19 and 82, respectively. The amount found in samples was 14.5 % for (A) and 0.7 % for (B).

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

108 081 L. CIESLA. M. HAJNOS, Monika WAKSMUNDZKA-HAJNOS* (*Department of Inorganic Chemistry, Medical University of Lublin, 4a Chodzki St., 20-093 Lublin, Poland; monika.hajnos@am.lublin.pl): Application of hydrophilic interaction TLC systems for separation of high polar glycoside compounds from the flowers of selected Verbascum species. J. Planar Chromatogr. 24, 295-300 (2011). TLC of Verbascum extracts (*e. g.* iridoids, and triterpene saponins) on silica gel in a horizontal chamber saturated with mobile phase for 10 min, by 2D separation with ethyl acetate - methanol - water 25 % ammonia 55:35:9:1 in the first direction and methanol - ethyl acetate - water - acetic acid 5:45:13:11 in a perpendicular direction. Detection by dipping in vanillin-sulfuric acid reagent for 1 s followed by heating for 10 min at 105 °C. The method

was validated for its specifity, precision (repeatability and intermediate precision), stability, and robustness. Use of an image-processing program for the construction of an ‚average' fingerprint.

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

108 082 J. CUI, (Cui Jian), Y. VUE (Yue Yongde)*, F. TANG (Tang Feng), J. WANG (Wang Jin) (*International Center for Bamboo and Rattan, NO. 8, Fuong Dongdajie, Chaoyang District, Beijing 100102, China; yueyd@icbr.ac.cn): HPTLC analysis of the flavonoids in eight species of Indocalamus leaves. J. Planar Chromatogr. 24, 394-399 (2011). TLC of leave extracts and six flavonoids as markers (vitexin, isovitexin, orientin, isoorientin, quercetin, and tricin) on silica gel, prewashed with methanol and methylene chloride, with methanol - ethyl acetate - acetone - methylene chloride in different ratios using automated multiple development. The developed plate was dried in air for 2 h and sprayed with 1 % aluminum trichloride in ethanol. Then the plate was left for 2 h for derivatization in a glass drying chamber. Quantitative determination by densitometry at 366 nm. The hR_F values of the six marker flavonoids were 22, 31, 38, 45, 57, and 88, respectively. Linearity was between 175 and 1750 ng/band. Instrument precision (n = 10) was between 0.2-0.9 %. The repeatability for standards and samples (n = 9), was 0.7 and 0.5, 0.8 and 0.5, 0.8 and 0.5, 0.8 and 0.4, 1.3 and 0.7, 1.1 and 0.3 % for isoorientin, orientin, isovitexin, vitexin, quercetin, and tricin, respectively. The limits of detection were 35, 40, 35, 50, 80, and 20 ng/zone for isoorientin, orientin, isovitexin, vitexin, quercetin, and tricin, respectively. The intra-day and inter-day precision was between 0.1-2.9 % and 0.3-2.4 % for all six marker flavonoids.

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

108 083 D. DENG*, D.R. LAUREN, J.M. COONEY, D.J. JENSEN, K.V. WURMS, J.E. UPRITCHARD, R.D. CANNON, M.Z. WANG, M.Z. LI (*HortResearch, Ruakura Research Centre, East Street, Private Bag 3123, Hamilton, New Zealand; ddeng@hortresearch.co.nz): Antifungal saponins from Paris polyphylla Smith. Planta Med. 74, 1397-1402 (2008). Analytical and preparative TLC of three steroidal saponins (25R)-spirost-5-ene-3ss, 17alpha-diol (pennogenin), 3-O-{O-alpha-L-rhamnopyranosyl-(1-2)-O-[O-ss-xylopyranosyl-(1-5)-alpha-L-arabinofuranosyl-(1-4)]-ss-D-glucopyranoside} on silica gel with dichloromethane - methanol - water 80:19:1 or 70:29:1. Detection by spraying with phosphomolybdic acid. Additional detection by antifungal bioassays.

traditional medicine, herbal, quality control, qualitative identification, preparative TLC 32e

108 084 M. ELLNAIN*, U. HUBICKA, B. ZUROMSKA, Z. JANECZKO, J. KRZEK (*Department of Pharmacognosy, Medical College, Jagiellonian University, 9 Medyczna Street, Cracow 30-688, Poland; marek1@farmacja.cm-uj.krakow.pl): Densitometric quantification of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) in extracts of fresh samples of Erigeron canadensis collected at different stages of growth. J. Planar Chromatogr. 24, 248-252 (2011). TLC of MGDG and DGDG on silica gel with chloroform - methanol -10 % acetic acid 40:10:1. Detection by spraying with a 25 % solution of sulfuric acid in methanol, followed by heating at 105 °C for 5 min. Quantitative determination by densitometry at 477 nm. The hR_F values were 48 for DGDG and 80 for MGDG. The limit of detection and quantification was 200 and 500 ng/band for DGDG and 500 and 1500 ng/band for MGDG. The linear range was 0.5-5.5 µg/band for DGDG and 1.5-5.5 µg/band for MGDG, respectively. The recovery (n= 9) was 95.7 % for DGDG and 93.5 % for MGDG. The repeatability and intermediate precision of results, as *%RSD*, was between 1.7-2.7 % for DGDG and 1.7-2.6 % for MGDG.

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

108 085 J. FENG (Feng Jinghui)*, D. SUN (Sun Dayong), J. LI (Li Junping), M. JIANG (Jiang Mingzhang) (*Yantai Dayang Pharm. Co. Ltd., Yantai 265500, China): (Study on quality standard for Wuyangbuxin capsules) (Chinese). J. of Qilu Med. & Pharm., 30 (2), 91-93 (2011). TLC of the extracts of Wuyangbuxin capsules 1) for *Polygonium multiflorum*, on silica gel with the upper phase of toluene - ethyl acetate - formic acid 10:1:1, detection under UV 365 nm; 2) for Licorice, on silica gel with petroleum ether (60-90 °C) - toluene - ethyl acetate - glacial acetic acid 20:40:14:1, detection by spraying with 10 % sulfuric acid in ethanol and heating until zones were detected; 3) for Milkvetch root, on silica gel with the lower phase of chloroform - methanol - water 13:6:2 at 10 °C, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until zones were detected; 4) for Epimedium herb on silica gel with ethyl acetate - butanone - formic acid - water 10:1:1:1, detection by spraying with $AlCl_3$ reagent, heating at 105 °C until zones were detected and viewing under UV 365 nm. Identification by fingerprint comparison with the standards of the individual drug components.

pharmaceutical research, quality control, herbal, traditional medicine, qualitative identification, comparison of methods 32e

108 086 J. FU (Fu Jingjuan)*, Zh. LIU (Liu Zhihui), F. QIAN (Qian Fang), X. CHANG (Chang Xingjie) (*The Affiliated Provincial Hosp. of Nanjing Trad. Med. & Pharmacy Univ., Nanjing 210029, China): (Study on the quality standard for Baibanting, Leukoplakia tincture) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. 1, 55-66 (2011). TLC of the extracts of Baibanting 1) for *Gardenia jasminoides Ellis*, on silica gel with ethyl acetate - acetone - methanol - water 5:5:1:1, detection under daylight and by spraying with 10 % sulfuric acid in ethanol and heating at 110 ºC; 2) for *Cuscuta chinensis Lam*, on polyamide phase with methanol - glacial acetic acid - water 4:1:5, detection by spraying with 3 % alumnium chloride in ethanol, evaluation under UV 366 nm; 3) for *Malaytea scurfpea* fruit, on silica gel with *n*-hexane - ethyl acetate 4:1, detection by spraying with 10 % KOH in methanol and evaluation under UV 366 nm. Quantification of gardenoside by HPLC. The procedures proved to be simple, accurate, reproducible, robust and suitable for the quality control of the medicine.

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

108 087 D.B. GANDHI, P.J. MEHTA* (*Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, S. G. Highway, Ahmedabad-382481, Gujarat, India; drpritimehta@nirmauni.ac.in): Simultaneous RP-HPTLC method for determination of levodopa, carbidopa, and entacapone in combined tablet dosage form. J. Planar Chromatogr. 24, 236-241 (2011). HPTLC of levodopa (LEV), carbidopa CAR), and entacapone (ENT) in a combined dosage form on RP-18 (prewashed with methanol) with acetonitrile - *n*-butanol - water - triethylamine 1:19:2:0.002, pH adjusted to 3.6 with phosphoric acid, in a twin-trough chamber saturated with mobile phase for 25 min. Quantitative determination by densitometry in absorbance mode at 282 nm. The hR_F values were 46, 64, and 87 for LEV, CAR, and ENT, respectively. Linearity was between 300-1500 ng/zone for LEV, 200-1000 ng/zone for CAR, and 200-2000 ng/zone for ENT. The intra-day and inter-day precision was below 1.8 % *RSD* for all drugs. The recovery for LEV, CAR, and ENT ($n = 3$) was between 101.0 and 102.4 %.

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

108 088 A. GANTAIT, A. SAHU, P. VENKATESH, P. K. DUTTA, P. K. MUKHERJEE* (*School of Natural Product Studies, Jadavpur University, Kolkata-700 032, India; naturalproductm@gmail.com): Isolation of taraxerol from *Coccinia grandis*, and its standardization. J. Planar Chromatogr. 23, 323-325 (2010). HPTLC of taraxerol on silica gel with hexane - ethyl acetate 9:1 in a saturated twin-trough chamber. Detection by spraying with anisaldehyde - sulfuric acid reagent followed by heating for 5 min at 80 °C. Quantitative determination by absorbance measurement at 540 nm. The average recovery was between 99.6 and 100.3 % with *%RSD* less than 2 %. For both intra-day and inter-day precision *%RSD* was less than 2 %. The LOD and LOQ were 47 and 140 ng/band, respectively. The linearity range was 0.5-2.5 µg/band. The hR_F value of taraxerol was 22.

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

108 089 B. GEHRMANN, M.F. MELZIG (*Einhorn-Rats-Apotheke, Markt 10-12, 25813 Husum, Germany): Optimization and qualitative determination of Mezereon homeopathic tincture by applying rapid horizontal TLC. Planta Med. 75, 1000 (2009). TLC and HPTLC of Mezereon (Daphne mezereum) bark extracts and scopoletin, umbelliferone, mezerein, and daphnetoxin on silica gel with various mobile phases containing toluene, ethyl acetate, and formic acid at different proportions. Detection under UV 254 and 366 nm and visible light. The applied procedure may be proposed for an updated and optimized TLC identification test of the homeopathic monograph of *Daphne mezereum L.*

herbal, quality control, qualitative identification, HPTLC 32e

108 090 D.G. GIMÉNEZ, E.G. PRADO, T.S. RODRÍGUEZ, A.F. ARCHE, R. DE LA PUERTA* (*Department of Pharmacology, School of Pharmacy, University of Seville, c/ Profesor Garcia Gonzales No 2, 41012 Seville, Spain; puerta@us.es): Cytotoxic effect of the pentacyclic oxindol alkaloid mitraphylline isolated from *Uncaria tomentosa* bark on human Ewing‘ sarcoma and breast cancer cell lines. Planta Med. 76, 133-136 (2010). TLC of mitraphylline on silica gel with (1) dichloromethane - acetone 5:4, (2) diethyl ether - ethyl acetate 1:1, and (3) dichloromethane - ethanol 19:1. The hR_F value in system (1) was 83, in (2) 73, and in (3) 68. Detection by spraying with sulfuric acid - acetic acid - water 1:20:4 followed by heating at 120 °C and by Dragendorff‘s reagent.

traditional medicine, herbal, clinical chemistry research, pharmaceutical research, qualitative identification 32e

108 091 Anna GUMIENICZEK*, T. INGLOT, A. KONCZAK (*Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland; anna.gumieniczek@umlub.pl): Classical densitometry and videoscanning in a new validated method for analysis of candesartan and losartan in pharmaceuticals. J. Planar Chromatogr. 24, 99-104 (2011). TLC of candesartan and losartan on silica gel with 1,4-dioxane - hexane - 99 % formic acid 50:50:1. Quantitative determination by densitometry at 258 nm for candesartan and at 243 nm for losartan, videoscanning at 254 nm for both drugs. The hR_F value for candesartan was 47 and for losartan 35. Linearity was between 0.2 and 1.4 µg/band for both drugs with correlation coefficients of 0.9997 and 0.9981 for candesartan, and 0.9986 and 0.9982 for losartan, for densitometry and videoscanning, respectively. Robustness (*%RSD*, peak area) was less than 1.9 and 0.8 % for candesartan, and 2.2 and 0.9 % for losartan in densitometry and videoscanning, respectively. The repeatability and intermediate precision (*%RSD*, two lowest amounts) were less than 3.6 and 4.7 % for candesartan and less than 4.7 and 5.3 % for losartan.

Mean recoveries for candesartan were 103.8-104.9 % for densitometry and 99.2-100.7 % for videoscanning; for losartan the respective values were 100.8-105.4 % and 98.6-99.2 %.

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

108 092 M. GUPTA, M. SINGH, H. MUKHATR, S. AHMAD* (* Faculty of Pharmacy, Jamia Hamdard, New Delhi, India): HPTLC fingerprinting of different leaf extracts of *Tylophora indica* (Burm f.) Merill. PHCOG J. 2(11), 381-385 (2010). TLC of Tylophora indica leaves, extracted with petroleum ether, chloroform and methanol, on silica gel with 1) *n*-hexane - ethyl acetate 2:3 for the petroleum ether extract, 2) chloroform - ethyl acetate - methanol 18:1:1 for the chloroform extract, and 3) chloroform - toluene - ethyl acetate 18:1:1 for the methanolic extract. Evaluation under UV 254 nm and 366 nm. 12 prominent bands were observed in all chromatographic fingerprints. The method is suitable for identification and authentication of the plant material.

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

108 093 G. HADAD*, R. SALAM, S. EMARA (*Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, University of Suez Canal, Ismailia, 41522, Egypt, ghhadad@yahoo.com): Validated stability-indicating HPTLC and HPLC methods for determination of pipazethate and its degradant. J. Liq. Chromatogr. Relat. Technol. 34, 1850-1869 (2011). HPTLC of pipazethate (1) and its degradant 10H-pyrido[3,2-b][1,4] benzothiadiazine-10-carboxylic acid (2) on silica gel with chloroform - diethylamine - methanol 94:1:5. Quantitative determination by absorbance measurement at 225 nm. The hR_F values of (1) and (2) were 35 and 28, respectively. Linearity was between 2-9 µg/zone for (1) and 1-6 µg/zone for (2). Limits of detection and quantification were found to be 53 and 180 ng/zone for (1), and 40 and 130 ng/zone for (2), respectively. Recoveries (by standard addition) were 100.1 % for (1) and 99.5 % for (2). Comparable results were obained with a validated HPLC method.

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

108 094 E. HAN (Han Erdemutu)*, SH. NA (Na Shengsang), X. HAN (Han Xiangyu), Y. MENG (Meng Yonghai), J. HAO (Hao Jianxun) (*Affiliated Hosp. of Inner Mongolia Med. Coll., Huhehaote 010059, China): (Study on the differentiation of Mongolian medicine Qi Shun E Er Dun by thin-layer chromatography) (Chinese). Tianjin J. of Trad. Chinese Med. & Pharm. 28 (2), 164-166 (2011). TLC of the extracts of the traditional Mongolian medicine on silica gel 1) for artificial cow-bezoar, with isooctane - ethyl acetate - glacial acetic acid 6:3:2, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones were detected under UV 365 nm, identification by comparison with the fingerprint of the individual drug components and cholic acid as reference; 2) for *Coptidis rhizoma*, with benzene - ethyl acetate - isopropanol - methanol - water 20:10:5:5:1, detection under UV 365 nm after exposure to ammonia vapour, identification by comparison of the fingerprint with the individual drug components and berberine hydrochloride as reference; 3) for light yellow *Sophora* root, with ethyl acetate - propanone - benzene - ammonia water 20:15:10:1, detection by spraying with 5 % potassium iodobismuthate solution, identification by comparison of the fingerprint with the individual drug components and with matrine as reference.

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

108 095 E.M. HASSAN*, A.A. SHAHAT, N.A. IBRAHIM, A.J. VLIETINCK, S. APERS, L. PIETERS (*Department of Medicinal and Aromatic Plants, National Research Centre, 12311 Dokki, Cairo, Egypt; emadnrc@yahoo.com): A new monoterpene alkaloid and other constituents of *Plumeria acutifolia*. Planta Med. 74, 1749-1750 (2008). Analytical and preparative TLC of a new monoterpene alkaloid plumerianine, (R)-4'-{(S)-1-hydroxyethyl)-5,6-dihydro-5'H-spiro[cyclopenta[c]pyridine-7,2'-furan}-5'-one, the iridoid 15-demethylplumeride, lupeol, uvaol, and ursolic acid on silica gel with toluene - ethyl acetate 8:2 or 8:3 and chloroform - methanol 9:1. Detection by spraying with vanillin-sulfuric acid reagent.

traditional medicine, herbal, quality control, qualitative identification, preparative TLC 32e

108 096 J. HUANG (Huang Jirong)*, Y. HAI (Hai Yinmei), W. BAO (Bao Wenling) (*Afiliated Hosp., Inner Mongolia Univ. for Nationalities, Tongliao 028000, China): (Identification of Mongolian medicinal herbs *Gentiana algida Pall.* and *Gentianella acuta* by thin-layer chromatography) (Chinese). J. of Inner Mongolia Univ. for Nationalities (Natural Sci. Edit.) 26(1), 71-72 (2011). TLC of the extracts of the title medicinal herbs on silica gel with 1) chloroform - methanol - water - formic acid 28:2:2:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C until the zones were detected; 2) chloroform - methanol - ammonia 40:10:1, detection by spraying with iodine solution. Identification by comparison of the fingerprint with the main components gentianine, isoorientin, flavone, isobellidifolin, swerchirin, and 1,5,8-trihydroxy-3,4-dimethoxyxanthone.

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

108 097 J. JAMES, I. DUBERY* (*Department of Biochemistry, University of Johannesburg, P. O. Box 524, Auckland Park, 2006, South Africa, idubery@uj.ac.za): Identification and quantification of triterpenoid centelloids in *Centella asiatica* (L.) Urban by densitometric TLC. J. Planar Chromatogr. 24, 82-87 (2011). TLC of crude extracts and madecassoside, asiaticoside, madecassic acid, and asiatic acid on silica gel with concentration zone with chloroform - glacial acetic acid - methanol - water 15:8:3:2; the hR_F value was 45, 55, 94, and 97, respectively. Detection by spraying with anisaldehyde-sulfuric acid reagent, followed by heating at 95 °C for 10 min or until the colored zones appeared. The correlation coefficients were between 0.9904 and 0.9982 and linearity was in the range of 1.25-10 nmol, corresponding to approximately 0.5-5 µg/zone for the acids and 1.2-10 µg/zone for the glycosides. The LOD and LOQ was 300 and 720 ng/zone for the sapogenin and saponin, respectively, and 500 and 1200 ng/zone. The average precision was less than 4 % for standards and between 4-6 % for samples.

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

108 098 E. KAALE*, P. RISHA, E. REICH, T. P. LAYLOFF (*Muhimbili University of Health and Allied Sciences, School of Pharmacy, Laboratory for Pharmaceutical Analysis, PO Box 65013, Dar es Salaam, Tanzania; elia.kaale@muhas.ac.tz.or elia.kaale@lycos.com): An interlaboratory investigation on the use of high-performance thin-layer chromatography to perform assays of lamivudine-zidovudine, metronidazole, neviparine, and quinine composite samples. J. AOAC Int. 93, 1836-1843 (2010). HPTLC of 1) lamivudine-zidovudine on silica gel with ethyl acetate - toluene - methanol 12:5:3, quantitative determination by absorbance measurement at 289 nm; of 2) metronidazole with ethyl acetate - ammonia 50:1, quantitative determination by absorbance measurement at 313 nm; of 3) neviparine with ethyl acetate - toluene 3:1, quantitative determi-

nation by absorbance measurement at 289 nm; and of 4) quinine with ethyl acetate - toluene - acetone 22:3:5, quantitative determination by absorbance measurement at 327 nm in a twin-trough chamber lined with wetted filter paper and saturated for 20 min. The average repeatability (within-laboratory) was 1.9 %, with 73 % less than 2 % and 97 % at 2.6 % or less. The average reproducibility (among-laboratory) was 2.7 %. Mean hR_F values for lamivudine, metronidazole, neviparine, quinine, and zidovudine were 19, 28, 34, 33, 57.

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

108 099 D. KALE, R. KAKDE* (*Department of Pharmaceutical Sciences, RTM Nagpur University, Amravati Road, Nagpur-440033, Maharashtra, India; drkakde@yahoo.com): Simultaneous determination of pioglitazone, metformin, and glimepiride in pharmaceutical preparations using HPTLC method. J. Planar Chromatogr. 24, 331-336 (2011). HPTLC of pioglitazone (PIO), metformin (MET), and glimepiride (GLI) in pharmaceutical preparations on silica gel, prewashed with methanol, with acetonitrile - methanol - propanol - ammonium acetate solution 7:2:1:1 in a twin trough chamber saturated for 10 min. Quantitative determination by densitometry at 240 nm. The hR_F value was 83, 21, and 89 for PIO, MET, and GLI, respectively. Linearity was in the concentration range of 300-1200 ng/band, 10-40 µg/band and 40-160 ng/band with correlation coefficients of 0.995, 0.996, and 0.998 for PIO, MET, and GLI, respectively. The LOD and LOQ was 57 and 171 ng for PIO, 6 µg and 18 µg for MET, and 12 and 36 ng for GLI. The *%RSD* for method and intermediate precision was below 2 %. The mean recovery (n = 5) was 98.2-99.5 % for PIO, 98.6-99.3 % for MET, and 98.7-99.7 % for GLI with *%RSD* between 0.4 and 1.3 %.

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

108 100 Juliane KASPER*, M.F. MELZIG (Freie Universität Berlin, Institute of Pharmacy, Königin-Luise-Str. 2 + 4, 14195 Berlin, Germany; jkasper@zedat.fu-berlin.de): HPTLC method for the quantification of isoflavones in nutritional supplements of Red Clover (*Trifolium pratense L.*). J. Planar Chromatogr. 24, 373-375 (2011). HPTLC of red clover capsule extracts and formononetin, biochanin A, daidzein, glycitein, and genistein on silica gel, prewashed with methanol, with dichloromethane - glacial acetic acid - ethyl acetate 12:2:1 in a horizontal chamber saturated for 15 min. Quantitative determination by densitometry at 260 nm. The hR_F value was 29, 34, 41, 48, and 59 for daidzein, glycitein, genistein, formononetin, and biochanin A, respectively. The two major isoflavones are formononetin and biochanin A. The limit of detection and quantification was 14 and 47 ng/band for formononetin and 12 and 40 ng per band for biochanin A, respectively. The recovery was 93.3-100.7 % for formononetin and 102.0-109.4 % for biochanin A.

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

108 101 A. KAUR, I.P. SINGH* (*Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S.Nagar, Punjab-160062, India; ipsing@niper.ac.in; ipsingh67@yahoo.com): Densitometric determination of antileishmanial phenylpropanoids of *Alpinia galanga* (Linn.) Willd. J. Planar Chromatogr. 24, 352-356 (2011). HPTLC of extracts of dried powdered rhizoms of A. galanga and three phenylpropanoids (1-acetoxychavicol acetate (1), acetoxyeugenol acetate (2), and trans-p-coumaryl diacetate(3)) on silica gel with *n*-hexane - ethyl acetate 4:1 with chamber saturation for 1 h. After drying second development with the same mobile phase. Quantitative determination by densitometry at the wavelength of maximum absorption. Linearity was between 0.6-1.8 µg/band, 0.4-1.5 µg/band and 0.1-0.3 µg/band for (1), (2), and (3), respectively. The LOD and LOQ was 150 and 500 ng/band for (1), 100 and 334 ng/

band for (2), and 23 and 77 ng/band for (3). The repeatability of application and repeatability of measurement (*%RSD*, $n = 6$) was 0.9 and 0.5 % for (1), 0.5 and 0.3 % for (2), and 1.8 and 0.9 % for (3). The intra-day and inter-day precision was below 5 % for all compounds. The hR_F value was 53, 37, and 43 for (1), (2), and (3), respectively.

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

108 102 L.D. KHATAL, A.Y. KAMBLE, M.V. MAHADIK, S. DHANESHWAR* (*Bharati Vidyapeeth University, Poona College of Pharmacy, Department of Pharmaceutical Chemistry, Pune, Maharashtra, India 411038; sunil.dhaneshwar@gmail.com): Validated HPTLC method for simultaneous quantitation of paracetamol, diclofenac potassium, and famotidine in tablet formulation. J. AOAC Int. 93, 765-770 (2010). HPTLC of paracetamol (PAR), diclofenac potassium (DCL), and famotidine (FAM) on silica gel (prewashed with methanol) with toluene - acetone - methanol - formic acid 500:200:200:1 in a twin-trough chamber after preconditioning for 30 min at room temperature (25 +/- 2°C) at a relative humidity of 60 +/- 5 %. Quantitative determination by absorbance measurement at 274 nm. The hR_F value of paracetamol was 62, of diclofenac potassium 75, and of famotidine 17. Linearity was between 1625-9750 ng/zone for PAR, 250-1500 ng/zone for DCL, and 100-600 ng/zone for FAM. The *%RSD* values for repeatability and intermediate precision were below 2 %. The LOD and LOQ were 50 and 100 ng/zone for PAR and DCL, and 10 and 50 ng/zone for FAM. The *%RSD* of peak areas was calculated for each parameter and was found to be less than 2 %. Recoveries (by standard addition) were in the range of 95-98 % at various added concentrations.

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

108 103 D. KIM, J. BAE, D. LEE, H. LEE, M. JOO, S. YOO* (*Department of Food Science & Technology and Carbohydrate Bioproduct Research Center, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul, 143-747, Korea, shyoo@sejong.ac.kr): Positive effects of glycosylated anthocyanin isolated from an edible berry fruit (*Acanthopanax sessiliflorum*) on its antioxidant activity and color stability. Food Research International 44, 2258-2263 (2011). HPTLC of sugar constituents linked to anthocyanidin in the fruits of *Acanthopanax sessiliflorum* on silica gel with *n*-propanol - water - 30 % ammonia 20:5:1 + 1 drop triethylamine. Detection by spraying with a solution of 0.3 % N-(1-naphtyl)-ethylenediamine and 5 % sulfuric acid in methanol, followed by heating at 121 °C for 10 min. The acidic hydrolysate consisted of the two monosaccharides, glucose and xylose.

herbal, qualitative identification, preparative TLC 32e

108 104 ANNE KLOEPPEL, W. GRASSE, F. BRUEMMER, Gertrud E. MORLOCK* (*University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany; gmorlock@uni-hohenheim.de): HPTLC coupled with bioluminescence and mass spectrometry for bioactivity-based analysis of secondary metabolites in marine sponges. J. Planar Chromatogr. 21, 431-436 (2008). HPTLC of sponge extracts (dienon, aeroplysinin-1, avarone and avarol as standards) on silica gel, prewashed with methanol, by automated multiple development in the AMD2 with a fifteen-step gradient based on methanol, dichloromethane, and *n*-hexane. Optional derivatization was performed by dipping (speed 4 cm/s, immersion time 1 s) the plate into sulfuric acid reagent followed by heating for 5 min at 110 °C. For bioluminescence detection the developed plate was dipped (speed 3.5 cm/s, immersion time 0 s) into a luminescent *Vibrio fisheri* bacteria suspension. Detection of bioluminescence with the Bioluminizer. Identification of zones of interest by MS. Visual LOD of avarol and avarone was 70 and 60 ng/band, respectively.

densitometry, HPTLC, quantitative analysis, AMD, biochemistry 32 e

108 105 A. LEHRI, J. BARTHWAL, A. NIRANJAN*, D.V. AMLA (*Central Instrumentation Facility, National Botanical Research Institute, Council of Scientifc and Industrial Research, Lucknow-226 001, India; abishek_niranjan@yahoo.co.in): Development and validation of HPTLC densitometric method for identification and quantification of geraniol in Palmarosa oil. J. Planar Chromatogr. 24, 316-319 (2011). HPTLC of Palmarosa oil extracts in toluene and geraniol on silica gel with toluene - ethyl acetate 37:3 in a twin-trough chamber saturated for 30 min at 25 +/- 2 °C. Detection by spraying with 3 % vanillin in ethanol - sulfuric acid 49:1 followed by heating at 100 °C for 5 min. Quantitative determination by densitometry in absorption mode at 400 nm. The instrumental precision and the repeatability ($n = 6$), was 0.3 and 3.2 %, respectively. LOD and LOQ was 1.4 and 2.8 µg/mL, respectively. The intra-day recovery was 99.8 % and the inter-day recovery 99.4 %. The hR_F value for geraniol was 36.

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

108 106 D. MAMMEN*, M. DANIEL, R.T. SANE (*M. S. University of Baroda, Vadodara-390 002, India; drdenni.mammen@gmail.com): Rapid and parallel analysis using HPTLC to detect seasonal and geographical variation in *Aerva lanata Juss.* ex Schultes. J. Planar Chromatogr. 24, 388-393 (2011). HPTLC of *p*-hydroxybenzoic acid in the whole plant of *Aerva lanata* collected during summer, monsoon and winter on silica gel, prewashed with methanol, with ethyl actate - toluene 7:3 in a twin trough chamber. Detection under UV light at 254 nm. The hR_F value of p-hydroxybenzoic acid was 73. Quantitative determination by densitometry at 252 nm. The calibration was linear in the range of 25-175 ng, with a regression coefficient of 0.9986. The *%RSD* for intra-day and inter-day precision was less than 2 %. The *%RSD* for the repeatability of sample application and measurement of area was 1.2 % and 0.9 %, respectively. The limit of detection and the limit of quantification was 0.5 and 1.4 ng, respectively. Recovery (by standard addition) was found to be 97.2 % ($n = 3$).

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

108 107 C. MATTLE, N. HEIGL, G. ABEL, G. K. BONN, C. W. HUCK* (*Institute of Analytical Chemistry and Radiochemistry,Leopold-Franzens University, Innrain 52a, 6020 Innsbruck, Austria; W.Huck@uibk.ac.at): Near-infrared diffuse reflection spectroscopy and multivariate calibration hyphenated with thin-layer chromatography for quality control of a phytomedicine and simultaneous quantification of methoxylated flavones. J. Planar Chromatogr. 23, 348-352 (2010). TLC of methoxylated flavones G1, G2, G3, G4 (3‘,4‘,5‘-trimethoxyflavone), and G5 on alumina with *n*-hexane - ethyl acetate 7:3 at ambient temperature with chamber saturation. Detection by visual inspection at 365 nm. The hR_F values of G1 (monomethoxyflavone), G2 (monomethoxyflavone), G3, G4 (trimethoxyflavone), and G5 (dimethoxyflavone) were 42, 30, 22, 17, and 12, respectively.

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

108 108 F. MELIANITA, J. WITHA, S. ARIFIN, W. KARTINASARI, G. INDRAYANTO* (*Department of R&D, Bernofarm Pharmaceutical Company, Sidoarjo, Surabaya, Indonesia, and Assessment Service Unit, Faculty of Pharmacy, Airlangga University, Jl. Dharmawangsadalam, Surabaya 60286, Indonesia, gunawanindrayanto@yahoo.com): Simultaneous densitometric determination

of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in some commercial gingers. J. Liq. Chromatogr. Relat. Technol. 32, 567-577 (2009). HPTLC of 6-gingerol (1), 8-gingerol (2), 10-gingerol (3), and 6-shogaol (4) in commercial gingers on Lichrosphere silica gel with toluene - ethyl acetate 3:1. Detection by spraying with anisaldehyde-sulfuric acid reagent, followed by heating at 120 ºC for 10 min. Quantitative determination by absorbance measurement at 577 nm. Linearity was between 96-480 ng for (1), 39-196 ng for (2), 49-242 ng for (3) and 50-256 ng for (4). Limits of detection and quantification were 26 and 77 ng/zone for (1), 16 and 47 ng/zone for (2), 17 and 50 ng/zone for (3) and 33 and 99 ng/zone for (4). *%RSD* of repeatability and intermediate precision were below 5 %. Recoveries were between 99.7 and 104 % for (1)-(4).

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

108 109 B. MOUSSA, M. MOHAMED*, N. YOUSSEF (*National Organisation for Drug Control and Research (NODCAR), 6 Abo Hazem Street, Pyramids Avenue, Post Office Box 29, Cairo, Egypt, 12553; mera_pharm2003@yahoo.com): Simultaneous densitometric TLC analysis of olmesartan medoxomil and hydrochlorothiazide in the tablet dosage form. J. Planar Chromatogr. 24, 35-39 (2011). HPTLC of olmesartan medoxomil (OLM) and hydrochlorothiazide (HTZ) on silica gel with chloroform - methanol - formic acid 16:3:1 in a chamber saturated for 1 h. Detection under UV light at 254 nm. Quantitative determination by densitometry at 260 nm for olmesartan medoxomil and 272 nm for hydrochlorothiazide. Linearity was between 0.05-1 mg/mL. Average recovery was 100.3 % and 99.9 % for OLM and HTZ, respectively. The LOD was 138 and 137 ng/zone for OLM and HTZ, respectively, and the LOQ was 459 and 456 ng/zone for OLM and HTZ, respectively. The intra-day and inter-day precision (*%RSD*, $n = 9$) was 1.2 and 1.4 % for OLM and 1.1 and 1.3 % for HTZ.

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

108 110 A.G. NAMDEO*, A. SHARMA, L. SATHIYANARAYANAN, D. FULZELE, K.R. MAHADIK (*Department of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth University, Paud Road, Erandwane, 411038 Pune, Maharashtra, India; ajay_namdeo@rediffmail. com): HPTLC densitometric evaluation of tissue culture extracts of Nothapodytes foetida compared to conventional extracts for camptothecin content and antimicrobial activity. Planta Med. 76, 474-480 (2010). HPTLC of camptothecin and plant material extracts (roots, stems, leaves,and fruits) on silica gel with chloroform - ethyl acetate - methanol 9:10:1 in a twin trough chamber at 25 +/- 2°C and 40 % relative humidity. Quantitative determination by densitometry at 360 nm. Linearity was between 80 and 480 ng/zone with a correlation coefficient of 0.998 +/- 0.020. Instrumental precision (*%RSD*) was 0.5 %. Repeatability (*%RSD*) of sample and standard were 1.1 and 1.0 %. The LOD and LOQ were found to be 40 and 80 ng/zone, respectively. The accuracy of the method was proven by the average percentage recovery of 99.1 %.

traditional medicine, pharmaceutical research, densitometry, HPTLC, quantitative analysis 32a

108 111 D.N. OLENNIKOV (Laboratory of Medical and Biological Research, Department of Biologically Active Substances, Institute of General and Experimental Biology, Siberian Division, Russian Academy of Sciences, Sakh'yanovoy St. 6, 670047, Ulan-Ude, Russian Federation; oldaniil@rambler.ru): Densitometric HPTLC analysis of kurarinone and sophoraflavanone G in *Sophora flavescens* root. J. Planar Chromatogr. 24, 121-124 (2011). HPTLC of kurarinone and sophoraflavanone G in the roots of *Sophora flavescens* on silica gel with chloroform - methanol 10:1 with chamber saturation for 30 min at 20 °C. Quantitative determination by absorbance measurement

at 285 nm. The intra-day and inter-day *%RSD* were 1.9-2.0 % and 2.2-2.4 %, respectively. Instrument precision and repeatability of the method were 0.4-0.6 and 1.81-1.84 %, respectively. Average recovery was 96.3-103.4 % for kuraninone and 96.8-102.9 % for sophoraflavanone G. The limits of detection and quantification were 27 and 80 ng/band for kurarinone and 12 and 36 ng/band for sophoraflavanone G. Linearity was in the range of 200-1200 ng/band for kurarinone and 90-550 ng/band for sophoraflavanone. The hR_F value was 31 and 50 for kurarinone and sophoraflavanone, respectively.

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

108 112 B.N. PATEL*, B.N. SUHAGIA, C.N. PATEL, H.J. PANCHAL (*Shri Sarvajanik Pharmacy College, Mehsana-384001, Gujarat, India; bhavi_pharma22738@yahoo.com): A simple and sensitive HPTLC method for quantitative analysis of darunavir ethanolate tablets. J. Planar Chromatogr. 24, 232-235 (2011). HPTLC of darunavir ethanolate in tablets on silica gel, prewashed with methanol, with toluene - ethyl acetate - methanol 7:2:1 in a twin-trough chamber lined with filter paper and saturated with mobile phase for 30 min at room temperature (25 +/- 2 °C). Quantitative determination by densitometry in absorbance mode at 267 nm. The hR_F of darunavir ethanolate was 47. Linearity was between 250 and 1750 ng/band with $r = 0.9994$. The limits of detection and quantification were 15 and 46 ng/band, respectively. The intra-day and inter-day precision was (*%RSD*, $n = 6$) between 0.5-0.9 % and 1.1-1.3 %. Recovery ($n = 6$) was 99.3-101.2 %.

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

108 113 K.G. PATEL*, V.G. PATEL, K.V. PATEL, T.R. GANDHI (*Anand Pharmacy College, Shri Ram Krishna Seva Mandal Campus, Near Town Hall, Anand, Gujarat, India; kalpana_jpatel@yahoo.com): Validated HPTLC method for quantification of myrecitin in the stem bark of Myrica esculenta Buch. Ham. Ex D. Don, Myricaceae. J. Planar Chromatogr. 23, 326-331 (2010). HPTLC of myrecitin and stem bark extracts on silica gel with toluene - ethyl acetate - formic acid - methanol 15:15:3:2. Quantitative determination by absorbance measurement at 268 nm. Linearity was between 0.4-2.0 µg/band. The limits of detection and quantitation were 93 ng and 284 ng/zone. The intra-day and inter-day precision of the method was in the range of 0.14-0.55 %. The recovery of myrecitin at three concentrations was in the range of 98.9-100.1 % and the average recovery was 99.3 %.

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

108 114 Alina PYKA*, D. NABIALKOWSKA, K. BOBER, M. DOLOWY (*Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellonska Street, 41-200, Sosnowiec, Poland, apyka@sum.edu.pl): Comparison of NP-TLC and RP-TLC with densitometry to quantitative analysis of tocopherol acetate in pharmaceutical preparation. J. Liq. Chromatogr. Relat. Technol. 34, 2548-2464 (2011). HPTLC of tocopherol acetate (1) and tocopherol (2) in oral fluid vitamin E on silica gel with chloroform - cyclohexane 11:9. Quantitative determination by absorbance measurement at 202 nm for (1) and 272 nm for (2). The hR_F values of (1) and (2) were 47 and 38, respectively. Linearity was in the range of 2-8 µg/band for (1) and (2). Limits of detection and quantification were found to be 50 and 150 ng/zone for (1). Precision was below 2 %. The intermediate/inter-day/intra-day precision was 0.4 % ($n = 6$). Recovery (by standard addition) was in the range between 99.8-101.5 %. Tocopherol acetate was better separated from tocopherol using normal phase TLC than by reversed phase TLC.

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

108 115 A.A. RAJOPADHYE, A.S. UPADHYE*, A.M. MUJUMDAR (*Agharkar Research Institute, G. G. Agarkar Road, Pune, India; upadhye.anuradha@gmail.com): HPTLC method for analysis of piperine in fruits of Piper species. J. Planar Chromatogr. 24, 57-59 (2011). HPTLC of piperine on silica gel with toluene - ethyl acetate - diethyl ether 6:3:1 in a saturated twin trough chamber. The hR_F of piperine was 40. Quantitative determination by densitometry at 337 nm. Linearity was between 15 and 75 ng/zone. LOD and LOQ was 5 and 15 ng/zone, respectively. The recovery was 94.5 %. The instrumental precision, repeatability, intra-day and inter-day precision (*%RSD*, $n = 6$) was 0.6 %, 0.8 %, 0.9 and 0.8 %, respectively.

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

108 116 A.S. RAO*, J. SHAO, T.J. SMILLIE, I.A. KHAN (*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, MS 38677, USA): Chemical fingerprinting of *Turnera diffusa* and closely related genera by high-performance thin-layer chromatography. Planta Med. 74, 352-353 (2008). HPTLC of tetraphyllin, turneradiffusin, beta-arbutin, terniflorin, echinaticin, turneradin and methanolic extracts of *Turnera diffusa* on silica gel with ethyl acetate - acetic acid - water 190:10:1. Quantitative determination by densitometric absorbance measurement at 254 nm.

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

108 117 K. RAVIKANTH, B. SINGH, A. GUPTA, A. SINGH, A. SHERMA, A. KUMAR* (*R & D Center, Ayurvet Limited, Village Katha, P. O. Baddi-173205, District Solan, Himachal Pradesh, India; abhishekkumaronline@gmail.com; krk@ayurvet.in): Development and validation of TLC method for analysis of Stresroak premix. J. Planar Chromatogr. 24, 66-71 (2011). HPTLC of extracts of *Stresroak premix* and gallic acid (1), mangiferin (2), and withanolide A (3) as standards on silica gel with A) ethyl acetate - formic acid - acetic acid - water 100:11:11:27, for (1) and (2), and B) chloroform - methanol 9:1 for (2) in a twin trough chamber. Quantitative determination by absorbance measurement at 280 nm for (1), 330 nm for (2), and 225 nm for (3). The hR_F values of (1), (2) and (3) were 76, 29, and 48, respectively. The average recoveries were 100.4 % (1), 99.3 % (2) and 98.0 % (3). The linear concentration range was 50-150 ppm for (1), and 40-100 ppm for (2) and (3). The LOD, defined as the amount of compound required to produce a signal at least three times the noise level, for gallic acid, mangiferin, and withanolide A was 80, 110, and 200 ng for (1), (2), and (3), respectively. The LOQ was 20, 27, and 38 µg, respectively.

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

108 001 E. REICH *et al.,* see section 1

108 118 K.K. ROUT, R.K. SINGH*, S.K. MISHRA (*Department of Chemistry, North Orissa University, Sriramchandra Vihar, Baripada, Mayurbhanjy-757003, Orissa, India; rajeshks2001@yahoo.com): Simultaneous quantification of two bioactive lupane triterpenoids from *Diospyros melanoxylon* stem bark. J. Planar Chromatogr. 24, 376-380 (2011). HPTLC of stem bark extracts from

D. melanoxylon and lupeol and betulin on silica gel, prewashed with methanol, with ethyl acetate - hexane 9:41 with chamber saturation for 3 min at 29 +/- 4 °C and 65 +/- 5 % relative humidity. The hR_F value was 46 and 25 for lupeol and betulin, respectively. Quantitative determination by densitometry in absorption mode at 560 nm for lupeol and 510 nm for betulin. Detection by derivatization with 5 % methanol-sulfuric acid reagent. The LOD and LOQ was 40 and 100 ng/zone for lupeol and 50 and 100 ng/zone for betulin, respectively. The instrument precision and repeatability (n = 6) were 0.8 and 1.3 % for lupeol and 1.1 and 1.2 % for betulin, respectively. The linearity range was 100-500 ng/zone for both lupeol and betulin. The intra-day and inter-day precision was 1.1-1.7 % and 1.3-2.0 % for lupeol and 0.8-1.9 % and 1.9-2.2 % for betulin.

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

108 119 C.S. RUMALLA, B. AVULA, Z. ALI, T. J. SMILLIE, V. FILION, A. CUERRIER, J. T. ARNASON, I. A. KHAN* (*National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA; ikhan@olemiss.edu): Quantitative HPTLC analysis of phenylpropanoids in Rhodiola species. J. Planar Chromatogr. 24, 116-120 (2011). HPTLC of rosin, rosarin, and rosavin in the roots of *Rhodiola rosea L.* and *Rhodiola sachalinensis Borissova* on silica gel, prewashed with methanol, with chloroform - methanol - water 130:70:21 in a twin-trough chamber lined with filter paper and saturated for 20 min, at 21-24 °C and 40-45 % relative humidity. Quantitative determination by densitometry at 250 nm. The calibrations were linear in the range of 100-500 ng/band with correlation coefficients of 0.9999, 0.9992, and 0.9992 for rosin, rosarin, and rosavin, respectively. Recovery of the three compounds was between 97 and 101 %. The LOD and LOQ were 30 and 100 ng/band, respectively, for all three compounds. The hR_F values for rosin, rosarin, and rosavin were 58, 45, and 42, respectively. Intra-day variation, as *%RSD,* was 2.7 % for rosin, 1.7 % for rosarin, and 2.8 % for rosavin, with standard errors of 0.3 %, 0.2 %, and 0.3 %, respectively. Inter-day variation, as *%RSD*, was 4.8 % for rosin, 4.2 % for rosarin, and 3.9 % for rosavin, with standard errors of 0.5 %, 0.5 %, and 0.4 %, respectively.

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

108 126 C. SALMON*, Y. SHAW, S. HIBBERT, C. GREEN, A. SMITH, L. WILLIAMS (*Scientific Research Council, Hope Complex, P.O. Box 350, Kingston 6, Jamaica, colleens@src-jamaica.org): Characterisation of cultivars of Jamaican ginger (*Zingiber officinale Roscoe*) by HPTLC and HPLC. Food Chemistry 131, 1517-1522 (2012). HPTLC fingerprinting of 6-gingerol (1), 8-gingerol (2), 10-gingerol (3) and 6-shogaol (4), in the rhizome of Jamaican ginger (*Zingiber officinale Roscoe*) on silica gel with hexane - ethyl acetate - formic acid 11:8:1. Detection by spraying with either 5 % ammonium molybdate in 10 % sulfuric acid, or 0.5 mL p-anisaldehyde in 50 mL glacial acetic acid and 1 mL 97 % sulfuric acid. Quantitative determination by densitometry at 366 nm. The hR_F values of (1) - (4) were 46, 49, 52 and 64, respectively. This method of chemical fingerprinting is a suitable analysis for rapid determination of the authenticity of the ginger product as a chemical composite.

herbal, traditional medicine, HPTLC, densitometry 32e

108 127 A. SARASWATHY, A. MEENA, R. SHAKILA, K. SUNIL, S. ARIYANATHAN (*CSMDRI for Ayurveda & Siddha Drug Development (CCRAS) Anna Hospital Campus, Arumbakkam, Chennai, (T.N.), India): Pharmacognostic studies on *Alangium salvifolium* (Linn.f.) Wang root bark.

PHCOGJ 2(11), 374-380 (2010). Root bark of *Alangium salvifolium* (Linn.f.) Wang is a reputed drug mentioned in the ancient books of Ayurveda and Siddha for the treatment of epilepsy, jaundice, hepatitis etc. TLC of ethanolic, chloroform, and ethyl acetate extracts of the root bark of *Alangium salvifolium* on silica gel with toluene - ethyl acetate - diethylamine 10:7:1. Evaluation under UV 254 nm and 366 nm.

traditional medicine, herbal, qualitative identification 32e

108 128 L. SATHIYANARAYANAN*, A. R. PARADKAR, K. R. MAHADIK (*Univ. of Bradford, Dep. of Pharm. Engineering Sci., Inst. of Pharm. Innovation, and IRC in Polymer Sci. & Technol., Bradford West Yorkshire UK): Development and validation of a densitometric HPTLC method for simultaneous analysis of wedelolactone and asiaticoside in a polyherbal formulation. Acta Chromatographica 22 (4), 651-663 (2010). HPTLC of wedelolactone (WED) and asiaticoside (ASI) in *Eclipta alba* and *Centella asiatica Linn.*, respectively, on silica gel with toluene - acetone - methanol - formic acid 60:40:40:1. The hR_F value was 26 and 75 for ASI and WED, respectively. Detection by spraying with 10 % methanolic sulphuric acid. Quantitative evaluation by absorbance measurement at 317 nm for WED and at 530 nm after derivatization for ASI. The linearity was in the range of 50-250 ng/band for WED and 150-550 ng/band for ASI with r = 0.999 for WED and 0.9989 for ASI. The recovery of WED was 99.3 % and of ASI 99.5 %.

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

108 129 L.P. SAWANT*, B.R. PRABHAKAR, N.S. PANDITA (*School of Pharmacy and Technology Management, SVKM's NMIMS, Vile Parle (W), Mumbai-400056. India; laxmanpsawant@gmail.com): HPTLC method for quantification of isovitexin in whole-plant powder of *Enicostemma littorale Blume.* J. Planar Chromatogr. 24, 301-305 (2011). HPTLC of a methanolic extract of *E. littorale Blume* and isovitexin on silica gel with acetonitrile - water 3:2 at room temperature (28 +/- 2 °C) in a twin-trough chamber saturated for 30 min. Quantitative determination by densitometry at 350 nm. Linearity was between 100-400 ng/band. The *%RSD* for instrumental precision, intra-day precision, and intermediate precision was less than 2 %. The recovery was 99.7 %. The limit of detection and quantification was 0.6 and 1.9 ng/band, respectively.

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

108 130 L.P. SAWANT*, B.R. PRABHAKAR, N.S. PANDITA (*School pf Pharmacy & Technology Management, SVKM's NMIMS, Vile Parle (W), Mumbai-400056, India; laxmanpsawant@gmail.com): A validated quantitative HPTLC method for analysis of biomarkers in *Enicostemma littorale Blume.* J. Planar Chromatogr. 24, 497-502 (2011). HPTLC of isoswertisin-5-O-beta-D-glucoside (1), swertiamarin (2), and swertisin (3) as biomarkers on silica gel with ethyl acetate - methanol - water 16:2:1 in a twin-trough chamber with saturation for 30 min. Quantitative determination by absorbance measurement at 287 nm. Linearity was between 25-75 µg/mL for (1), 200-600 µg/mL for (2), and 100-300 µg/mL for (3). The relative standard deviation for instrumental precision, intra-assay precision, and intermediate precision was below 2 %. The average recovery was 99.9 % for (1), 99.6 % for (2), and 99.1 % for (3). The hR_F values were 32 for (1), 41 for (2), and 52 for (3). The limit of detection was 570 ng, 740 ng, and 300 ng for (1), (2), and (3), respectively.

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

108 131 A.A. SHIRKHEDKAR*, S.S. SURANA (*R. C. Patel College of Pharmacy, Department of Pharmaceutical Chemistry, Shirpur, Dist. Dhule (M. S.), India 425 405; atulshirkhedkar@rediffmail.com): Development and validation of a reversed-phase high-performance thin-layer chromatography-densitometric method for determination of atorvastatin calcium in bulk drugs and tablets. J. AOAC Int. 93, 798-803 (2010). HPTLC of atorvastatin calcium on RP-18 with methanol - water 7:3 in a twin-trough chamber saturated for 30 min at room temperature (25 +/- 2 °C) at a relative humidity of 60 +/-5 %. Quantitative determination by absorbance measurement at 246 nm. The hR_F value was 62. The LOD and LOQ were 6 and 18 ng/band, respectively. The linearity was between 100 and 800 ng/band. The recovery was between 99.4-100.7 % with *%RSD* between 0.6-1.4 %. The *%RSD* of intra-day and inter-day precision ($n = 3$) was 0.7-1.7 % and 0.6-1.9 %, respectively; the *%RSD* of repeatability of application ($n = 6$) was 1.3 %.

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

108 132 A. SINGH*, N.I. ALVI (*Customs Laboratory, JN Custom House, Nhava Sheve, Navi-Mumbai-400 707, India; amarsinghhpc@yahoo.com): High-performance thin-layer chromatographic quantification of yohimbine in the stem bark of *Pausinystalia yohimbe*. J. Planar Chromatogr. 24, 253-256 (2011). HPTLC of yohimbine on silica gel, prewashed with methanol, with toluene - ethyl acetate - diethyl amine 7:2:1 in a twin trough chamber saturated with mobile phase for 10 min at 25 +/- 2 °C. Quantitative determination by densitometry in absorbance mode at 285 nm. Linearity was between 400 and 1200 ng/band. The hR_F value was 39. The repeatability as system precision and method precision ($n = 6$) was 0.9 and 0.8 % CV. The limit of detection and quantification was 80 ng and 260 ng. The instrument precision ($n = 6$), intra-day and inter-day precision ($n = 3$, *%RSD*) were 0.2, 0.1, and 0.1 %, respectively.

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

108 133 Anna SOBANSKA*, E. BRZEZINSKA (*Department of Analytical Chemistry, Medical University of Lodz, Ul. Muszynskiego 1, 90-151 Lodz, Poland; a.sob@poczta.onet.pl): Normal-phase TLC analysis of UV filter avobenzone and octocrylene in sunscreen preparations. J. Planar Chromatogr. 24, 154-159 (2011). TLC of avobenzone (methoxydibenzoylmethane), octocrylene, Uvinul T 150 (ethyl hexyl triazone, ET), and ethylparaben on silica gel with cyclohexane - diethyl ether 1:1 (method A), or with cyclohexane - piperidine 15:1 (method B), in a chamber lined with filter paper and saturated for 20 min. Quantitative determination by densitometry at 380 nm for avobenzene and at 300 nm for octocrylene. LOD and LOQ were 170 and 510 ng/zone for avobenzone (method A) and 180 and 537 ng/zone for octocrylene (method B), respectively. The linearity range was 200-1800 ng/zone for avobenzone and octocrylene.

cosmetics, quality control, densitometry, quantitative analysis 32 a

108 135 H. TAVALLALI*, S. F. ZAREIYAN J., M. NAGHIAN (*Payame Noor University, Department of Chemistry, 19395-4697, Tehran, Iran, Tavallali@pnu.ac.ir, Tavallali@yahoo.com): An efficient and simultaneous analysis of caffeine and paracetamol in pharmaceutical formulations using TLC with a fluorescence plate reader. J. AOAC Int. 94, 1094-1099 (2011). TLC of caffeine and paracetamol in capsules and tablets on silica gel with *n*-hexane - ethyl acetate - ethanol 25:15:4. Detection at 254 nm. Quantitative determination by densitometry at 254 and 270 nm. The hR_F value of caffeine and paracetamol was 48 and 73, respectively. Linearity was between 0.2-1.9 for caffeine and 0.03-1.5 µg/L for paracetamol. The detection limit of caffeine was 25 ng/L and of paracetamol 32 ng/L. The precision was 1.9 % (n=6). Recovery (by standard addition) was 98-

99.5 % for both compounds.

quality control, pharmaceutical research, quantitative analysis 32a

108 136 J. VAIJANATHAPPA, S. BADAMI* (*Sree Siddaganga College of Pharmacy, Tumkur 572 102, Karnataka, India; shribadami@rediffmail.com): Antiedematogenic and free radical scavenging activity of swertiamarin isolated from *Enicostemma axillare*. Planta Med. 75, 12-17 (2009). HPTLC of swertiamarin and the ethyl acetate extract of *Enicostemma axillare* on silica gel with ethyl acetate - butanol 1:1 in a twin trough chamber. Quantitative determination by densitometric absorbance measurement at 254 nm.

traditional medicine, herbal, clinical chemistry research, HPTLC, quantitative analysis, densitometry 32e

108 137 A. VARMA, H. PADH, N. SHRIVASTAVA* (*B. V. Patel Pharmaceutical Education & Research Development (PERD) Centre, Sarkhej - Gandhinagar Highway, Thaltej, Ahmedabad - 380054, Gujarat, India; neetashrivastava_perd@yahoo.co.in): Ecogeographical phytochemistry of Adhatoda vasica Nees in relation to quantitative variations of alkaloids. J. Planar Chromatogr. 24, 406-411 (2011). HPTLC of plant extracts and vasicine on silica gel with ethyl acetate - methanol - ammonia (17.3 % ammonia) 40:10:1 in a twin-trough chamber saturated for 15 min. Quantitative determination by densitometry at 298 nm. The hR_F value of vasicine was 47. The limit of detection and the limit of quantification was 40 and 100 ng/zone, respectively. The average intra-day and inter-day precision was 0.7 and 2.2 %, respectively. Linearity was between 100-1300 ng/zone with a correlation coefficient of 0.999 and a *%RSD* of 2.3 %. Average recovery was 98.9 %.

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

108 138 B. WONGPAN, O. VALLISUTA*, N. RUANGWISE, A. MITREVEJ (*Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-Ayuthaya Rd., Ratchatewi, Bangkok 10400, Thailand; pyoln@mahidol.ac.th): TLC-densitometric method for the quantification of crebanine in *Stephania venosa (BI) Spreng*. J. Planar Chromatogr. 24, 264-267 (2011). TLC of crebanine on silica gel with toluene - ethyl acetate - methanol 14:3:3 in a twin-trough chamber saturated for 1 h at 25 +/- 2 °C. Detection under UV light at 254 nm. Quantitative determination by densitometry in absorbance mode at 286 nm. The method precision (*%RSD*, $n = 3$) and the instrument precision (*%RSD*, $n = 6$) were 0.9 and 0.5 %, respectively. The repeatability (*%RSD*, $n = 5$) was 0.8; the accuracy as average recovery was 100.1 %. The limit of detection and quantification was 10 and 15 ng/zone, respectively. Linearity was between 100-500 ng/zone. The hR_F value was 54.

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

108 139 G. WU* (Wu Guodong), D. ZHANG (Zhang Dong), ZH. WANG (Wang Zhenwang), J. LI (Li Jiaoshe), M. LI (Li Manhui), H. ZHANG (Zhang Huiwen), B. GAO (Gao Bowen) (*Baotou Med. College, Baotou 014060, China): (Methodology study of the quality criteria of Xinnao Tongtai capsules) (Chinese). Chinese J. of Ethnopharm. 2, 57-59 (2011). TLC of the extracts of Xinnao Tongtai capsules 1) for Lobed Kudzuvine Root, on silica gel with chloroform - methanol - water 28:10:1, detection under UV 366 nm; 2) for Szechwan Lovage Rhizome and *Angelica sinensis*, on silica gel with *n*-hexane - ethyl acetate 9:1, detection under UV 366 nm. Quantification of gastrodin by HPLC. The procedures proved to be simple, accurate, reproducible, robust and suitable

for the quality control of the medicine.

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

108 140 H. YAN (Yan Hua)*, Q. WANG (Wang Baoqin), J. LU (Lu Jing) (*National Inst. for the Contr. of Pharm. & Biolog. Products, Beijing 100050, China): (Separation and identification of oleanolic acid and ursolic acid in the herbal drugs and preparations by thin-layer chromatography) (Chinese). Chinese J. of Pharm. Anal. 29 (12), 2168-2179 (2009). TLC of the extracts of 11 varieties of herbal drugs and preparations on silica gel (conditioned with 1 % iodine in dichloromethane) with cyclohexane - dichloromethane - ethyl acetate - glacial acetic acid 200:50:80:1. Detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C. Identification of oleanolic acid and ursolic acid by comparison of the hR_F values (hR_F 54 for oleanolic acid and 38 for ursolic acid, respectively) with standards.

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

108 141 D. YANG (Yang Donghua)*, F. LIU (Liu Feng), J. MA (Ma Jiutai), X. LU (Lu Xinyi), Y. DANG (Dang Yanni), C. HAN (Han Cui) (*Shaanxi Coll. of Chinese Med., Shaanxi, Xianyang, 712046 China): (Study on the improvement of the quality standard for Kelu Oral Liquid) (Chinese). Chinese J. of Northwest Pharm. 26 (5), 324-327 (2011). TLC of the extracts of Kelu Oral Liquid on silica gel 1) for *Ephedrae herba*, with chloroform - methanol - concentrated ammonia 40:7:1, detection by spraying with 5 % ninhydrin in ethanol and heating at 105 °C, identification by fingerprint comparison with ephedrine/pseudoephedrine hydrochloride; 2) for *Scutellariae radix*, with ethyl acetate - butanone - formic acid - water 5:3:1:1, detection by spraying with 10 % $FeCl_3$ in ethanol and heating at 105 °C, identification by fingerprint comparison with astragaloside IV; 3) for menthol and Tatarian Aster root, with petroleum ether (60-90 °C) - ethyl acetate 9:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, identification by fingerprint comparison menthol and shionone; 4) for *Glycyrrhizae radix*, with ethyl acetate - formic acid - glacial acetic acid - water 15:1:1:2, detection under UV 365 nm after spraying with 10 % sulfuric acid in ethanol and heating at 105 °C, identification by fingerprint comparison with glycyrrhizic acid ammonium salt; 5) for Loquat leaf, with cyclohexane - chloroform - ethyl acetate - glacial acetic acid 40:10:16:1, detection by spraying with 20 % phosphomolybdic acid in ethanol and heating at 105 °C, identification by fingerprint comparison with ursolic acid; 6) for *Fritillaria cirrhosa D. Don*, with ethyl acetate - methanol - concentrated ammonia 18:2:1, detection by spraying with 5 % potassium iodobismuthate and 0.2 % sodium nitrite solution, identification by fingerprint comparison with peiminine.

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

108 142 J. YANG (Yang Jing), L.-L. CHOI (Choi Lei-lei), D.-Q. LI (Li De-Qiang), F.-Q. YANG (Yang Feng-Qing), L.-J. ZENG (Zeng Ling-Jie), J. ZHAO (Zhao Jing), S.-P. LI (Li Shao-Ping)* (*State Key Laboratory for Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China; Lishaoping@hotmail.com): Simultaneous analysis of hydrophilic and lipophilic compounds in *Salvia miltiorrhiza* by double-development HPTLC and scanning densitometry. J. Planar Chromatogr. 24, 257-263 (2011). HPTLC of hydrophilic and lipophilic constituents of *Salvia miltiorrhiza* and standards protocatechuic acid and aldehyde, salvianolic acid A and B, dihydrotanshinone I, rosmarinic acid, caffeic acid, cryp-

totanshinone, tanshinone II A, tanshinone I, and miltirone on silica gel with dichloromethane - ethyl acetate - formic acid 11:12:5 for the first development and petroleum ether - ethyl acetate - cyclohexane 15:11:14 for the second development with chamber saturation for 30 min. The first mobile phase separated the hydrophilic constituents salvianolic acid B, salvianolic acid A, rosmarinic acid, caffeic acid, protocatechuic acid, and protocatechuic aldehyde. Detection under UV light at 254 and 365 nm. After documentation the plates were placed in a second chamber and development with the low polarity mobile phase which separated dihydrotanshinone I, cryptotanshinone, tanshinone I and II A, and miltirone. Detection under UV light at 254 and 365 nm. Quantitative determination by densitometry in absorbance mode at 260 or 290 nm. The linear range was between 0.1-0.3 and 0.7-8.3 µg/zone. Instrumental precision was less than 4 % ($n = 6$). Precision on one plate was below 5 % ($n = 6$) and on different plates below 14 %. Depending on the substance, the limits of detection and quantification were between 14-22 and 69-276 ng/zone, respectively. The repeatability ($n = 6$) was between 1.3-3.4 %. Some of the compounds had similar hR_F values: for rosmarinic acid 44, for salvianolic acid 43, for caffeic acid 49, for protocatechuic acid 49, for dihydrotanshinone 65 and for cryptotanshinone 63. Additional detection by spraying with 5 % sulfuric acid in ethanol.

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

108 143 J. YANG (Yang Jun-Li), R. WANG (Wang Rui), L. LIU (Liu Lei-Lei), Y. SHI* (Shi Yan-Ping) (*State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, people's Republic of China;Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China. shiyp@licp.cas.cn): Sesquiterpenoids from *Inula britannica*. Planta Med. 77, 362-367 (2011). Analytical and preparative TLC of 19 sesquiterpenoids (e.g., ivalin, 6ss-hydroxytomentosin, 4-epipulchellin 2-O-acetate, and bigelovin) on silica gel with chloroform - ethyl acetate 8:1 and chloroform - methanol 15:1. Detection under UV 254 nm and by spraying with 98 % sulfuric acid - ethanol 1:19, followed by heating.

traditional medicine, herbal, qualitative identification, preparative TLC 32e

108 144 J. YANG (Yang Jun-Li), L. LIU (Liu Lei-Lei), Y. SHI* (Shi Yan-Ping) (*Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China; shiyp@licp.cas.cn): Limonoids and quinoline alkaloids from *Dictamnus dasycarpus*. Planta Med. 77, 271-276 (2011). Analytical and preparative TLC of limonoids and quinoline alkaloids (1',2'-didehydro-7,8-dimethoxyplatydesmine, 3-chloro-8,9-dimethoxygeibalansine, dasylactone A, dasylactone B, dictamnine, 7,8-dimethoxymyrtopsine, isofraxinellone, fraxinellone, obacunone, limonoic acid, rutaevine, and rutaevine acetate) on silica gel with chloroform - methanol 30:1, 10:1 and 6:1. Detection under UV 254 nm and by spraying with 98 % sulfuric acid - ethanol 1:19 followed by heating.

traditional medicine, herbal, qualitative identification, preparative TLC 32e

108 145 L. YANG (Yang Liu), S. XU* (Xu Shunjun), Q. FENG (Feng Qianru), H. LIU (Liu Hepin), R. TIAN (Tian Runtao), P. XIE (Xie Peishan) (*Macau Institute for Applied Research of Medicine and Health, Macau, shijxu2002@hotmail.com): A simple thin-layer chromatographic fingerprint method for distinguishing between *Radix paeoniae Rubra* and *Radix Paeoniae Alba*. J. Liq. Chromatogr. Relat. Technol. 32, 2893-2905 (2009). HPTLC of albiflorin, paeoniflorin, (beta)-cate-

chin, benzoyloxypaeoniflorin, benzoylpaeoniflorin, beta-sitosterol in the roots of *Radix paeoniae Rubra* (1) and *Radix Paeoniae Alba* (2) on silica gel with chloroform - ethyl acetate - methanol - formic acid 30:5:10:1 for high polarity components and toluene - ethyl acetate - methanol - formic acid 20:4:2:1 for lipophilic components. Detection by spraying with vanillin - sulphuric acid - ethanol 1:5:95, followed by heating at 105 ºC for 10 min. Qualitative determination by densitometry at 366 nm. The HPTLC fingerprints allowed differentiation between the roots of (1) and (2).

traditional medicine, herbal, HPTLC, qualitative identification 32e

108 146 D. ZALUSKI*, H. D. SMOLARZ, M. SZPILEWSKA (*Medical University of Lublin, Department of Pharmaceutical Botany, 1 Chodzki St, 20-093 Lublin, Poland; d.zaluski@umlub.pl): Eleutherosides in aerial parts of Eleutherococcus species cultivated in Poland. J. AOAC Int. 94, 1422-1426 (2011). HPTLC of eleutherosides B, E, and E1 in the fruits and leaves of E. senticosus and five other species of Eleutherococcus species after extraction with SPE, on silica gel in the horizontal chamber with saturation for 20 min, with a two-step development with 1) chloroform - methanol - water 70:30:4 and 2) chloroform - methanol - toluene - ammonium hydroxide 9:6:3:2. Detection by immersion in Liebermann-Burchard reagent for 1 s and heating at 110 °C for 10 min. The hR_F values for the eleutherosides E, B, and E1 were 43, 53, and 66, respectively.

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

108 147 J. ZENG* (Zeng Jianghong), C. LIU (Liu Canhui) (*The Second People's Hosp. of Shaodong, Shaoyang, Hunan 422811, China): (Study on the quality standard of Biyan syrup) (Chinese). J. of China Pharm. 20 (9), 16-17 (2011). TLC of the extracts of Biyan syrup 1) for *Herba Ephedrae*, on silica gel with chloroform - methanol - ammonia 40:10:1, detection by spraying with ninhydrin reagent and heating at 105 ºC; 2) for *Flos Magnoliae*, on silica gel with chloroform - diethyl ether 5:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 ºC; 3) for *Radix Astragali*, on silica gel with ethyl acetate - butanone - formic acid - water 5:3:1:1, detection by spraying with 5 % iron(III) chloride in ethanol; 4) for *Angelica dahurica* (Fisch.) Benth. et Hook, on silica gel with toluene - petroleum ether (60-90 ºC) - ethyl acetate 6:3:1, detection under UV 366 nm.

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

35. Other technical products and complex mixtures

108 148 Birgit BOECKEL (Bayer Weimar GmbH & Co. KG, Product Supply Pharma, QC Raw Materials, Doebereiner Str. 20, 99427 Weimar, Germany, birgit.boeckel@bayer.com: Cleaning validation using HPTLC. CBS 107, 2-4 (2011). To ensure effective cleaning of the reaction vessels in pharmaceutical production wool swabs are used to swipe a defined area of the vessel. The wool swabs are extracted with chloroform and the extracts are applied as rectangles of 4x3 mm. HPTLC on silica gel with toluene - ethyl acetate 3:2 with chamber saturation for 10 minutes. Identification of substances by densitometric spectra recording between 200-350 nm followed by 3-level calibration. Detection by spraying or immersion in methanol - sulfuric acid 9:1 and heating for 5 min at 105 °C. Evaluation under visible light and UV 366 nm.

clinical routine analysis, quality control, HPTLC, densitometry, quantitative analysis 35d

37. Environmental analysis

108 028 H. FARAJI *et al.*, see section 7

108 149 Andrea SEIGEL, Alexandra SCHROCK, R. HAUSER, B. SPANGENBERG* (*University of Offenburg, Institute of Process Engineering, Badstrasse 24, 77652 Offenburg, Germany, spangenberg@FH-Offenburg.de): Sensitive quantification of diclofenac and ibuprofen using thin-layer chromatography coupled with a *Vibrio fisheri* bioluminescence assay. J. Liq. Chromatogr. Relat. Technol. 34, 817-828 (2011). HPTLC of diclofenac (1) and ibuprofen (2) in aqueous environmental samples, on cyano phase with dichloromethane - methanol - cyclohexane 19:1:8. Detection by dipping into a *Vibrio fisheri* bacteria suspension for 3 sec. Then a glass plate was placed on top of the layer and a light-sensitive camera was used to measure the luminescence for 1 to 10 min. Linearity was between 10 and 2000 ng/zone. Limits of detection and quantification were 89 and 129 ng/band for (1), and 20 and 26 ng for (2). The coupling of HPTLC with a luminescent bacteria assay is suitable to determine drugs in aqueous environmental samples. *[Note of the editor: The linearity starts below the LOD which arises questions.]*

environmental, quantitative analysis, densitometry, HPTLC 37c

38. Chiral separation

108 150 M. DABROWSKA, J. KRZEK* (*Jagiellonian University, Collegium Medicum, Department of Inorganic and Analytical Chemistry, Medyczna 9, 30-688 Kraków, Poland; jankrzek@cm-uj.krakow.pl): Chiral separation of diastereomers of cefuroxime axetil by high-performance thin-layer chromatography. J. AOAC Int. 93, 771-777 (2010). HPTLC of the diastereomer A and B of cefuroxime axetil on cellulose (activated by heating at 60 °C) with 1 % aqueous beta-cyclodextrin - methanol 15:1. Quantitative determination by absorbance measurement at 285 nm. The hR_F of diastereomer A was 87 and for diastereomer B 93. Linearity was between 100-450 ng/zone for both diastereomers. Precision was good with *%RSD* values below 2.67 %. The recovery was in the range of 96.6-104.2 %. The LOD for both diastereomers was 40 ng/zone.

quality control, densitometry, HPTLC, quantitative analysis 38

Chromatographischer Fingerprint von Biopolymeren (Polysacchariden)

20

F. Gamlich und D. Schick

Die hier beschriebene HPTLC-Methode zur schnellen Analytik pflanzlicher Polymere wurde am Institut für Lebensmittelchemie, Universität Hohenheim, Stuttgart, entwickelt. Die Arbeit entstand durch Zusammenarbeit von Lebensmitteltechnologe Frank Gamlich in einem Praktikum bei Prof. Morlock und Dinah Schick während ihrer Wissenschaftlichen Abschlussarbeit bei Prof. Schwack.

Einleitung

Der Grossteil pflanzlicher Polymere besteht aus Polysacchariden, die bestimmte Aufgaben übernehmen: Sie binden Wasser und dienen als Energiespeicher oder zur Strukturgebung. Polysaccharide werden meist aus Landpflanzen oder Algen gewonnen, sind aber auch als organische Modifizierungen kommerziell erhältlich. In Nutrazeutika, Lebensmitteln, Pharmazeutika oder anderen Produkten werden sie als Dickungsmittel, Hydrokolloide (zur Gelstabilisierung) oder Stabilisatoren für Emulsionen oder Suspensionen eingesetzt.

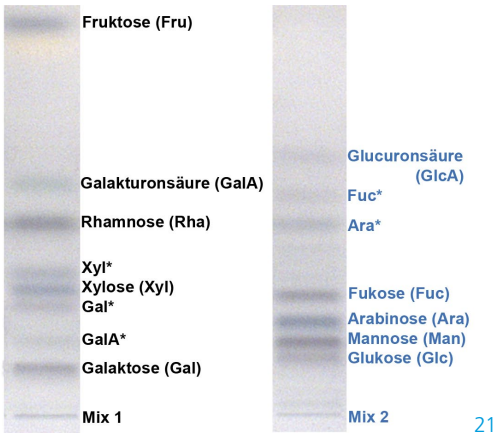
Die Analytik von Dickungsmitteln – in der Regel mit GC [1] – ist eine anspruchsvolle Aufgabe. Eine als Ergänzung zur GC einsetzbare HPTLC-Methode wurde entwickelt und validiert, mit der die unterschiedlichen Dickungsmittel-Typen auf einfache Weise unterschieden werden können. Die Genauigkeit der sehr effektiven, schnellen und kostensparenden neuen HPTLC-Methode wurde erfolgreich durch den Vergleich mit der GC-Methode verifiziert.

Schicht

HPTLC-Platten Kieselgel 60 (Merck), 20 × 10 cm

Standardlösungen

Zucker oder Uronsäuren (je 10 mg) wurden in je ein 10 mL Reaktionsröhrchen eingewogen und in 1 mL methanolischer Salzsäure (0.5 oder 2 mol/L [1]) gelöst. Nach Methanolyse bei 100 °C für 4 h wurden die Derivate mit 50 µL Pyridin versetzt. Dann wurden je 30 µL (Fructose 90 µL) in zwei Gemischlösungen in je einen 2 mL Messkolben pipettiert und mit Methanol bis zur Marke aufgefüllt (150 ng/µL; Fructose 450 ng/µL).



21

Nach der Methanolyse waren die aus den Zuckern und Uronsäuren entstandenen Methylglykoside und Methylglykosid-Methylester in beiden Gemischlösungen sichtbar. Aber auch Nebenprodukte, deren Struktur nicht bekannt ist, wurden gebildet (markiert*).

Probenvorbereitung

Die Aufbereitung der Dickungsmittel und Lebensmittelproben erfolgte gemäss [1].

Probenauftragung

Bandförmig mit DC-Probenautomat 4, 21 Bahnen, Bandlänge 8 mm, Bahnabstand 9 mm, Abstand vom unteren Rand 8 mm, Auftragevolumen 1–7 µL (Proben) und 2–15 µL (Standards)

Chromatographie

Automatische Entwicklungskammer (ADC 2) mit 10 mL *i*-Propylacetat – Ethylacetat – Methanol – Wasser

5:4:1:0.1 (v/v/v/v), Laufstrecke 60 mm, Trocknungszeit 30 s vor und 2 min nach Entwicklung

Densitometrie

TLC-Scanner 3 mit winCATS-Software, Spektrenaufnahme von 200–800 nm, Absorptionsmessung bei 370 und 630 nm (Mehrwellenlängenscan), Spaltgrösse 6 x 0.45 mm, Messgeschwindigkeit 20 mm/s; Peakflächen-Auswertung mit polynomer oder linearer Regression

Postchromatographische Derivatisierung

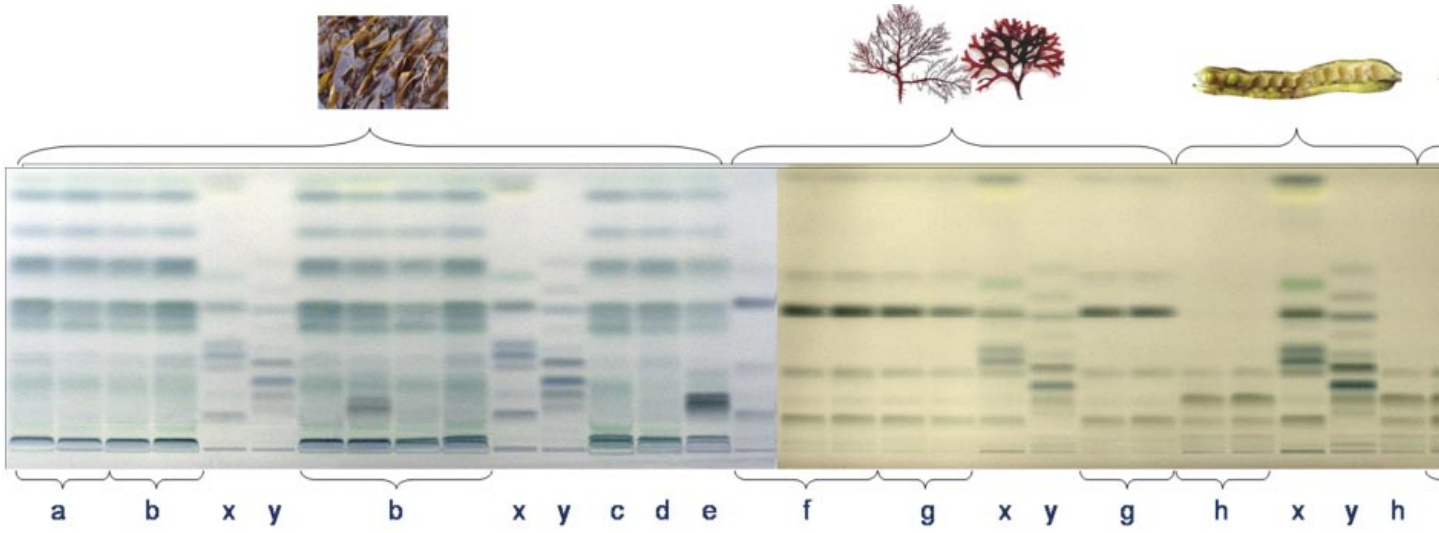
Durch Tauchen mittels Chromatogramm-Tauchvorrichtung (Eintauchgeschwindigkeit 3.5 cm/s, Eintauchzeit 1 s) in Anilin-Diphenylamin-*o*-Phosphorsäure-Reagenz (Zugabe von 20 % *o*-Phosphorsäure (85 %) zu einer 1:1 Mischung von 2 %igen-Lösungen von Diphenylamin und Anilin in Aceton); anschliessend Erhitzen auf dem DC-Plattenheizer (110 °C, 5 min). Das Reagenz ist im Kühlschrank mehrere Monate stabil.

Dokumentation

Die Chromatogramme wurden unter Weisslicht-Beleuchtung (Transmissionsmodus) im TLC Visualizer dokumentiert.

Ergebnisse und Diskussion

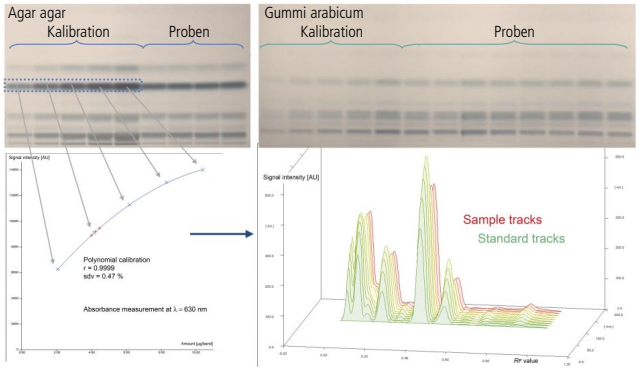
Nach einer reduzierten Probenvorbereitung wurden 21 Proben, z.B. die Methylglykoside und Methylglykosid-Methylester der unterschiedlichen Biopolysaccharide sowie beide Standardgemischlösungen, in nur 20 min getrennt. Die Platten wurden nach Derivatisierung mit dem Anilin-Diphenylamin-*o*-Phosphorsäure-Reagenz dokumentiert. Alginsäure und ihre Natrium-, Kalium- und Ammoniumsalze (Bahnen a–d) zeigten nur geringfügig unterschiedliche Fingerprints. Nur Propylenglykol-Alginat zeigte ein anderes Muster durch den typischen, organischen Rest (Bahn e). Agar agar und Carrageen bestehen aus den gleichen monomeren Bausteinen; somit war ihr Muster nahezu gleich (Bahnen f und g). Die Galaktomannane, z. B. Carubin und Guaran, unterschieden sich jedoch deutlich in ihrem Verhältnis von Mannose zu Galaktose (~ 4:1 bei Carubin und ~ 2:1 bei Guaran, Bahnen h und i) und konnten differenziert werden, obwohl sie die gleichen monomeren Bausteine enthielten. Ein Pulvergemisch von Guaran und mikrokristalliner Cellulose wurde auch analysiert (Bahn j). Die einzelnen Pflanzenexsudate (Traganth, Gummi arabicum, Karaya; Bahnen k, l und n) konnten deutlich unterschieden werden. Bahn m zeigt zum Vergleich das von Bakterien produzierte Xanthan. Das Muster der verschiedenen Pektine war charakteristisch (Bahn o: Pektin C, Bahn p: Pektin A, Bahn q: nicht spezifiziertes Pektin) und konnte von einer Formulierung mit geringem Pektingehalt unterschieden werden (Gehalt nur 20 %, Bahn p*), die Arabinose als monomeren Hauptbaustein aufwies. Glucose ist der monomere Baustein von mikrokristalliner Cellulose und Stärke, die das gleiche Muster zeigten (Bahnen t und u). Natriumcarboxymethyl-Cellulose (Na-CMC, Bahn r) zeigte ein zusätzliches Band darüber, wohingegen Hydroxypropylmethyl-Cellulose (HPMC, Bahn s) ein komplexes Muster aufwies.



HPTLC-Trennung von unterschiedlichen pflanzlichen Polysacchariden nach der Methanolyse mit den charakteristischen monomeren Bausteir

Zusammenfassend zeigten alle planzlichen Polysaccharide aufgrund ihrer unterschiedlichen monomeren Bausteine einen charakteristischen Fingerabdruck und konnten mit der neuen HPTLC-Methode differenziert werden.

Die pflanzlichen Polysaccharide wurden verschiedenen Lebensmittel-Matrices zugesetzt (z. B. Milch und Apfelsaft), anschliessend daraus isoliert und methanolysiert. Markerverbindungen wurden für die Quantifizierung ausgewählt (rot), und nach der Spektrenmessung wurde die Absorptionsmessung bei 370 und 630 nm durchgeführt. Standen Markerverbindungen nicht zur Verfügung (3,6-Anhydrogalaktose, Mannuron- (ManA) und Guluronsäure (GulA)), so wurde die jeweils intensivste Zone für die Quantifizierung verwendet.



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Analytik von Agar agar, der aus Apfelsaft isoliert wurde (links; Kalibration darunter) und von Gummi arabicum (aus Milch isoliert, rechts)

Zur Verifizierung der Ergebnisse wurde die HPTLC mit der GC verglichen, für die eine zusätzliche Silylierung erforderlich war [1]. Die mittlere Wiederfindung der aus Milch (blau) und Apfelsaft (orange) isolierten pflanzlichen Polysaccharide zeigte, dass die HPTLC zur Quantifizierung gleich gut geeignet ist wie die GC. Die Probenanzahl variierte je nach Anzahl der Polysaccharide. Diese wurden von C.E. Roeper, Biesterfeld, FMC BioPolymer, Provisco, AppliChem und Harke kostenlos zur Verfügung gestellt.

Analyse mit		GC		HPTLC	
Polysaccharide	Monomere Bausteine	*n*	Mittlere Wiederfindung ± SD (%)	*n*	Mittlere Wiederfindung ± SD (%)
Carubin	Gal, Man, (Ara)	4	82 ± 12	8	108 ± 26
Guaran	Gal, Man, (Ara)	7	90 ± 36	12	87 ± 36
Traganth	GalA, Xyl, Fuc, Gal, Ara	3	51 ± 12	4	44 ± 3
Gummi arabicum	GlcA, Man, Gal	4	78 ± 16	4	99 ± 13
Xanthan	GlcA, Man, Glc	4	65 ± 13	6	54 ± 13
Pektin	GalA, Gal, Ara	16	53 ± 22	23	83 ± 20
Alginsäure	ManA, GulA	4	38 ± 22	4	35 ± 12
Alginate	ManA, GulA	14	66 ± 30	16	80 ± 33
Agar agar	Gal, 3,6-AnhydroGal	4	54 ± 19	4	41 ± 7
Carrageen	Gal, 3,6-AnhydroGal	8	117 ± 33	8	86 ± 6

Fazit

Die HPTLC-Ergebnisse sind mit den GC-Ergebnissen vergleichbar. Jedoch ist die HPTLC-Methode im Vergleich effektiver hinsichtlich Probendurchsatz, Robustheit, Kosten und vor allem der Analysenzeit; bei gleicher Probenzahl ist die HPTLC-Methode ca. 8 mal schneller.

[1] BgVV, Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB, Methode L 00.00-13, Beuth Verlag, Berlin, Köln, November 1986.

Weitere Informationen sind von der Autorin erhältlich.

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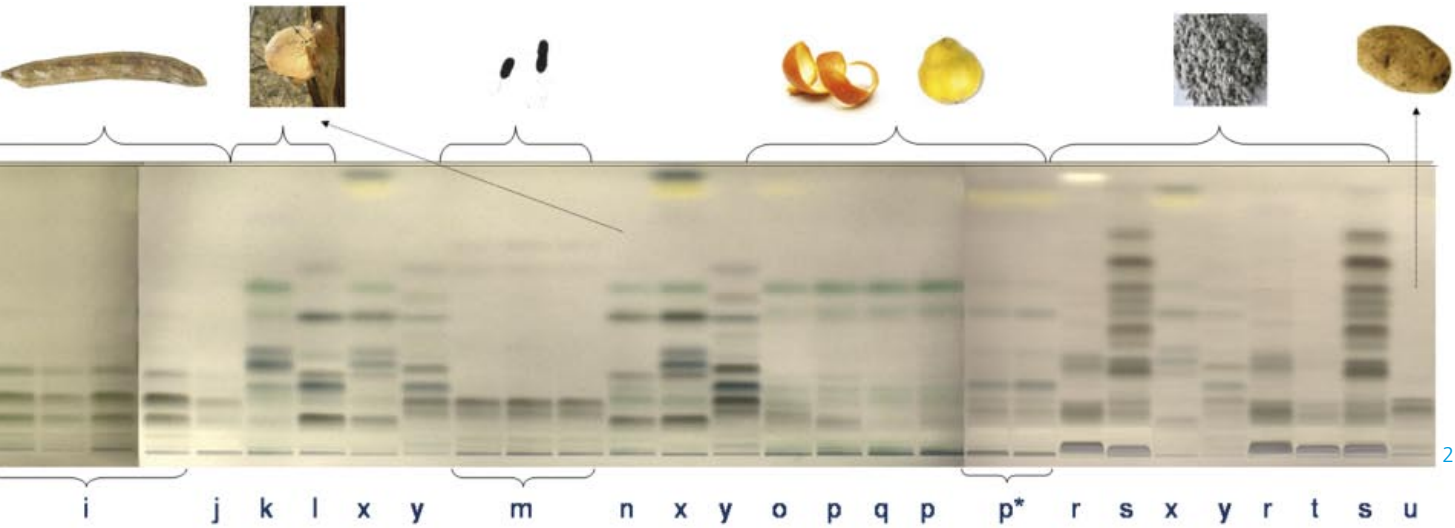

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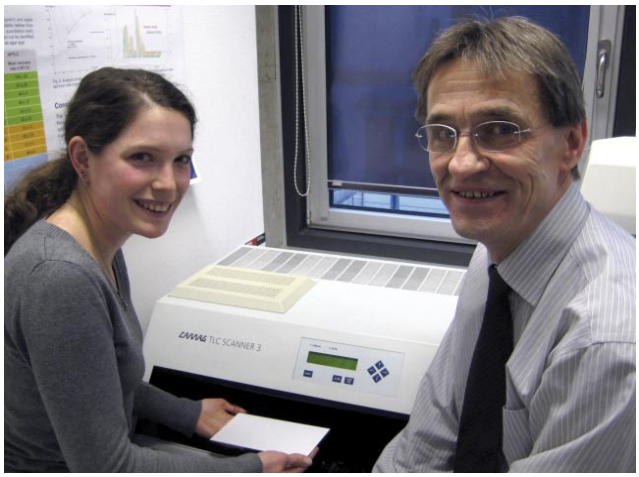
…nen (siehe Diskussion); Mix 1 (x) und Mix 2 (y)

Bestimmung von PAK in Spielzeug mittels HPTLC



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Sophie Behringer und Prof. Dr. Wolfgang Schwack

Am Institut für Lebensmittelchemie der Universität Hohenheim in Stuttgart wird die Planar-Chromatographie aufgrund ihrer Vorteile in der Lebensmittelanalytik eingesetzt. Sophie Behringer nutzte diese Technik im Rahmen ihrer Wissenschaftlichen Abschlussarbeit für die Analytik von Bedarfsgegenständen.

Einleitung

Die Anwesenheit polycyclischer aromatischer Kohlenwasserstoffe (PAK) in Spielzeug stellt aufgrund ihrer Kanzerogenität und leichten dermalen Aufnahme ein großes Problem dar. Durch die kürzlich erlassene EU Spielzeug-Richtlinie mit neuen Grenzwerten für CMR-Stoffe* wurde die Bestimmung von PAK wieder zu einem aktuellen Thema [1]. Für die Vergabe des deutschen GS-Prüfzeichens** muss der Gehalt von jedem der 16 EPA-PAK in Spielzeug unter einem Grenzwert von 0,2 mg/kg liegen. Zur Konformitätsprüfung werden PAK in Spielzeug routinemässig mit zeitaufwändigen GC-MS und HPLC-FLD Methoden bestimmt.

Planar-Chromatographie als matrix-robustes Analysenverfahren erwies sich als schnelle, effiziente und kostengünstige Alternative mit geringen laufenden Kosten und geringem Lösungsmittelbedarf zur Bestimmung von PAK in Spielzeug. Innerhalb von 2 h können bis zu 14 Spielzeugproben parallel analysiert werden. Die intensive Eigenfluoreszenz der PAK ermöglicht eine selektive und sensitive Detektion. Die entwickelte HPTLC-Methode erlaubt die Bestimmung von 14 PAK in den typischen Spielzeugmaterialien aus Polypropylen, Polyvinylchlorid, Polyester oder lackiertem Holz.

Standardlösungen

Standardmix aus kommerziellen Stammlösungen in Acetonitril (10 µg/mL): Anthracen (ANT), Benzo[b]-fluoranthen (BBF), Benzo[k]fluoranthen (BKF), Pyren (PYR) (jeweils 0,25 µg/mL) sowie Acenaphthen (ACE), Benzo[a]anthracen (BAA), Benzo[a]pyren (BAP), Benzo[ghi]perylen (BPE), Chrysen (CHR), Dibenzo[a,h] anthracen (DBA), Indeno[1,2,3-c,d]pyren (IND), Fluoren (FLU), Fluoranthen (FLA) und Phenanthren (PHE) (jeweils 0,5 µg/mL)

Probenvorbereitung

Extraktion von ca. 0.5 g der auf 3 mm zerkleinerten Spielzeugprobe mit 5 mL Tetrachlorkohlenstoff für 1 h im Ultraschallbad bei 60 °C; clean-up des Extraktes (1 mL) auf einer SPE-Kartusche (250 mg SiO_2), Elution mit 2 mL Tetrachlorkohlenstoff; Einengung des Eluates mit einem Stickstoffstrom auf 1 mL

Schicht

HPTLC-Platten Silica gel 60 RP-18 (Merck), 20 x 10 cm

Probenauftragung

Bandförmig mit DC-Probenautomat 4, Bandlänge 8 mm, Bahnabstand 10 mm, seitlicher Randabstand 13 mm, Abstand vom unteren Rand 8 mm, Auftragevolumen 20 µL Probelösung bzw. 0,5, 5, 10, 20 µL Standardlösung

Chromatographie

Dreifachentwicklung im AMD 2-System, Laufstrecke 45, 55 und 65 mm unter Stickstoff mit Acetonitril – Wasser 9:1, Trocknungsdauer 2 min

Dokumentation

Im Digistore 2 unter UV 254 und 366 nm

Detektion

Fluoreszenz-Messung mit TLC-Scanner 4 bei verschiedenen Anregungswellenlängen/Kantenfiltern; nach Tauchen der Platte in Nitromethan wird die Fluoreszenz alternierender PAK unterdrückt, und die Bestimmung nicht-alternierender PAK wird möglich.

EPA-PAK	hR_F	Anregung [nm] (Lampe)	Kantenfilter [nm]
ACE	81	220 (D2)	320
ANT*	75	250 (D2)	320
BAA*	58	366 (Hg)	400
BAP*	37	366 (Hg)	400
BBF	43	270 (D2)	400
BPE*	26	270 (D2)	400
BKF	43	270 (D2)	400
CHR	58	270 (D2)	400
DBA*	37	270 (D2)	400
FLA	69	270 (D2)	400
FLU	81	250 D2)	320
IND	26	270 (D2)**	400
PHE*	75	250 (D2)	320
PYR*	63	270 (D2)	320

* alternierend
**nach Tauchen in Nitromethan

Warum war bei dieser Methode AMD vorteilhaft, obwohl nur drei Läufe gemacht und kein Gradient benutzt wurde?

Durch den Einsatz der AMD – unter Stickstoff – ergab sich ein Optimum an Wiederfindungen. PAK sind sehr luft- und lichtempfindlich, insbesondere die Leitkomponente Benzo[a]pyren.

Eine Dreifachentwicklung ist auch in einer Trogkammer durchführbar, jedoch erkennt man nach jeder Entwicklung eine Abnahme der blauen Fluoreszenz infolge Oxidation. Um diese klein zu halten, muss man lichtgeschützt arbeiten und die Platte ohne Fön trocknen lassen, was viel Zeit in Anspruch nimmt. Arbeiten unter Schutzgas wäre ebenfalls sehr aufwändig. Letzteres gilt auch für die ADC.

Ergebnisse und Diskussion

Während auf Coffein-imprägnierten Kieselgelschichten nur 6 der 16 EPA-PAK detektierbar sind [2], erwies sich zur Chromatographie eine Umkehrphase (RP-18) als optimal. Einerseits zeigen alle PAK auf dieser lipophilen Phase intensive Fluoreszenz, andererseits wurde damit die weitestgehende Trennung von 14 PAK in 8 scharfe Zonen erreicht. Dies gelang mit einer Dreifachentwicklung, für die das AMD 2-System unter Stickstoff zu bevorzugen ist, um Oxi-

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Das Prinzip

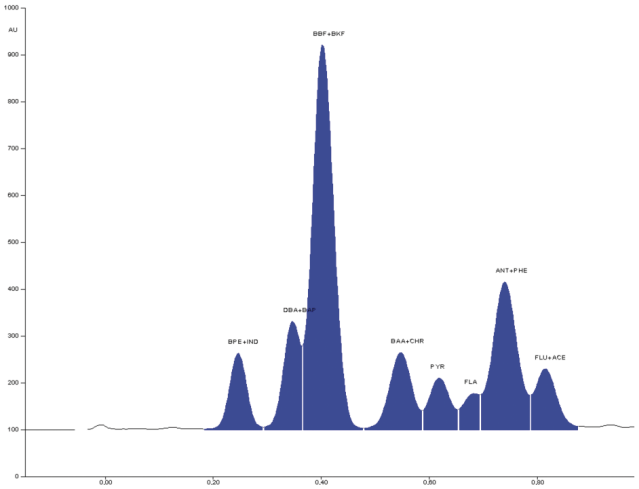
- Die HPTLC-Platte wird mehrfach in der gleichen Richtung entwickelt.
- Jeder Einzellauf führt über eine grössere Strecke als der vorangegangene.
- Für jeden Entwicklungsschritt wird die Elutionsstärke des Fliessmittels verringert.
- Zwischen den Einzelläufen wird die Platte vollständig getrocknet.

Das Ergebnis

- Auf diese Weise erfolgt eine Gradientenelution mit gleichzeitiger Fokussierung der Trennzonen.
- Typische Peakbreiten liegen bei 1 mm, so dass auf der zur Verfügung stehenden Trennstrecke von 80 mm bis zu 40 Komponenten Basislinien getrennt werden können.
- Das sichert die höchste Trennleistung, die mit der Planar-Chromatographie erreicht werden kann.

Das nebenstehende Anwendungsbeispiel zeigt, dass das AMD-Verfahren auch aus anderen Gründen als zur Erzielung der bestmöglichen Auftrennung komplexer Probengemische mit grossen Polaritätsunterschieden entscheidende Vorteile bieten kann. In diesem Falle sind es: Arbeitsablauf unter Schutzgasatmosphäre, Schutz vor Lichteinfluss und schneller Ablauf der gesamten Chromatographie einschliesslich Trocknung.

dationen zu vermeiden. Naphthalin und Acenaphthylen sind leider zu flüchtig und nach der Entwicklung nicht mehr detektierbar.



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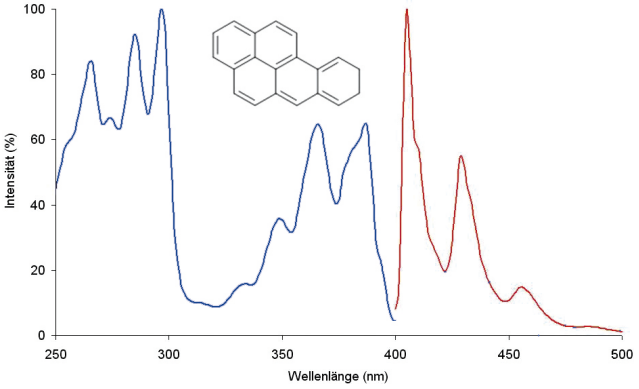
Fluoreszenz-Messung eines Standard-Mix beispielhaft bei 250 nm/>320 nm

Zur Auflösung der nicht getrennten 6 PAK-Paare und zur Quantifizierung aller 14 PAK, insbesondere der Leitkomponente Benzo[a]pyren, wurde eine selektive Detektion entwickelt. Aus Fluoreszenzanregungsspektren, aufgenommen von der RP18-Schicht an einem Spektralfluorimeter, konnten geeignete Anregungswellenlängen ermittelt werden, mit denen es möglich war, eine der Substanzen des nicht getrennten Paares durch gezielte Anregung und die Auswahl des passenden Kantenfilters selektiv zu quantifizieren.



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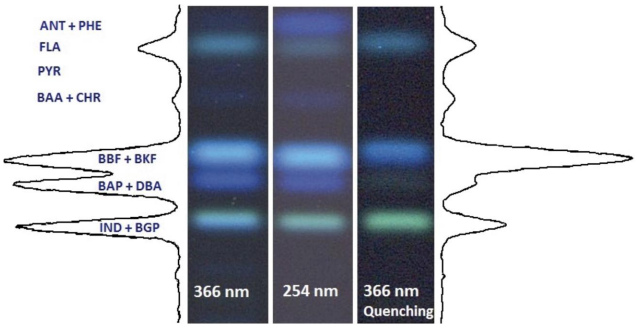
Fluoreszenz-Anregungsspektrum (blau, λ_{ex} = 366 nm) und Fluoreszenz-Emissionsspektrum (rot, λ_{em} = 405 nm) von Benzo[a]pyren

Bei einer zweiten Anregungswellenlänge erfolgte die Summenbestimmung der zwei nicht getrennten PAK. Die Anwendung eines substanz- und wellenlängenspezifischen Faktors auf die gemeinsame Peakfläche ermöglichte durch einen rechnerischen Schritt die präzise Quantifizierung des anderen PAK des nicht getrennten Paares. Zusätzlich wurde die Bestimmung nicht getrennter Substanzen mit der Methode der partiellen Fluoreszenz-Unterdrückung ermöglicht. In Gegenwart von Nitromethan wird die Fluoreszenz der alternierenden PAK gelöscht, so dass die selektive Quantifizierung der nicht getrennten, nicht alternierenden PAK möglich wird. Dieses Verfahren war schliesslich die einzige Chance zur Bestimmung von IND neben BPE, während das nicht getrennte Paar BBF/BKF generell nur als Summe bestimmbar war. Da beide toxikologisch gleich bewertet werden, entsteht aus der Summenbestimmung auch kein Nachteil. Dasselbe gilt für das nicht getrennte Paar ANT/PHE. Zwar ließ sich ANT neben PHE selektiv bei 350 nm anregen, jedoch ist die Empfindlichkeit zu gering. Daher wurde auch das nicht getrennte Paar ANT/PHE bevorzugt als Summe bestimmt.



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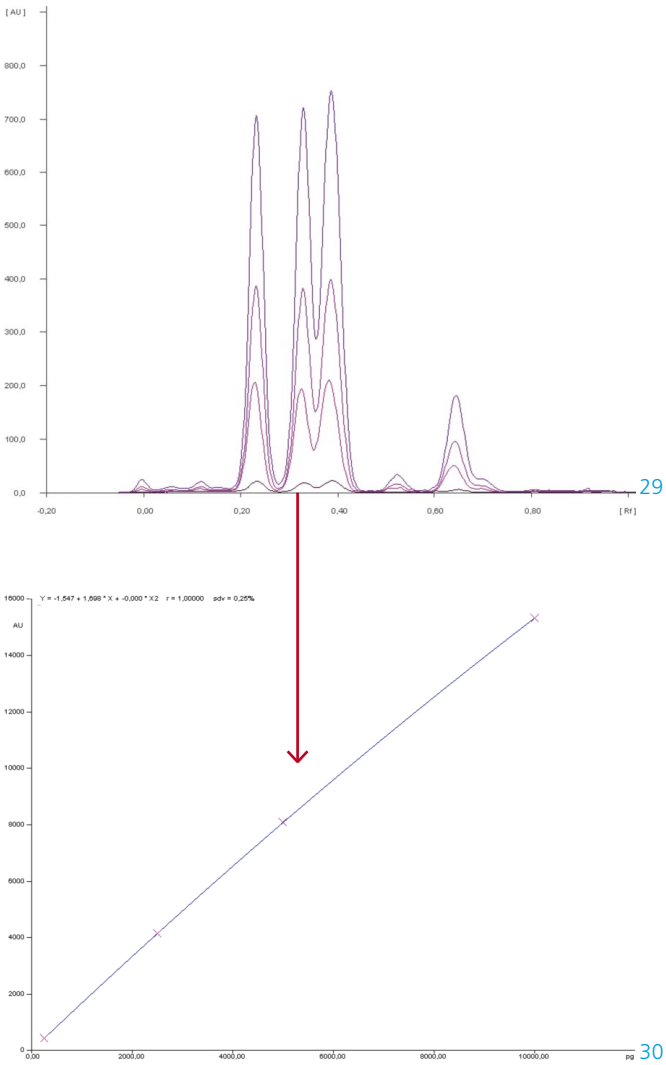
Plattenbilder eines entwickelten Standard-Mix (10 ng/Zone) bei verschiedenen Wellenlängen sowie nach Fluoreszenz-Unterdrückung durch Nitromethan; Fluoreszenz-Messungen bei 366 nm/>400 nm

Einige der untersuchten Probenextrakte zeigten nach Entwicklung starke Verunreinigungen durch Polymere und polare fluoreszierende Matrixkomponenten, welche die Chromatographie und Detektion störten. Ein einfaches Clean-up an einer SPE-Kartusche beseitigte diese Störungen. Durchgeführt wurde die Aufreinigung in einer speziell entwickelten Vorrichtung unter Stickstoff, um Verluste durch Oxidation, insbesondere von Benzo[a]pyren, zu verhindern, wodurch sehr gute Wiederfindungen erzielt wurden.

Die polynomen Kalibrierfunktionen besassen ein hohes Bestimmtheitsmass und geringe Standardabweichungen; die Variationskoeffizienten für die Wiederholpräzision und Vergleichspräzision lagen weitgehend unter 10 %.



Kalibrierung von Benzo[a]pyren (0,25–10 ng/Zone), Fluoreszenz-Messung bei 366 nm/> 400 nm

Die entwickelte HPTLC-Methode erlaubt die Bestimmung von 14 der 16 EPA-PAK. Mit Bestimmungsgrenzen von 0,1–0,2 mg/kg sind die Anforderungen für die Vergabe des GS-Prüfzeichens** bestens erfüllt. Die Validierungsdaten sowie der geringe Zeitaufwand und der hohe Probendurchsatz zeigen, dass die neu entwickelte HPTLC-Methode im Vergleich zu üblicherweise genutzten Methoden für die PAK-Bestimmung eine effiziente Alternative darstellt. Eine erste Gegenüberstellung der Untersuchungsergebnisse von GC-MS und HPTLC derselben Proben zeigte, dass die HPTLC-Methode gut vergleichbare Werte liefert.

* CMR-Stoffe: Einstufung als cancerogen, mutagen oder reproduktionstoxisch

** GS-Prüfzeichen: bescheinigt geprüfte Sicherheit gemäß dem deutschen Produktsicherheitsgesetz, ähnlich dem CE-Zeichen der Europäischen Union.

[1] Richtlinie 2009/48/EC, Amtsbl. Eur. Union (2009) L 170, 1–37

[2] G. Morlock, S. Kopacz, J. Liq. Chromatogr. Rel. Technol. 31 (2008) 1925

Weitere Informationen sind vom Autor auf Anfrage erhältlich.

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