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CAMAG LITERATURDIENST PLANAR-CHROMATOGRAPHIE

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In diesem Heft:
Reinigungsvalidierung von Industrieanlagen,
Probenvorbereitung mittels Planar-Chromatographie,
Prüfung von Honig auf Frische
und weitere Themen

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107

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Planar-Chromatographie
Herausgegeben von Gerda Morlock
cbs@camag.com
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IN DIESER AUSGABE

Verfahren, Anwendungen

In dieser Ausgabe hervorgehobene Produkte

Rubrik: Kennen Sie CAMAG?

CAMAG (Schweiz)
Sonnenmattstr. 11 • CH-4132 Muttenz 1
Tel. +41 61 467 34 34 • Fax +41 61 461 07 02
info@camag.com

CAMAG (Deutschland)
Bismarckstr. 27–29 • D-12169 Berlin
Tel. +49 30 516 55 50 • Fax +49 30 795 70 73
info@camag-berlin.de

www.camag.com

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Reinigungsvalidierung mittels HPTLC

Die Bayer Weimar GmbH und Co. KG ist ein pharmazeutisches Produktionsunternehmen mit Sitz in Weimar. Hier werden hormonhaltige feste Arzneiformen (Empfängnisverhütung, Hormonsubstitution) für die Bayer AG hergestellt und verpackt.

Die Steuerung und Überwachung der qualitätsentscheidenden Prozesse der Arzneimittelherstellung erfolgt durch unmittelbar vor Ort angesiedelte In-Prozess-Kontrollbereiche. Zu diesen Prozessen zählt auch die Reinigungsvalidierung. Für die Analytik zuständig ist Frau Dipl.-Chem. Birgit Böckel. Bei produktberührenden Herstellungsanlagen muss sichergestellt sein, dass das angewandte Reinigungsverfahren (CIP-Reinigung, aber auch manuelle Reinigung) Rückstände soweit reduziert hat, dass die Qualität der nachfolgend hergestellten Produkte nicht beeinflusst wird.

Seit 1998 werden in Weimar Prüfungen zur Reinigungsvalidierung mittels HPTLC durchgeführt. Die Auswahl des Analyseverfahrens wurde durch folgende Faktoren beeinflusst:

- **Vergleichbare Ergebnisse zwischen HPTLC und HPLC**
- **Auswertung der HPTLC-Platten mit Scanner, Derivatisierung, R_F-Wert**
- **Nachweis von sehr geringen Mengen/Nebenzonen**
- **Eine grosse Anzahl von Proben ist simultan verarbeitbar**
- **Deutliche Zeit- und Kostenersparnis gegenüber der HPLC**

Zur Anwendung kommt bevorzugt das Swab-Verfahren (direkte Probenahme mittels Wischtest). Dabei wird eine definierte Oberfläche mit geeignetem Probenahmematerial in vorgeschriebener Weise abgewischt, üblicherweise eine Fläche von 200 cm². Dafür werden 2–3 cm lange Swabs aus Schurwatte benutzt, die zuvor mit Chloroform durch Soxhletextraktion gereinigt werden.

Probenvorbereitung

Die benutzten Swabs werden 3-mal mit je 50 mL Chloroform (z. Spektroskopie) 15 min mit Ultraschall behandelt, die Extrakte vereinigt und am Rotationsverdampfer bei ca. 60 °C zur Trockne eingedampft. Der Rückstand wird mit Chloroform versetzt und für einige Minuten mit Ultraschall behandelt. Sofern die Lösung nicht klar ist, wird sie filtriert (0,45 µm) oder zentrifugiert. Eine Blindlösung wird in gleicher Weise hergestellt.

Standardlösungen

Die Kalibrierung wird mit 3 unterschiedlichen Konzentrationen im Bereich der Bestimmungsgrenze bis zum 20-fachen der Konzentration der Bestimmungsgrenze durchgeführt.

Schicht

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

Probenauftragung

Mit DC-Probenautomat 4 (ATS 4), Flächenauftragung 4 x 3 mm, je 100 µL Prüflösung sowie Blindlösung und 3 Standardlösungen zur Realisierung 3 unterschiedlicher Konzentrationen (s. oben). Die Auftragegeschwindigkeit beträgt 250 nL/s.

Chromatographie

Automatische Entwicklungskammer ADC mit Toluen – Ethylacetat 3 : 2 nach 10 min Kammersättigung

Densitometrie

TLC Scanner 3 mit winCATS Software. Die Identifizierung der Wirkstoffe erfolgt durch Spektrenaufnahme im Bereich 200–350 nm und anschliessend ihre quantitative Bestimmung über eine Dreipunktkalibrierung.

Postchromatographische Derivatisierung

Die HPTLC-Platte wird mit Methanol – Schwefelsäure (9:1) durch Sprühen oder Tauchen und anschliessendem Erhitzen 5 min auf 105 °C derivatisiert. Tauchen empfiehlt sich im Falle der Quantifizierung.

Auswertung

Die Auswertung erfolgt im UV-Bereich mittels Densitogramm und Bilddokumentation mit hoch auflösender 12 bit CCD Kamera sowohl unter sichtbarem Licht als auch unter UV 366 nm.

Ergebnisse und Diskussion

Es wurde nachgewiesen, dass bei der Reinigungsvalidierung mittels HPTLC die richtigen Werte erhalten werden.

Die R_f-Werte, die UV-Absorptionsspektren und die Farbe bzw. Fluoreszenz nach der Derivatisierung dienen zur Identifizierung.

Bei der Prüfung von Selektivität und Spezifität wurde gezeigt, dass die Wirkstoffe von den Hilfsstoffen gut getrennt werden und eine genügend grosse Auftrennung von allen zu untersuchenden Wirkstoffen untereinander erreicht wird.

Je nach Konzentrationsbereich kann sich die Kalibrierfunktion (Konzentration zu Messsignal) als linear oder polynom erweisen. Es werden nur solche akzeptiert, deren Korrelationskoeffizient mindestens 0,990 beträgt.

1

CAMAG Automatische Entwicklungskammer (ADC 2)

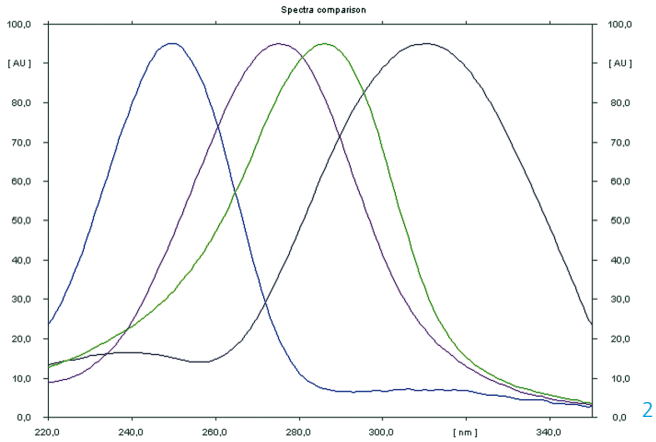
In der Automatischen Entwicklungskammer ADC 2 erfolgt die Entwicklung von 20 x 10 cm HPTLC-Platten vollautomatisch unter reproduzierbaren, von Umwelteinflüssen unabhängigen Bedingungen. Vorkonditionierung der Schicht, relative Feuchte und Sättigung der Kammer, Laufstrecke sowie die abschliessende Trocknung können in der ADC 2 vorgegeben und automatisch überwacht werden. Es sind zwei Betriebsarten möglich: Stand-alone mit Eingabe der Programmparameter via Tastatur oder Remote-Betrieb mit PC und winCATS Software.

Hinweis: Müssen wie beim nebenstehenden Verfahren grosse Probenvolumina flächenförmig aufgetragen werden, so können diese mit Vorteil vor der eigentlichen Chromatographie mit einem Fliessmittel hoher Elutionsstärke zu schmalen Banden fokussiert werden. Dieser Schritt einschliesslich der nachfolgenden Zwischentrocknung kann bei der ADC 2 in den automatisierten Ablauf einbezogen werden.

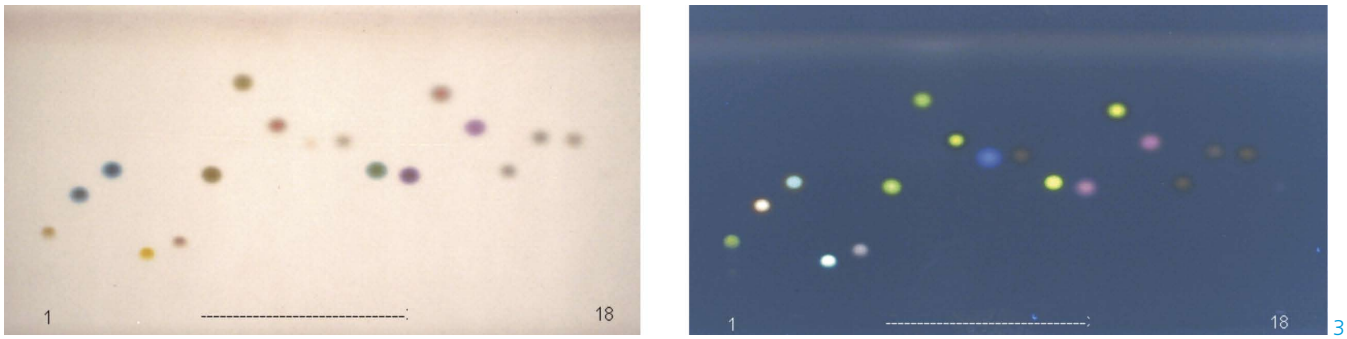
Vollständige Informationen finden Sie im Spezialprospekt »Automatische Entwicklungskammer ADC 2« oder unter **www.camag.com/adc2**

Die Nachweisgrenze betrug für alle Wirkstoffe mindestens 0,5 µg/Swab. Die auf den verschiedenen Rohstoffmaterialien (Glas- bzw. Stahloberflächen usw.) ermittelten Wiederfindungsraten lagen zwischen 70 und 100 %. Diese Wiederfindungsraten konnten auch bei einer Standzeitkontrolle der Swabs bestätigt werden.

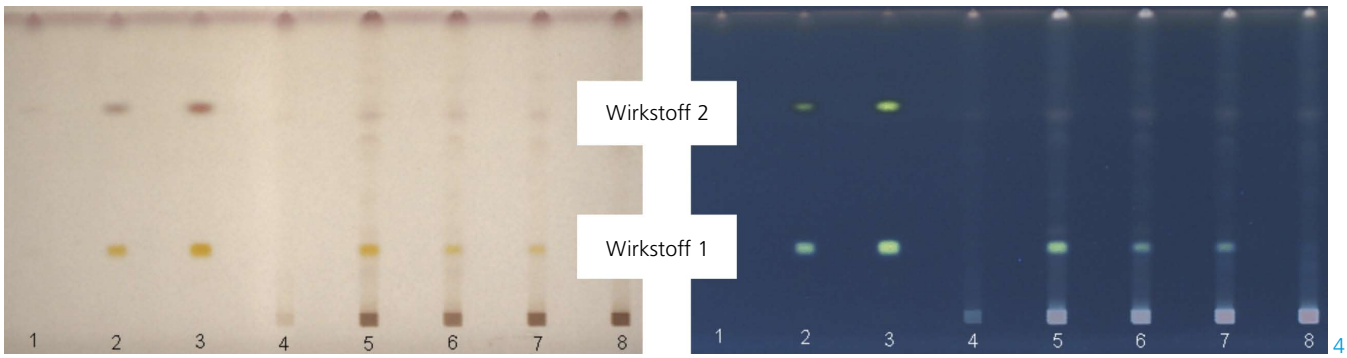
Die in einer Risikoanalyse festgelegten Probenahmestellen wurden mittels der freigegebenen Swabs abgereinigt und anschliessend analysiert. Als Beispiel wurde eine Prüfung gewählt, bei der noch ein Wirkstoffrest nachweisbar war. Über die Kalibrierung und die Einbeziehung der gesamten produktberührenden Flächen wurde der Anteil an Wirkstoffrest berechnet. Im gewählten Beispiel lag die berechnete Menge mit < 0,5 % vom Grenzwert deutlich unterhalb der erlaubten Werte.



2 *Absorptionsspektren verschiedener im Unternehmen vorkommender Wirkstoffe*



3 *HPTLC-Platte nach der Derivatisierung zur Überprüfung der Selektivität der unterschiedlichen Wirkstoffe im sichtbaren Licht und bei 366 nm*



4 *HPTLC-Platte nach der Derivatisierung: Bahn 1 bis 3: Mischung der Wirkstoffe 1 und 2 zur Erstellung der Kalibrierkurve, Bahn 4 Blindprobe, Bahn 5 bis 8: 4 Probenahmestellen zur Überprüfung einer Anlage*

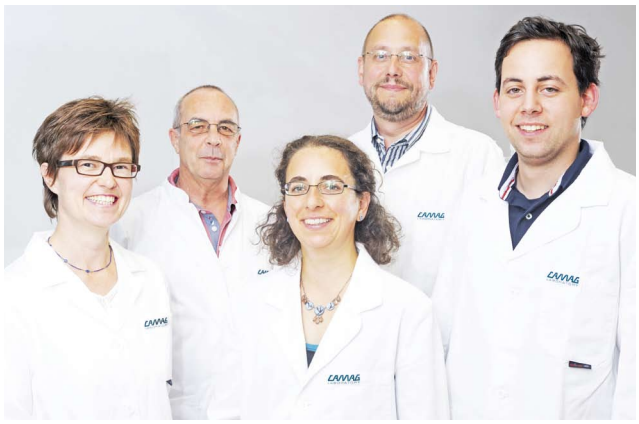
Fazit

Mit der HPTLC kann in der Reinigungsvalidierung für hormonelle Wirkstoffe eine Bestimmung an Restverunreinigungen mit der erforderlichen Genauigkeit durchgeführt werden. Das Prüfverfahren ist einfach und schnell. Es liefert über die quantitative densitometrische Auswertung genaue Ergebnisse.

Weitere Informationen sind von der Autorin auf Anfrage erhältlich.

Kontakt: Frau Dipl.-Chem. Birgit Böckel, Bayer Weimar GmbH & Co. KG, Product Supply Pharma, QC Rohstoffe, Döbereiner Str. 20, 99427 Weimar, birgit.boeckel@bayer.com

Content Uniformity Test (CUT) von Coenzym Q10 in Weichgelatinekapseln mittels HPTLC



5

CAMAG Labor, Dr. Anita Ankli, Daniel Handloser, Valeria Widmer, Dr. Eike Reich, Eliezer Ceniviva

Im CAMAG-Labor ist ein hochqualifiziertes Team von Chemikern, Pharmazeuten und Laboranten tätig, das auf langjährige HPTLC-Erfahrung zurückblicken kann. Unser Ziel ist es, die Vorteile und die Flexibilität der HPTLC sowie die Reproduzierbarkeit und Zuverlässigkeit von HPTLC-Methoden aufzuzeigen. Außerdem entwickeln wir Problemlösungen für unsere Kunden. Sprechen Sie mit uns!

Einleitung

Coenzym Q10 kommt natürlicherweise im menschlichen Körper vor und ist an der Energieproduktion beteiligt. Daher weisen Herz, Lunge und Leber die höchsten Q10 Konzentrationen auf. Die Substanz ist vielseitig aktiv und wirkt vor allem als mögliches Immunstimulans und als Antioxidans, das die Zellschädigung als Radikalfänger verhindert. Als Nahrungsergänzungsmittel wird Q10 vor allem in Form von Weichgelatinekapseln verkauft. In allen bedeutenden Arzneibüchern der Welt ist für einzeldosierte Arzneizubereitungen der Content Uniformity Test (CUT, Prüfung auf Gleichförmigkeit des Gehalts) verankert. Die Festlegungen sind in der internen Qualitätskontrolle des Herstellers, als Bestandteil der Zulassungsunterlagen sowie im nationalen und internationalen Warenverkehr von Bedeutung.

Das Ziel bestand darin, eine schnelle HPTLC-Methode für die Analyse einer grossen Zahl einzelner Coenzym Q10-Kapseln zu entwickeln. Die Anforderungen an die Methode lagen in einfacher Probenvorbereitung und unproblematischer, schneller chromatographischer Entwicklung. Die Auswertung der Resultate sollte unkompliziert und zuverlässig sein.

Probenvorbereitung

Eine Weichgelatinekapsel wurde zusammen mit 50.0 mL Toluen in einen Erlenmeyerkolben gegeben. Eingetaucht im Lösungsmittel wurde die Kapsel mit einem Skalpell zerschnitten. Anschliessend wurde der Kolben 15 Minuten maschinell geschüttelt. Je nach deklariertem Gehalt der Kapsel wurde ein Aliquot des Extraktes auf eine Konzentration von ungefähr 15 µg/mL verdünnt. Die Probelösungen wurden vor direkter Lichteinstrahlung geschützt.

Standardlösung

Eine Standardlösung von 1 mg/mL Coenzym Q10 in Toluen wurde hergestellt und mit Toluen auf 10, 15 und 20 µg/mL verdünnt. Die Standardlösungen wurden vor direkter Lichteinstrahlung geschützt. Sie sind etwa eine Woche bei 4 °C stabil.

Schicht

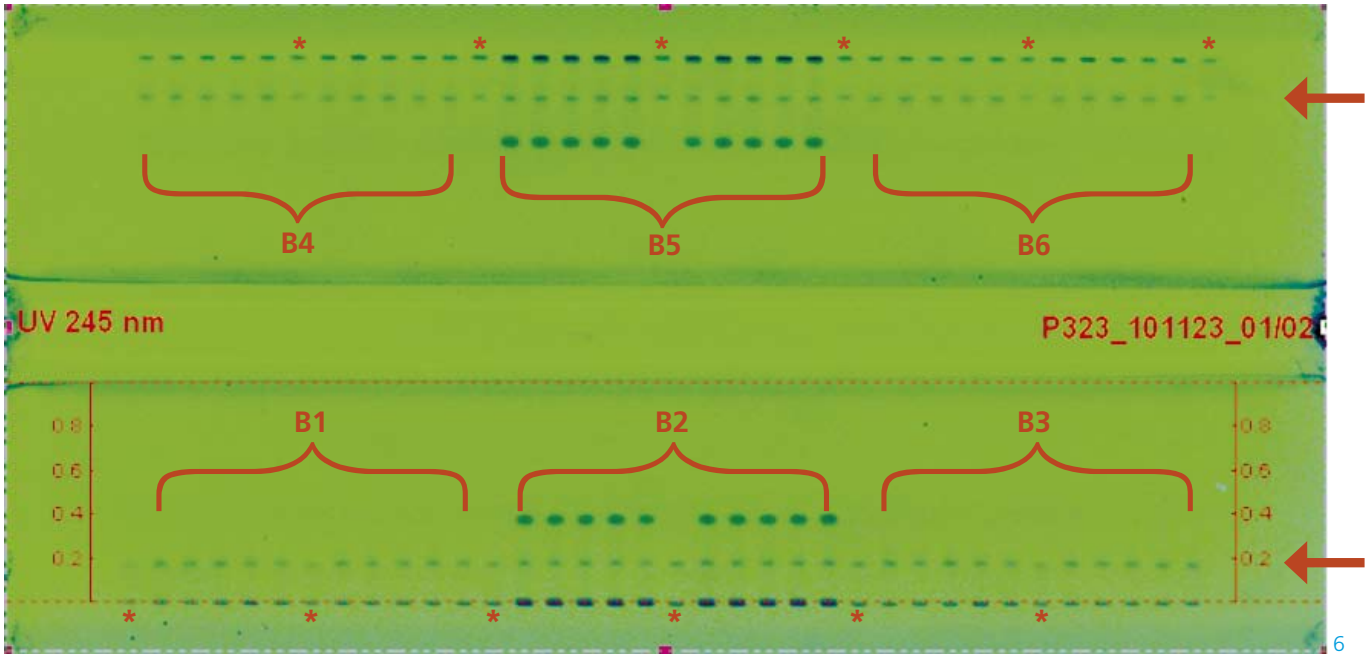
HPTLC-Platten Kieselgel 60 F_{254} (Merck), 20 × 10 cm, wurden mit Methanol in der Horizontal-Entwicklungskammer vorgewaschen und anschliessend im Trockenschrank bei 120 °C 30 min getrocknet.

Probenauftragung

Bandförmig mit dem DC-Probenautomat 4 (ATS 4), Bandlänge 2 mm, Bahnabstand 5 mm, Abstand vom unteren Plattenrand 8 mm, Abstand vom linken Rand 20 mm, Auftragevolumen 2 µL von Standard- und Probelösungen.

Chromatographie

In der Horizontal-Entwicklungskammer 20 × 10 cm mit 8 mL Toluen auf jeder Seite (36/72 Proben), der Doppeltrogkammer 20 × 10 cm oder in der Automatischen Entwicklungskammer (ADC 2) ohne Kammersättigung mit 10 mL Toluen.



6

60 Proben (6 Chargen, B1–B6) und 12 Standards wurden auf die HPTLC-Platte aufgetragen; Coenzym Q10 ist bei ca. $R_F \approx 0.20$ zu erkennen. Die Q10-Chargen auf den mittleren Bahnen enthalten auch Vitamin B2 und Vitamin E ($R_F \approx 0$ und 0.4); (Hinweis: Zur besseren Darstellung wurden die Auftragevolumina in diesem Bild auf 6 µL erhöht).*

Densitometrische Auswertung

TLC Scanner 4 mit winCATS Software, Absorptionsmessung bei 282 nm; Spaltgrösse: 3.00 x 0.20 mm; Auswertung über Peakhöhe, lineare Regression (20–50 ng) bzw. alternativ polynome Regression (20–150 ng).

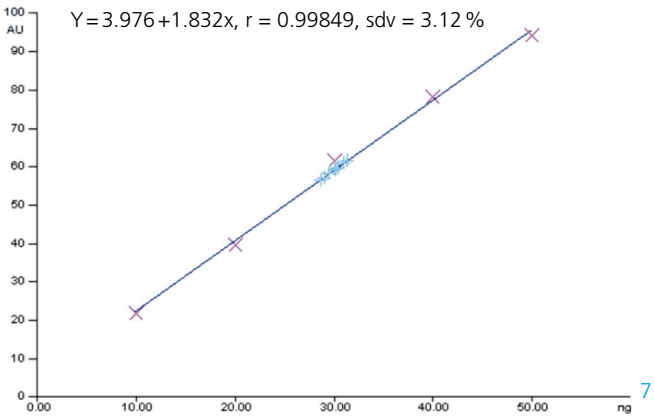
Ergebnisse und Diskussion

60 Proben und 12 Standards wurden auf eine HPTLC-Platte aufgetragen und mittels einer Horizontal-Entwicklungskammer von beiden Seiten zur Mitte entwickelt. Als Beispiel wurden je 10 Proben von 6 verschiedenen Chargen sowie Standards in verschiedenen Konzentrationen parallel chromatographiert. Die Durchführung der Analyse war sehr einfach, da Toluen sowohl als Lösungsmittel als auch als mobile Phase für die Chromatographie verwendet wurde. Der am meisten Zeit beanspruchende Schritt war die Probenauftragung. Die Entwicklung und die Auswertung der Chromatogramme waren »extrem« schnell. Die gesamte Analyse von 72 Proben wurde in 86 min durchgeführt.

CUT-Zeitaufwand für je 10 Proben von 6 verschiedenen Chargen und 12 Standards auf einer HPTLC Platte

Schritte	[min]
Auftragung von 72 Bahnen mit DC-Probenautomat 4 (ATS 4)	64
Chromatographie in der Horizontal-Entwicklungskammer	6
Trocknungszeit der Platte	10
Auswertung mit dem TLC Scanner 4	6
Totaler Zeitaufwand	**86 min**

Die Linearität der Kalibrierkurve im Arbeitsbereich 20–50 ng zeigt untenstehende Abbildung.



7

Kalibrierfunktion für Coenzym Q10 bei UV 282 nm. Messung von 10 Proben und 5 Standards in einem linearen Bereich

Der CUT wurde gemäss Europäischem Arzneibuch [1] bzw. Amerikanischem Arzneibuch (USP) [2] mit 10 Proben je Charge durchgeführt. Der Gehalt der Weichgelatinekapsel betrug gemäss Deklaration 30 mg. Der durchschnittlich gemessene Gehalt der Probe war 30.08 mg. Der Mittelwert der Einzelgehalte, ausgedrückt als Prozentsatz des deklarierten Gehalts, wurde berechnet und betrug $\bar{x}$ = 100.25 mit einer Standardabweichung von s = 2.76. Gemäss [1, 2] ist die Akzeptanzkonstante k von 10 Proben mit k = 2.4 definiert. Für die Endbestimmung des Akzeptanzwertes (AV) muss der Referenzwert M bekannt sein; für diesen Fall 1 war M = $\bar{x}$ [1, 2]. Der berechnete AV-Wert von 6.6 war kleiner als der maximal erlaubte AV von 15. Somit ist der gemessene Gehalt der Weichgelatinekapseln als gleichförmig zu bezeichnen.

CUT-Berechnung gemäss PhEur [1] respektive USP 34 [2]. Probengrösse n = 10

X(Average)	X(Average)	Zielgehalt T gemäss Deklaration	30 mg
29.53 ng	29.53 ng	Durchschnitt (berechnet)	30.08 mg
X(Average)	X(Average)	Mittelwert der Einzelgehalte ausgedrückt als Prozentsatz $\bar{x}$ (berechnet)	$\bar{x}$ = 100.25
31.28 ng	31.28 ng		
X(Average)	X(Average)	Standardabweichung der Probe s (berechnet)	s = 2.76
30.17 ng	30.17 ng	Akzeptanzkonstante k (n = 10)	k = 2.4
X(Average)	X(Average)	M (case 1), wenn 98.5 % ≤ $\bar{x}$ 101.5 %	M = $\bar{x}$
30.05 ng	30.05 ng	Akzeptanzwert AV	AV = |M – $\bar{x}$ | + ks
X(Average)	X(Average)	AV (berechnet)	**AV = 6.6**
30.43 ng	30.43 ng	Max. zulässiger AV, L1 = 15.0	15.0

Weitere Informationen sind bei den Autoren auf Anfrage erhältlich (Lab@camag.com).

[1] European Pharmacopoeia 7.0, 2.9.40. Uniformity of Dosage Units.

[2] USP 34 /NF29, The United States Pharmacopeial Convention 12601 Twinbrook Parkville, MD 20852, <905> Uniformity of Dosage Units, S. 403–406.

8

CAMAG DC-Probenautomat (ATS 4)

Automatisches Probenauftragen ist der Schlüssel für hohe Präzision und Produktivität im Routinebetrieb. Mit dem ATS 4 (Automatic TLC Sampler 4) werden die Proben wahlweise punktförmig durch Kontaktübertragung (0.1–5 µL) oder strich- bzw. rechteckförmig (0.5 – >50 µL) durch Sprühen aufgetragen. Strichförmig aufgetragene Startzonen gewährleisten die bestmögliche Trennleistung, die mit dem gewählten chromatographischen System erreichbar sind. Aufsprühen in Rechteckform mit nachfolgender Fokussierung erlaubt ein präzises, die Schicht schonendes Aufbringen grösserer Volumina mit den Vorzügen der strichförmigen Startzonen.

In dem nebenstehend beschriebenen Beispiel aus der Praxis wurde quasi-punktförmig aufgetragen, um möglichst viele Proben auf der Platte unterzubringen. Um die Auftragezonen möglichst klein zu halten, sollte das Sprühverfahren angewendet werden. Dazu wurde 2 mm Bandlänge gewählt, die kürzest mögliche, die das ATS 4-Programm im Sprühmodus akzeptiert.

Bei dem Praxisbeispiel Reinigungsvalidierung (S. 2–4) wurde Auftragung in Rechteckform angewendet, allerdings ohne Fokussierung.

Neuer Präsident des Verwaltungsrates


9

Auf Empfehlung des CAMAG Stiftungsrates wurde Herr Dr. Konstantinos Natsias von der Generalversammlung am 21. Juni 2011 einstimmig zum Präsidenten des Verwaltungsrates gewählt, nachdem Herr Christian Gfeller seinen Rücktritt von diesem Posten erklärt hatte.

Dr. Natsias gehört seit mehr als 20 Jahren zu unserem Unternehmen. Er trat am 1. August 1989 bei CAMAG Berlin als deren Vertriebsleiter ein und wurde 2001 zum Geschäftsführer berufen. Im Juni 2010 wurde er in den Verwaltungsrat des Mutterhauses gewählt und ist nun dessen Präsident. Die ganze »CAMAG-Familie« ist überzeugt, in ihm einen kompetenten und tatkräftigen Präsidenten gefunden zu haben, der sowohl für Fortschritt als auch Wahrung der Tradition des Hauses CAMAG einsteht. Alle Mitarbeiterinnen und Mitarbeiter schätzen seine liebenswürdige und kollegiale Art.

Herrn Gfeller danken wir für 45 Jahre treuen und erfolgreichen Einsatz für CAMAG und sind froh, dass er uns noch weiterhin als Verwaltungsrat zur Verfügung steht.

Dr. Dieter Jänchen
Präsident des Stiftungsrates

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PLANAR CHROMATOGRAPHY

CBS

Liebe Freunde

Das International Symposium for High-Performance Thin-Layer Chromatography, in Basel, 6.–8. Juli 2011 (HPTLC 2011) begann mit einer Aufführung des traditionellen Basler Morgenstraichs, dem einmaligen Auftakt zur Basler Fasnacht. Damit war sichergestellt, dass die mehr als 310 Teilnehmer hellwach waren, um Neuigkeiten aus dem Gebiet der Planar-Chromatographie aus aller Welt aufzunehmen. Der internationale Charakter der Veranstaltung war gesichert, indem Wissenschaftler aus ca. 40 Ländern an dem Kongress teilnahmen. 50 Plenarvorträge und ca. 160 Posterpräsentationen wurden im Rahmen des Programms gehalten (siehe www.hptlc.com).

Sehr erfreulich war das grosse Interesse der jungen Generation, denn 22 % der Teilnehmer waren Studenten. Insgesamt kamen etwa gleich viele Kongressbesucher aus dem universitären Bereich und aus der Industrie, was die aktuelle praktische Bedeutung der HPTLC unterstreicht.

Das Symposium gab den Teilnehmern reichlich Gelegenheit, Wissen auszutauschen und Forschungsprojekte zu vereinbaren bzw. zu erweitern. Allgemein wurde das hohe wissenschaftliche Niveau der Veranstaltung gewürdigt und besonders die ausgezeichneten Präsentationen der jüngeren Vortragenden begrüsst.

Von vielen Seiten wurde der Wunsch geäussert, das nächste Symposium dieser Reihe im Jahr 2013 zu organisieren, wobei verschiedene Städte sowohl in Europa als auch in Asien vorgeschlagen wurden. Ich hoffe, Sie werden mit dabei sein!

Herzlichst

Gerda Morlock

Gerda Morlock
cbs@camag.com

Dear friends

The International Symposium for High-Performance Thin-Layer Chromatography, Basel, 06–08 July 2011 (HPTLC 2011) began with a unique arrangement and presentation of the famous opening of the Basel carnival, Basler Morgenstraich. The more than 310 participants were surely awake and ready to listen to the latest information about HPTLC from all around the world. A truly international flavour was evident as scientists from about 40 countries were attracted by this congress. In the final program 50 oral lectures and about 160 posters were presented (see www.hptlc.com).

The interest of the younger generation was very encouraging, as 22 % of the participants were students. The attendees were about equally distributed between academia and industry, which underlines the practical importance of HPTLC today.

The meeting gave ample opportunity to exchange knowledge and to arrange or extend research cooperation. All in all, the scientific level of the symposium was greatly appreciated, due in large part to excellent presentations by young speakers as well as the introduction of interesting new research fields that utilized planar chromatography.

Numerous requests were made for arranging the next International HPTLC Symposium, assumedly in 2013. Various locations in Europe as well as in Asia were suggested, all of them appearing highly attractive. Hope to see you there, wherever "there" turns out to be.

Sincerely,

Gerda Morlock

Gerda Morlock
cbs@camag.com

CAMAG SEPTEMBER 2011 107

THE CBS CLASSIFICATION SYSTEM

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

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

1. Reviews and books

107 001 S.C. CHENG*, M.Z. HUANG, J. SHIEA (*Inst. of Forensic Med., Ministry of Justice, Taipei, Taiwan): Thin layer chromatography/mass spectrometry. J. Chromatogr. A 1218 (19), 2700-2711 (2011). A review on TLC coupled with mass spectrometry (MS) for direct identification and structural characterization of the analytes on TLC plates through an interface. According to differences in their operational processes of the TLC/MS techniques reported in the literature the existing TLC/MS systems can be classified into two categories: 1) indirect mass spectrometric analyses, performed by scraping, extracting, purifying, and concentrating the analyte from the TLC plate and then directing it into the mass spectrometer‘s ion source for further analysis; 2) direct mass spectrometric analyses, where the analyte on the TLC plate is characterized directly through mass spectrometry without the need for scraping, extraction, or concentration processes. Direct MS is conventionally performed under vacuum, but the development of ambient mass spectrometry has allowed analytes on TLC plates to be characterized under atmospheric pressure. Thus, TLC/MS techniques can also be classified into two other categories according to the working environment of the ion source: vacuum-based TLC/MS or ambient TLC/MS.

quantitative analysis, qualitative identification, review 1, 4e

107 002 Irena CHOMA*, Edyta GRZELAK (*Dep. of Chromatogr. Methods, Univ. of Maria Curie - Sklodowska, M. Sklodowska Sq. 3, 20-031 Lublin, Poland): Bioautography detection in thin-layer chromatography. J. of Chromatogr. A 1218 (19), 2684-2691 (2011). Review on TLC/bioautography. Discussion of three versions of bioautography, i.e. contact, immersion and direct bioautography. The focus is put on direct bioautography and many applications are quoted, not only for testing various groups of compounds, but also for investigating biochemical processes and factors influencing bacterial growth. Various related methods can be included into direct bioautography, of which TLC-bioluminescence screening is the most promising one.

HPTLC, review, autoradiography, qualitative identification, quantitative analysis, postchromatographic derivatization 1, 3e

107 003 T.H. DZIDO*, P.W. PLOCHARZ, A. CHOMICKI, Aneta HALKA-GRYSINSKA, Beata POLAK (*Dep. of Phys. Chem., Med. Univ. of Lublin, Chodzki 4a, 20-093 Lublin, Poland): Pressurized planar electrochromatography. J. Chromatogr. A 1218 (19), 2636-2647 (2011). Presentation of theoretical backgrounds, development, examples of separations, constructional details and principle of action of devices of pressurized planar electrochromatography (PPEC). Description of the development mode in respect of operating variables (composition of the mobile phase, pressure exerted on adsorbent layer, mobile phase flow velocity, temperature of separating system, etc.) influencing separation efficiency (kinetic performance, repeatability, separation time), and the advantages of PPEC such as high kinetic performance, short separation time and different separation selectivities, especially relative to conventional TLC, and its challenge as well.

review 1, 3d

107 004 K. FERENCZI-FODOR*, Z. VÉGH, B. RENGER (*Gedeon Richter Plc., P.O.B. 27, H-1475 Budapest, Hungary): Impurity profiling of pharmaceuticals by thin-layer chromatography. J. Chromatogr. A 1218 (19), 2722-2731 (2011). Review on the features of TLC in the different areas of pharmaceutical analysis, like in-process and intermediate control, illustrated by impurity testing of active ingredients and final products, as well as its application in pharmaceutical research and development. Based on examples reported in the last five years it is shown that TLC is still a very popular and frequently used analytical method in the pharmaceutical industry, although there is a

tendency in current pharmacopoeias for favouring HPLC.

pharmaceutical research, comparison of methods, review, HPTLC 1

107 005 B. FRIED*, J. SHERMA (*Lafayette College, Department of Chemistry, Easton PA 18042-1782, USA): Thin layer chromatography in helminthology: a review. Revista Iberica de Parasitologia 65 (1-4), 21-36 (2005). Review on the TLC literature in helminthology from 1996 to 2004. Principles and practices of modern TLC for the analysis of lipids, amino acids, carbohydrates and pigments in helminths such as various species of trematodes, cestodes and nematodes are described.

pharmaceutical research, HPTLC, qualitative identification, quantitative analysis, preparative TLC, comparison of methods, postchromatographic derivatization, densitometry, review 1

107 006 Beate FUCHS, Rosmarie SUESS, Kristin TEUBER, Mandy EIBISCH, J. SCHILLER (*Univ. of Leipzig, Med. Dep., Inst. of Med. Phys. & Biophys., Härtelstr. 16/18, 04107 Leipzig, Germany): Lipid analysis by thin-layer chromatography - A review of the current state. J. Chromatogr. A 1218 (19), 2754-2774 (2011). HPTLC for lipid analysis is particularly useful for smaller, apolar compounds and offers some advantages over HPLC. Description of stationary phases, solvent systems and detection methods for the individual lipid classes (cholesterol and its derivates, glycerides, sphingo- and glycolipids, phospholipids). In comparison with common staining methods the combination of HPTLC and mass spectrometric detection methods is a very powerful method to investigate the identities of the HPTLC zones in detail.

HPTLC, review, qualitative identification, quantitative analysis, comparison of methods 1, 11

107 007 E. KAALE*, P. RISHA, T. LAYLOFF (*Muhimbili Univ. of Health and Allied Sciences, Dar es Salaam, Tanzania): TLC for pharmaceutical analysis in resource limited countries. J. Chromatogr. A 1218 (19), 2732-2736 (2011). A review on the sustainability and robust advantages of TLC and the parameters which are critical to the successful performance of product quality assessments in resource limited areas including field applications. The training required for successful performance of HPTLC assessments is much lower than that of other technologies with comparable reproducibility such as HPLC, because of the robustness and ease of use for HPTLC. Presentation of some of the successful applications of planar chromatography in resource limited countries. In practice in finished pharmaceutical products there are generally few active ingredients which are assessed making the HPTLC adequate for these analyses.

pharmaceutical research, quality control, HPTLC, quantitative analysis, qualitative identification, comparison of methods, review 1

107 008 A. MARSTON (Chem. Dep., Univ. of the Free State, Bloemfontein 9300, South Africa): Thin-layer chromatography with biological detection in phytochemistry. J. of Chromatogr. A 1218 (19), 2676-2683 (2011). A review on bioautography on TLC plates as an important means of detecting the biological activity of a sample. The technique requires only small amounts of sample, is ideal for the investigation of plant constituents which often occur as complex mixtures, and can be used for the target-directed isolation of these constituents. In contrast to HPLC, many samples can be run at the same time on TLC, and organic solvents, which cause inactivation of enzymes or death of living organisms, can be completely removed before biological detection. Many bioassays are compatible with TLC and antimicrobial, radical scavenging, antioxidant activities and enzyme inhibition tests can be applied.

quantitative analysis, qualitative identification, review, autoradiography, postchromatographic derivatization, HPTLC 1, 3e

107 009 C. NEUMANN*, R. RAMOTOWSKI, T. GENESSAY (*Forensic Science Program, Eberly College of Science, The Pennsylvania State Univ., 107 Whitmore Lab, Univ. Park, PA 16802, USA): Forensic examination of ink by high-performance thin layer chromatography - The United States Secret Service Digital Ink Library. J. Chromatogr. A 1218 (19), 2793-2811 (2011). A review on the forensic examination of writing ink on documents. The focus in ink analysis is on screening questioned samples and on verifying their compounds in relation to control ink samples. Description of a project designed to develop improved standardization procedures to ensure the best possible reproducibility between analyses run on different HPTLC plates. HPTLC of ink samples (punched from written documents and extracted with tetrahydrofuran - water 4:1) on silica gel with *n*-butanol - ethanol - water 10:2:3 without chamber saturation. Detection by densitometric measurement of absorption intensities of each point of the elution track directly at 31 wavelengths between 200 and 700 nm.

HPTLC, qualitative identification, review, forensic science 1, 35

107 010 Salwa POOLE*, C.F. POOLE (*Detroit District Lab., US Food and Drug Admin., 300 River Place, Suite 5900, Detroit, MI 48207, USA): High performance stationary phases for planar chromatography. J. of Chromatogr. A 1218 (19), 2648-2660 (2011). Review on the kinetic performance of stabilized particle layers, particle membranes, and thin films for TLC. Forced flow and pressurized planar electrochromatography is best suited to overcome the limited performance achieved by capillary flow for stabilized particle layers. For conventional and high performance plates band broadening is dominated by molecular diffusion at low mobile phase velocities typical of capillary flow systems and by mass transfer with a significant contribution from flow anisotropy at higher flow rates typical of forced flow systems. There are few possible changes to the structure of stabilized particle layers that would significantly improve their performance for capillary flow systems while for forced flow a number of avenues for further study. New media for ultra TLC shows possibilities for miniaturized high performance systems but the realization of their true performance requires improvements in instrumentation for sample application and detection.

review, HPTLC 1, 3

107 011 J. SHERMA*, B. FRIED (*Lafayette College, Department of Chemistry, Easton PA 18042-1782, USA): Studies of echinostomes using chromatography and atomic spectrometry. B. Fried, R. Toledo (Eds): The biology of echinostomes. Springer 2009. Chapter 10. Review on TLC and HPTLC for qualitative and quantitative determination of lipids, amino acids, carbohydrates and pigments in echinostomes. the goal of this work was to better understand the chemical composition of larval and adult echinostomes and of the host tissues infected by these digeneans.

pharmaceutical research, HPTLC, densitometry, preparative TLC, quantitative analysis, qualitative identification, review 1b

107 014 CH. TISTAERT*, Bieke DEJAEGHER, Y. VANDER HEYDEN, (*Department of Analytical Chemistry and Pharmaceutical Technology, Center for Pharmaceutical Research (CePhaR), Vrije Universiteit Brussel-VUB, FABI, Laarbeeklaan 103, 1090 Brussels, Belgium): Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. Anal. Chim. Acta 690 (2), 148-161 (2011). Chromatographic fingerprinting has been generally accepted as

analytical method for the quality control of herbal medicines. This review describes the evolution of the regulations and guidelines on the quality control of herbal medicines, and reviews the established analytical techniques in TLC, HPLC, UHPLC, hydrophilic interaction chromatography, and GC. Emphasis is put on the most recent developments, such as miniaturized techniques, new stationary phases, analysis at high temperatures and multi-dimensional chromatography. The new chemometric data handling techniques are discussed.

pharmaceutical research, traditional medicine, quality control, herbal, HPTLC, quantitative analysis, qualitative identification, review 1, 32e

107 015 J.D. VASTA, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton PA 18042-1782, USA): Effects of estivation on selected metabolites in pulmonate snails as determined by chromatography. Trends in Chromatography 6, 1-10 (2010). Review on TLC, HPTLC and other chromatographic methods for the analys of selected metabolite classes such as neutral and polar lipids, amino acids, carbohydrates, carboxylic acids, lipophilic pigments, and purine bases in estivating pulmonate snails.

pharmaceutical research, review, quantitative analysis, qualitative identification, HPTLC 1

2. Fundamentals, theory and general

107 016 L. KOMSTA (Med. Univ. of Lublin, Chair and Dep. of Med. Chem., Faculty of Pharmacy, Jaczewskiego 4, 20-090 Lublin, Poland): A new general equation for retention modeling from the organic modifier content of the mobile phase. Acta Chromatographica 22 (2), 267-279 (2010), DOI:10.1556/AChrom.22.2010.2.9. Presentation of a general equation for modeling retention, using the organic modifier content of the mobile phase, which is based on the Box-Cox transform of modifier concentration. Both the semilogarithmic relationship (Soczewinski-Wachtmeister equation) and logarithmic relationship (Snyder-Soczewinski equation) are found to be special cases of the proposed equation. The equation can be fitted easily with free software and an additional coefficient can be interpreted as closeness to the previous models. The equation enables extrapolation to zero modifier content even with strong closeness to log-log dependence. Discussion of a case study on nine drug-like substances, with comparison of 14 previously proposed retention equations found in the literature.

pharmaceutical research 2

107 017 L. KOMSTA (Medical University of Lublin, Department of Medicinal Chemistry, Jaczewskiego 4, 20-090 Lublin, Poland): Extending equal-spreading criteria to two-dimensional thin-layer chromatography - the points in a unit square problem revisited. Acta Chromatographica 20(3), 309-327 (2008). Presentation and discussion of an approach based on the distances to the closest spot and to the top or bottom of the plate in the optimization of two-dimensional thin-layer chromatographic separation. This is different to the method of relying on selection of the two most orthogonal chromatographic systems which best co-operate in the separation, which is mainly achieved by investigating the correlation between hR_F values or scoring the distances between the spots. The theory arises from a well-known geometrical problem about equal-spreading of the points inside a unit square, proposing two coefficients, sensitive and insensitive, to complete separation. This is the two-dimensional version of the previously proposed criteria retention uniformity and retention distance, which describes the equal-spreading of the spots in one-dimensional chromatography. The coefficients range from 0 to 1 and their distribution as a random variable is well defined and not affected by the number of separated compounds.

pharmaceutical research 2

107 018 L KOMSTA*, K. SZEWCZYK (*Med. Univ. of Lublin, Chair and Dep. of Med. Chem., Fac. of Pharm., Jaczewskiego 4, 20-090 Lublin, Poland): The kernel density estimate as a measure of the performance of one and two-dimensional TLC systems with large retention datasets in the context of their use in fingerprinting. Acta Chromatographica 21(1), (2009). Introduction of a new objective chromatographic response function RK, based on the kernel density estimation, for evaluation of the fingerprinting performance of a particular TLC system (uniformity of retention) for which a large set of experimental hR_F values of possible components of the mixture is available. The RK criterion is insensitive to large numbers (hundreds or thousands) of hR_F values, can be applied to one and two-dimensional TLC and is easily computed.

pharmaceutical research, quality control, traditional medicine, quantitative analysis 2b

107 019 L. KOMSTA*, R. SKIBINSKI, A. GUMIENICZEK, A. WOJNAR (*Med. Univ. of Lublin, Chair and Dep. of Med. Chem. Faculty of Pharm., Jaczewskiego 4, 20-090 Lublin, Poland): Multi-way analysis of retention of model compounds in thin-layer chromatography. Acta Chromatographica 22(1), 27-36 (2010). Investigation of the TLC retention of 35 model compounds with ten screening mobile phases on six normal-phase and seven reversed-phase adsorbents. The retention factors formed two cubes with dimensions 35x10x6 and 35x10x7, respectively, which enabled three-way analysis by PARAFAC, having a one-component PARAFAC model as the optimum in both cases and two-component models performed worse. The one-component model explained 78.8 % of the variance in NP-TLC and 94.2 % of the variance in RP-TLC. The major variability of the retention factor can be modelled as the product of three factors related to the substance itself, the mobile phase, and the adsorbent. Rf modelling was substantially better than using k or RM (rate mobility) values.

pharmaceutical research 2

107 020 A. PETRUCZYNIK*, K. SLIWKA, M. WAKSMUNDZKA-HAJNOS (*Med. Univ., Dep. of Inorg. Chem., 20-081 Lublin, Poland): Effect of the vapour phase on the separation of isoquinoline alkaloids by thin-layer chromatography. Acta Chromatographica 22 (3), 391-404 (2010). Examination of the effect of conditioning of the silica layer by mobile phase vapor, diethylamine vapor and its aqueous and methanolic solutions, and ammonia vapor on the retention of alkaloids eluted with multicomponent non-aqueous mobile phases. Investigation of the effect of conditioning time and vapor phase composition on system efficiency and peak symmetry, and as well the effect of vapor phase composition on separation selectivity.

pharmaceutical research, clinical chemistry research, quantitative analysis, qualitative identification, densitometry 2

107 021 B. RENGER*, Z. VÉGH, K. FERENCZI-FODOR (*Bernd Renger Consulting, Fritz-Reichle-Ring 2, 78315 Radolfzell, Germany): Validation of thin-layer and high-performance thin-layer chromatographic methods. J. Chromatogr. A 1218 (19), 2712-2721 (2011). Presentation of a guidance on how to adopt international accepted formal requirements and guidelines for validation of different TLC/HPTLC procedures. Analytical validation is a key requirement to asses and to prove a method‘s reliability and suitability for intended different applications, ranging from simple screening tests to sophisticated instrumental quantitative assays of analytes in complex matrices. In addition description of selected parameters for robustness testing and for on-going quality assurance of analytical performance based on control charts.

HPTLC 2f

107 022 S. SEGAN, F. ANDRIC, A. RADOICIC, D. OPSENICA, B. SOLAJA, M. ZLATOVIC, D. MILOJKOVIC-OPSENICA* (*Institute of Chemistry, Technology, and Metallurgy, University of Belgrade, 11158 Belgrade, Serbia, dusankam@chem.bg.ac.rs): Correlation between structure, retention and activity of cholic acid derived cis-trans isomeric bis-steroidal tetraoxanes. J. Sep. Sci. 34, 1-9 (2011). Quantitative structure-retention (QSRR) and quantitative structure-activity relationship (QSAR) studies were performed to correlate the molecular characteristics of seven pairs of cis-trans isomeric bis-steroidal tetraoxanes with their reversed-phase thin-layer chromatography retention and their antiproliferative activity. TLC on 1) RP-18 with mobile phases of 0-14 vol % water in methanol (increment 2 %), 10-30 vol % water in acetone (increment 5 %) and 10-35 vol% water in dioxane (increment 5 %) and on 2) cyano phase with mobile phases of 10-30 vol% water in methanol (increment 5 %), 10-40 vol% water in acetone (increment 5 %). Detection by spraying with sulfuric acid 50 %, followed by heating. In all instances, it was found that the retention of the investigated compounds decreased with increasing concentrations of the organic modifier in the mobile phase.

pharmaceutical research, quantitative analysis 2c

107 023 T. SLAWIK*, R. SKIBINSKI, B. PAW, G. DZIALO (*Med. Univ. of Lublin, Dep. of Med. Chem., Pharm. Faculty, Jaczewskiego 4, 20-090 Lublin, Poland): Reversed-phase TLC study of the lipophilicity of some 3-hydroxy-1,2-benzisoxazoles substituted in the benzene ring. Acta Chromatographica 21(2), 251-258 (2009). Study of the relative lipophilicity, RM0, and specific hydrophobic surface area of eleven 3-hydroxy-1,2-benzoisoxazoles substituted in the benzene ring (two isomeric fluoro, three isomeric chloro, three isomeric bromo and dibromo derivatives, and a nitro derivative) by TLC on RP-18 with methanol - water mixtures. Comparison of lipophilicity RM0 with computed partition coefficients IAlogP, A logPs , clogP, milogP, logPKOWIN , and xlogP, and the best correlation (r> 0.9) was found between RM0 and logPKOWIN and xlogP values. Comparison of RM0 values with computed partition coefficients by principal-components analysis and comparison of the chromatographic behavior of 3-hydroxy-1,2-benzisoxazoles with that of their bioisosteric analogues 1,2-benzisothiazolonoles. It was found that the experimental RM0 values for both groups of compounds were in accordance with the equation RM0 = aRM0 + b (r>0.9).

pharmaceutical research 2

107 024 A. ZIEBA*, W. PRUS (The Med. Univ. of Silesia, Dep. of Org. Chem., ul. Jagiellonska 4, 41-200 Sosnowiec, Poland): Determination of the lipophilicity of new azaphenothiazines by reversed-phase thin-layer chromatography. Acta Chromatographica 21(3), 369-378 (2009). Determination of the lipophilicity RM0 and log PTLC of thirteen novel, potentially biologically active, 12 H-quino[3,4- b] [1,4] benzothiazinium salts by TLC on RP-18 with methanol - aqueous Tris buffer mixtures. RM values were linearly dependent on methanol concentration, and extrapolation of these to 0 % methanol gave the lipophilicity RM0. log PTLC was obtained from RM0 by use of a calibration curve obtained for five standards of known experimental lipophilicity (log P). The lipophilicity log Pcalcd was calculated for the thirteen quinobenzothiazines by use of nine software products. The chromatographic lipophilicity RM0 can be used as a measure of the lipophilicity of the azaphenothiazine derivatives investigated.

pharmaceutical research 2

3. General techniques

107 025 R. AKKAD*, W. SCHWACK (*Inst. of Food Chem., Univ. of Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Germany): Effect of bromine oxidation on high-performance thin-layer chro-

matography multi-enzyme inhibition assay detection of organophosphates and carbamate insecticides. J. Chromatogr. A 1218 (19), 2775-2784 (2011). A multi-enzyme inhibition assay (HPTLC-EI) based on rabbit-liver esterase (RLE) and cutinase following HPTLC allows detection of thiophosphate pesticides. Because choline esterase inhibition is more effective after conversion of thiophosphate thions into their corresponding oxons, a pre-oxidation step was added to the HPTLC-EI assay by using bromine vapor. Bromine was more effective than iodine or UV irradiation for oxidation. It increased the inhibitory strength of parathion, parathion-methyl, chlorpyrifos, chlorpyrifos-methyl, and malathion by 2 orders of magnitude. In contrast, bromine oxidation of organophosphate and carbamate insecticides resulted in a slight reduction in their inhibition factors, due to partial bromination and degradation of the parent compounds. Bromine oxidation increased the inhibition factors for demeton-S-methyl and propoxur. The HPTLC-EI system was applied to the analysis of apple juice and water samples spiked with paraoxon (0.001 mg/L), parathion (0.05 mg/L), and chlorpyrifos (0.5 mg/L) and the mean recoveries were 95-106 % and 91- 102 % for RLE and cutinase, respectively.

agricultural, HPTLC, postchromatographic derivatization, effect-directed analysis 3e, 29

107 026 Vera BAUMGARTNER*, CH. HOHL, W. SCHWACK (*State Laboratory Basel-City, Basel, Switzerland): Rolling - A new application technique for luminescent bacteria on high-performance thin-layer chromatography plates. J. Chromatogr. A 1218 (19), 2692-2699 (2011). HPTLC coupled with bioluminescence detection can be used for screening for unknown substances. So far the HPTLC plate was dipped in an aqueous solution of *Vibrio fischeri* bacteria. However polar substances may be dissolved during this process, which leads to blurring and tailing of the zones on the plate. This was overcome by application of the bacteria solution by rolling. A rolling device was made of commercially available household articles and tested using octhilinone and methylparaben. Comparison of rolling with dipping showed that despite the manual steps involved in the rolling process, the results were reproducible. Depending on the substance and its amount on the HPTLC plate, with rolling peaks were narrower, up to a factor of 4 higher and showed a higher signal-to-noise ratio than with dipping.

HPTLC, comparison of methods, biodetection 3c

107 002 Irena CHOMA et al., see section 1

107 003 T.H. DZIDO et al., see section 1

07 027 L. KOMSTA (Med. Univ. of Lublin, Dep. of Med. Chem., Faculty of Pharm., Jaczewskiego 4, 20-090 Lublin, Poland): Dealing with charged-coupled device noise in thin-layer videodensitometry. Optimization of several image-denoising techniques. Acta Chromatographica 21(3), 355-367 (2009). Different techiques for videoscan denoising are presented. Due to the charged-coupled devices (CCD) noise can be a serious problem during videoscanning, especially when scanning dark plates with weakly fluorescent spots. Optimization of several kind of filters (averaging, circular, Gaussian, Savitzky-Golay, median, Wiener, FIR) and wavelet shrinkage (twelve mother wavelets from the Daubechies, Symmlet, and Coiflet family, five decomposition levels, and soft/hard thresholding) against noise autocorrelation or mean-squared error to the reference image obtained by grabbing and averaging 256 CCD frames. The median filter provided the best results. The other filters except Gaussian and wavelet shrinkage at high decomposition level were also sufficient. The Gaussian filter and wavelet shrinkage at low decomposition level could not be recommended.

quantitative analysis, qualitative identification 3f

107 008 A. MARSTON, see section 1

107 028 A.J. OKO*, S.R. JIM*, M.T. TASCHUK, M.J. BRETT (*Univ. of Alberta, Dep. of ECE, 2nd Floor ECERF, Edmonton, AB, Canada T6G 2V4): Analyte migration in anisotropic nanostructured ultrathin-layer chromatography media. J. Chromatogr. A 1218 (19), 2661-2667 (2011). Investigation of the performance of highly anisotropic nanostructured thin film ultrathin-layer chromatography (UTLC) media with porosity and architecture engineered using the glancing-angle deposition (GLAD) process. The anisotropic structures resemble nanoblades, producing channel-like features that partially decouple analyte migration from development direction, offering new separation behaviours. Study on GLAD-UTLC plate performance in terms of migration distance, plate number, retention factor and a figure of merit specific to GLAD-UTLC, track deviation angle, showing that migration distances increase with porosity by a factor of two for all feature orientations (up to a maximum of 22 mm) over the range of porosities considered in this study. Plate numbers approaching 1100 are observed for GLAD-UTLC plates when the nanoblade features are aligned with the development direction. The theoretical model describing mobile phase flow in anisotropic GLAD-UTLC media was in good agreement with experimental results. The plates provide channel features that reduce transverse spot broadening while providing the wide pores required for rapid migration and high separation performance, which may enable a greater number of parallel separations on miniaturized GLAD-UTLC plate formats. The small sizes should also make them compatible with the office chromatography concept in which office peripherals (inkjet printers and flatbed scanners) replace conventional TLC instruments.

quantitative analysis, qualitative identification 3

107 010 Salwa POOLE et al., see section 1

107 029 P. SAMTEN, P. WETWITAYAKLUNG, N. KITCHAROEN, U. SOTANAPHUN* (*Silpakorn Univ. Dep. of Pharmacognosy, Nakhon-pathom 73000, Thailand): TLC image analysis for determination of the piperine content of the traditional medicinal preparations of Bhutan. Acta Chromatographica 22 (2), 227-236 (2010). TLC of piperine, the bioactive constituent of black pepper (*Piper nigrum*), on silica gel with dichloromethane - ethyl acetate 9:1 at 30 °C in a twin-trough chamber saturated for 30 min. Detection under UV light at 254 nm and documentation with a digital camera. Based on the image a density profile plot was established by Scion Image software, which allowed to calculate the concentration of piperine by comparison of the peak areas of samples and piperine standards. The linearity was in the range of 24-84 ng/zone (r^2=0.9927). The limits of detection and quantitation were 0.35 and 1.05 ng/zone, respectively. Precision (repeatability, n=6) and intermediate precision (2 days, n=12) both are below 2.6 *%RSD*. Recovery is between 96.7-101.4 %.

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

107 030 P.K. ZARZYCKI*, Magdalena M. SLACZKA, Magdalena B. ZARZYCKA, Elzbieta WLODARCZYK, M.J. BARAN (*Section of Toxicology and Bioanalytics, Department of Civil and Environmental Engineering, Koszalin University of Technology, Sniadeckich 2, 75-453 Koszalin, Poland): Application of micro-thin-layer chromatography as a simple fractionation tool for fast screening of raw extracts derived from complex biological, pharmaceutical and environmental samples. Anal. Chim. Acta 688 (2), 168-174 (2011). Demonstration of the separation and detection capability of micro-TLC technique involving simple one step liquid extraction of complex materials without need for multi-step sample preparation. Isolation of the target components

(cyanobacteria pigments, lipids and fullerenes) from complex matrices including spirulina dried cells, birds‘ feathers and fatty oils as well as soot samples derived from biomass fuel and fossils-fired home heating systems. The isocratic separation protocol required less than 1 mL of one component or binary mobile phases. Development was achieved within 5-8 min. Detection by exposure to iodine vapors or by spraying with phosphomolybdic acid reagent.

quality control, environmental, agricultural, toxicology, quantitative analysis, qualitative identification 3

4. Special techniques

107 031 F. BRETIN, F. MAQUIN* (*Sanofi-Aventis, Centre de Recherche, 13 quii Jules Guesde, 94403 Vitry-sur-Seine, France, francis-maquin@sanofi-aventis.com): TLC/HPTLC-ELSD-MS coupling. CBS 105, 2-4 (2010). TLC and HPTLC of reaction samples from small molecule lead development, on silica gel with mixtures of methanol and dichloromethane/ethyl acetate or ethyl acetate and heptane/cyclohexane (ratios depending on the compound mixtures). Detection with primuline or berberine reagent. Direct elution into the MS with the TLC-MS interface. Substances not detected by DAD can successfully be measured by ELSD detection coupled to TLC.

pharmaceutical research, HPTLC, quantitative analysis 4e

107 001 S.C. CHENG et al., see section 1

107 032 D. GONSALVES, R. COUTO, E. CONCEISAO, N. REIS, E. GIL* (*Faculty of Pharmacy, Goias Federal University, Goiania, Brazil, ericsgil@gmail.com) : Solid state differential pulse voltammetry (DPV) from spots of thin-layer chromatography (TLC): a new method for analysis of antioxidant phytoactives. Quim. Nova. 34, 330-334 (2011). TLC of rosmarinic acid in preparations of Rosmarinus officinalis on silica gel with acetone - formic acid - methylene chloride 50:17:170. Detection under UV 366 nm. Quantitative determination by solid state differential pulse voltammetry (DPV). Linearity was between 0.694x10-3 to 0.526x10-3 mol/L. The limits of detection and quantification were 1.2x10-5 and 3.6x10-5 mol/L, respectively. The intermediate/interday/intraday precisions were 3.03 % and 2.2 %, respectively. Recovery (by standard addition) was 96.3 % for rosmarinic acid. The method presented high recovery levels compared to an HPLC method.

quality control, herbal, HPTLC, quantitative analysis, comparison of methods 4e, 11a

5. Hydrocarbons and halogen derivatives

107 033 S. GRASHORN, L. SCHUELE, Gerda MORLOCK* (*University of Hohenheim, Institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany, gerda.morlock@uni-hohenheim.de): HPLC-MS or simply HPTLC for analysis of sucralose in water? CBS 106, 7-10 (2011). HPTLC of sucralose on silica gel (pre-washed by development with methanol, followed by drying at 100 °C for 15 min) with isopropyl acetate - methanol - water 15:3:1 up to 60 mm (migration time 15 min). Detection by dipping in aniline diphenylamine o-phosphoric acid reagent followed by heating at 120 °C for 20 min, evaluation under white light and UV 366 nm. Quantitative determination by absorbance measurement at 400 nm. Via the TLC-MS Interface the respective zones were eluted and transferred into a single-quadrupole mass spectrometer. Electrospray ionization mass spectra were recorded in full scan mode. The recovery of sucralose in drinking water was 84 ± 7 % (n=3). The limit of detection was 6 ng/band. The calibration curve (10-300 ng/band, r=0.9999, 1.3 *%RSD*) was suited to analyze sucralose at concentrations of 0.1-5 µg/L.

environmental, agricultural, HPTLC, densitometry, quantitative analysis 5c

7. Phenols

107 034 K. MUKHERJEE, M. VENTKATESH, B. SAHA, P. MUKHERJEE* (*School of Natural Product Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032, India, naturalproductm@gmail.com): Effect of soy phosphatidyl choline on the bioavailability and nutritional health benefits of resveratrol. Food Research International 44, 1088-1093 (2011). HPTLC of resveratrol (1) and the resveratrol complex with hydrogenated soy phosphatidyl choline (2) on silica gel with dichloromethane - methanol 4:1. The hR_F values of (1) and (2) were 87 and 92, respectively.

food analysis, quality control, HPTLC, qualitative identification 7

107 035 M. SZAUFER-HAJDRYCH*, W. BYLKA, I. MATLAWSKA, M. WÓJCIAK-KOSIOR, G. MATYSIK, J. JODYNIS-LIEBERT (*Poznan University of Medical Sciences, Department of Pharmacognosy, Swiecickiego 4, 61-771 Poznan, Poland): Densitometric HPTLC and HPLC analysis of phenolic acids from Aquilegia vulgaris. Acta Chromatographica 20(4), 685-695 (2008). Determination of p-coumaric and protocatechuic acids in an ether fraction from a methanolic extract of Aquilegia vulgaris L. by HPTLC on silica gel with mixtures of heptane, dichloromethane, diisopropyl ether, formic acid, and water in various ratios. Satisfactory separation of the phenolic acids was achieved by use of the multiple gradient development technique. HPTLC results of the quantities of p-coumaric and protocatechuic acids were somewhat higher (0.396 and 2.584 mg/g dry plant material, respectively), than those determined by HPLC (0.374 and 2.283 mg/g dry plant material, respectively). Both methods were satisfactory in the precision, expressed as relative standard deviation, and are useful for quality control of Aquilegia vulgaris extracts.

pharmaceutical research, herbal, traditional medicine, quality control, HPTLC, quantitative analysis, qualitative identification,comparison of methods 7

8. Substances containing heterocyclic oxygen

107 036 G. CHAKRABORTHY*, P. GHORPADE (*SVKM'S, NMIMS University, School of Pharmacy & Technology Management, Shirpur Campus, Dist Dhulia, Shirpur, Maharashtra, 425405, India, phdgs77@indiatimes.com): Determination of quercetin by HPTLC in Calendula officinalis extract. International Journal of Pharma and Bio Sciences 1(1), 2-4 (2010). TLC of methanol 50 % extracts of cut dried flowers of Calendula officinalis on silica gel with chloroform - methanol 19:1. The hR_F value of quercetin was 43. Quantitative determination by densitometry at 366 nm. The method was linear in the range of 1-5 µg/band. The identity of quercetin in the sample was confirmed by comparing hR_F values and UV spectra of sample and standard.

quality control, herbal, densitometry, quantitative analysis 8b

107 037 M. DASZYKOWSKI*, M. HAWRYL, M. WAKSMUNDZKA-HAJNOS, B. WALCZAK (*Silesian University Department of Chemometrics, Institute of Chemistry, 9 Szkolna Street, 40-006 Katowice, Poland): Identification of similar and orthogonal chromatographic thin-layer systems for two-dimensional separations of flavonoids and their analogues. Acta Chromatographica 20(3), 283-307 (2008). TLC of twenty flavonoids and their analogues on different stationary phases (non-polar and polar bonded stationary phases, silica gel, amino phase, diol phase) developed with a variety of binary mobile phases (aqueous and non-aqueous). Evaluation of similarities and differences among the chromatographic systems by principal component analysis and hierarchical clustering. Application of scoring indices to the separation power of a given system or a pair of systems allowed selection of the most suitable systems either to perform two-dimensional separations or to enhance the overall resolution by merging two stationary phases. On the basis of the investigation relatively efficient two-dimensional system on amino phase were developed.

TLC with tetrahydrofuran - water 9:1 in the first dimension and acetonitrile - water 9:1, 4:1, 3:1, or 7:3 in the second dimension was found to be suitable for the separation of the compounds. Theoretically the compounds were best separated by combining diol and amino phases and using methanol - water 3:2 and acetonitrile - water 9:1, respectively.

qualitative identification, quantitative analysis 8a

107 038 Monika JADHAO (Dept. of Pharmaceutical, Vidya Bharti College of Pharmacy-Amravati District-Amravati, M.S., India 444602, monikajadha02006@yahoo.co.in): Estimation of andrographolide in herbal powder and polyherbal Asava by HPTLC. International Journal of Pharma and Bio Sciences 1(4), 242-245 (2010). HPTLC of andrographolide in Andrographis paniculata and a polyherbal Asava formulation on silica gel with benzene - ethyl acetate 1:1. The hR_F value of andrographolide was 10. Quantitative determination at 220 nm. The method was linear in the range of 360-660 ng/band. The andrographolide content of the sample of Andrographis paniculata was 237.2 µg/100 mg, where as Asava contained 41.8 µg/5 mL. The average recovery of andrographolide by standard addition method was 97.7 %.

quality control, herbal, densitometry, quantitative analysis 8b

07 039 M. MEHTA*, D. PATEL, K. GINPREET, C. MEENA (*SVKM's NMIMS School of Pharmacy and Technology Management, 400056, India): Simultaneous estimation of curcumin, piperine and quercetin in ayurvedic combinatorial extract by HPTLC and UV visible spectrophotometric method. 62nd Indian Pharmaceutical Congress Abstract No. F-324 (2010). TLC of curcumin, piperine and quercetin in ayurvedic extract on silica gel with chloroform - toluene - ethyl acetate - methanol 4:4:1:1. The results obtained by the chromatographic method were comparable with a UV-VIS photometric method. All three compounds did not show any mutual interference.

traditional medicine, quality control, herbal, densitometry, comparison of methods, quantitative analysis 8b, 32e

107 040 M. PHALE*, Purnima HAMRAPURKAR, Manasi CHACHAD, Priti PATIL, S. PAWAR (*Dept. of Pharmaceutical Analysis, Prin. K. M. Kundani College of Pharmacy, Jote Joy Bldg., Rambhau Salgaonkar Rd., Cuffe Parade, Coloba, Mumbai 400005, India): Precise and sensitive HPTLC method for quantitative estimation of wedelolactone in Eclipta alba Hassk. Pharmacophore 1(2), 103-111 (2010). HPTLC of wedelolactone in powdered dried aerial parts of Eclipta alba Hassk, extracted with methanol, on silica gel with toluene - ethyl acetate - formic acid 50:50:1. Quantitative determination by absorbance measurement at 351 nm.

herbal, densitometry, quantitative analysis 8b

107 041 A. SUNEETHA*, K. KUMAR, M. NAVEENA (*Hindu College of Pharmacy, Amaravati Rd., Guntur, A.P., 522002, India): Densitometric method for the estimation of escitalopram oxalate in bulk and pharmaceutical dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-240 (2010). TLC of escitalopram oxalate on silica gel with n-butanol - acetic acid - water 3:1:1. Quantitative determination by absorbance measurement at 240 nm. The method was linear in the range of 100-600 ng/band.

pharmaceutical research, quality control, densitometry, quantitative analysis 8b

10. Carbohydrates

107 042 Meghan CICCHI, B. FRIED, J. SHERMA* (*Lafayette College, Department of Chemistry, Eas-

ton PA 18042-1782, USA): Effects of estivation on the concentrations of glucose and maltose in two strains of Helisoma trivolvis snails as determined by TLC-densitometry. Acta Universitatis Cibiniensis, Seria F Chemia 12, 41-48 (2009) Analysis of glucose and maltose in the digestive gland-gonad complex and hemolymph of estivated Helisoma trivolvis snails. TLC on silica gel with ethyl acetate - glacial acetic acid - methanol - water 12:3:3:2. Detection with alpha-naphtol - sulfuric acid reagent and quantitative determination by absorbance measurement at 515 nm. A significant decrease of glucose and maltose concentrations was observed after 2-5 days of estivation.

HPTLC 10

107 043 I. UNTERIESER, J. CUERS, K. VOIGES, J. ENEBRO, Petra MISCHNICK* (*Technische Universität Braunschweig, Institut für Lebensmittelchemie, Schleinitzstr. 20, 38106 Braunschweig, Germany, p.mischnick@tu-braunschweig.de): Quantitative aspects in electrospray ionization ion trap and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of malto-oligosaccharides. Rapid Commun. Mass Spectrom. 25, 2201-2208 (2011). HPTLC of an equimolar mixture of malto-oligosaccharides, derivatized with p-aminobenzoic acid, on silica gel with acetonitrile - water - acetic acid 8:2:1. Quantitative determination by fluorescence measurement at 366 nm. The relative molar composition of the oligomers, determined by HPTLC, was used as a reference data for mass spectrometric analyses. For both electrospray ionization and matrix-assisted laser desorption/ionization methods, the instrumental parameters significantly influence the signal intensities and areas.

pharmaceutical research, HPTLC, quantitative analysis, densitometry, comparison of methods 10a

11. Organic acids and lipids

107 044 R. ARORA*, S. JAIN (*Noida Institute of Engineering & Technology Dept. of Pharmaceutical Sciences 19, Knowledge park, Phase 2 Greater Noida, Uttar Pradesh, India, ritu.wadhwa84@gmail.com): Quantification of p-(para)methoxy cinnamic acid ethyl ester (PMCAEE) from Hedychium spicatum by HPTLC. International Journal of Pharma and Bio Sciences 1(3), 1-4 (2010). The presence of p-methoxy cinnamic acid ethyl ester (PMCAEE) in Hedychium spicatum (Zingiberaceae), a spicy annual herb, was confirmed by TLC and other qualitative tests. HPTLC of PMCAEE on silica gel with n-hexane - acetone 4:1. The hR_F value of PMCAEE was 43. Quantitative determination by absorbance measurement at 310 nm. The method was linear in the range of 1-5 µg/band. The alcoholic extract of the plant was found to contain 0.81 % of PMCAEE.

quality control, herbal, densitometry, quantitative analysis 11a

107 045 A. BATTEWAR*, P. SAYL, B. KUCHEKAR, V. CHOUDHARY (*MAEER'S Maharashtra Institute of Pharmacy, Paud Raod, Kothrud, Pune 411038, MS, India): Development and validation of a HPTLC method for simultaneous estimation of thiocolchicoside and aceclofenac in combined dosage form 62nd Indian Pharmaceutical Congress Abstract No. F-247 (2010). TLC of aceclofenac and thiocolchicoside on silica gel with methanol - chloroform - water 48:1:1. The hR_F values were 70 and 83 for thiocolchicoside and aceclofenac, respectively. Quantitative determination by absorbance measurement at 254 nm. The method was linear in the range of 30-180 ng/band for thiocolchicoside and 750-4500 ng/band for aceclofenac. The recovery was in the range of 99.2-100.0 % for both drugs.

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

107 046 S. BOHARUPI*, A. TATED, F. KHAN, A. CHANDEWAR (*Dept. of Pharmaceutical Chemistry, P. Wadhwani College of Pharmacy, Yavatmal 445001, India): Formulation, HPTLC method deve-

lopment and validation of gallic acid in health drinks. 62nd Indian Pharmaceutical Congress Abstract No. F-259 (2010). Health drinks usually contain several phytopharmaceuticals with immunomodulatory and antioxidant activities. TLC of gallic acid on silica gel with toluene - ethyl acetate - methanol - formic acid 15:15:1:4. The gallic acid content was established and the identity of the gallic acid zone in sample and standard was confirmed by UV spectra comparison.

traditional medicine, herbal, densitometry, quantitative analysis 11a

107 047 V.L. CEBOLLA*, Carmen JARNE, Pilar DOMINGO, A. DOMÍNGUEZ, A. DELGADO-CAMÓN, Rosa GARRIGA, J. GALBÁN, L. MEMBRADO, Eva M. GÁLVEZ, F.P. COSSÍO (*Instituto de Carboquímica, Consejo Superior de Investigaciones Cientificas (CSIC), C/Miguel Luesma, 4, 50018 Zaragoza, Spain): Fluorescence detection by intensity changes for high-performance thin-layer chromatography separation of lipids using automated multiple development. J. of Chromatogr. A 1218 (19), 2668-2675 (2011). Use of the changes in emission of berberine cation, induced by non-covalent interactions with lipids on silica gel for detection and quantification of lipids using fluorescence densitometry in HPTLC/AMD. Three different HPTLC/AMD gradients were developed for the separation of 1) neutral lipid families and steryl glycosides, 2) different sphingolipids, and 3) sphingosine-sphinganine mixtures. Rationalization of fluorescent molar responses of studied lipids, and differences in response among different lipid families in the light of a previously proposed model of FDIC response, which is based on ion-induced dipole interactions between the fluorophore and the analyte, likewise, application of computational calculations using molecular mechanics as a complementary useful tool to explain high FDIC responses of cholesteryl and steryl-derivatives, and moderate responses of sphingolipids. Proposal of an explanation for the high FDIC response of cholesterol, whose limit of detection is 5 ng.

HPTLC, densitometry, AMD, qualitative identification, quantitative analysis 11

107 048 Jessica COUNIHAN, B. FRIED*, J. SHERMA (*Department of Biology, Lafayette College, Easton PA 18042-1782, USA): Effects of Echinostoma caproni infection on the neutral and polar lipids of intestinal and non-intestinal organs in the BALB/c mouse as determined by HPTLC. Parasitol. Res. 107, 947-953 (2010). HPTLC of neutral lipids on silica gel (prewashed by development with dichloromethane - methanol 1:1 and dried for 30 min at 120 °C) with petroleum ether - diethyl ether - glacial acetic acid 80:20:1, detection by spraying with 5 % ethanolic phosphomolybdic acid reagent and heating at 115 °C for 10 min. HPTLC of polar lipids (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin) with chloroform - methanol - deionized water 65:25:4, detection by spraying with 10 % cupric sulfate in 8 % phosphoric acid and heating at 140 °C for 30 min.

pharmaceutical research, HPTLC, quantitative analysis, qualitative identification, densitometry 11

107 049 M. DESHPANDE*, S. CHAUDHRI, V. KASTURE (*Amrutvahini College of Pharmacy, Sangamner, Dist. Ahemadnagar, MS., India): HPTLC determination of cefixime and ambroxol in human plasma by liquid-liquid extraction. 62nd Indian Pharmaceutical Congress Abstract No. F-239 (2010). TLC of cefixime and ambroxol (extracted from human plasma with acetonitrile - methanol 3:1, centrifuged and dried at 40 °C, then dissolved in methanol) on silica gel with acetonitrile - methanol - triethylamine 41:5:4. The hR_F values were 27 and 54 for cefixime and ambroxol. Quantitative determination by absorbance measurement at 254 nm. The recovery from plasma was in the range of 69.5-74.4 % for ambroxol and 83.5-87.9 % for cefixime.

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

107 006 Beate FUCHS et al., see section 1

107 032 D. GONSALVES et al., see section 4

107 050 Kiran KAMBLE*, P. KULKARNI, L. SATHIYANARAYANAN, K. MAHADIK (*Dept. of Q. A. Technique, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune 411038, India): Simultaneous HPTLC-densitometric analysis of alizarin and betulinic acid in polyherbal formulation. 62nd Indian Pharmaceutical Congress Abstract No. F-252 (2010). TLC of alizarin and betulinic acid on silica gel with toluene - ethyl acetate - formic acid 18:3:1. The hR_F values were 53 and 58 for betulinic acid and alizarin, respectively. Quantitative determination by absorbance measurement at 287 nm. The method was linear in the range of 60-160 ng/band for alizarin and 300-800 ng/band for betulinic acid. The average recovery was in the range of 99.4-99.6 % for both compounds.

herbal, densitometry, quantitative analysis 11a

107 051 J. KUMAR, V. KUMAR* (*Neuropharmacology Research Laboratory, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Uttar Pradesh, India, vikas.phe@itbhu.ac.in): Acute and sub-chronic toxicity study of standardized extract of *Fumaria indica* in rodents. J. Ethnopharmacol. 134, 992-995 (2011). HPTLC of fumaric acid and fumaric acid conjugates (as dimethyl fumarate) in the aerial parts of *Fumaria indica* on silica gel with formic acid - chloroform - butanol - heptane 3:4:8:11. Quantitative determination by absorbance measurement at 260 nm.

quality control, traditional medicine, HPTLC, densitometry, quantitative analysis 11a

107 052 K. LADANI*, K. DESAI, M. PATEL, U. CHHALOTIYA, C. NAGDA (*Indukaka Ipcowala College of Pharmacy, Beyond GIDC, New V. V. Nagar 388121, Gujarat, India): Development and validation of HPTLC method for ampicillin and dicloxacillin in bulk and their combined pharmaceutical dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-335 (2010). TLC of ampicillin and dicloxacillin on silica gel with *n*-butanol - water - formic acid 63:6:4. Quantitative determination by absorbance measurement at 220 nm. The hR_F values of ampicillin and dicloxacillin were 85 and 69 respectively. The linearity was in the range of 1-6 µg/zone for both ampicillin and dicloxacillin. The recovery for ampicillin was 98.5-101.9 % and that for dicloxacillin was 98.3-101.3 %.

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

107 053 V. LEELA*, L. KOKILA, R. LAVANYA, A. SARASWATHY, P. BRINDHA (*Dept. of CARISM, SASTRA Univeristy, Thyanjavur, T.N., India, leelevadivelu@gmail.com): Determination of gallic acid in Acacia nilotica Linn by HPTLC. International J. Pharm. & Tech 2(2), 285-292 (2010). TLC of gallic acid in acetone extracts of bark powder of Acacia nilotica on silica gel with toluene - ethyl acetate - formic acid 15:10:2. The hR_F value of gallic acid was 36. Quantitative determination by absorbance measurement at 280 nm. The method was linear in the range of 100-350 ng/band with recovery of 97.5 %.

traditional medicine, quality control, herbal, densitometry 11a

107 054 Deepali MHASKE*, S. DHANESHWAR, S. SHAH, A. PADGILWAR (*Dept. of Q. A. Techniques and Pharm. Chem., Bharati Vidyapeeth University, Centre for Advanced Pharmsceutical

Research, Erandwane, Pune 411038, (MS), India): Stability indicating HPTLC method for determination of camylofin dihydrochloride in pharmaceutical dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-273 (2010). TLC of camylofin dihydrochloride on silica gel with toluene - methanol - chloroform - 10 % ammonia 8:5:6:1. The hR_F value was 35. The sample was subjected to different stress conditions (acid, base, oxidative, thermal, photolysis). The proposed method could effectively separate the drug from its degradation product.

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

107 055 S. MULGUND*, K. CHIDRAWAR, D. RANE, K. JAIN (*Dept. of Q. A. Techniques, Sinhgad College of Pharmacy, Vadgaon (BK) Pune 411041, M.S., India): Stability indicating HPTLC method for simultaneous estimation of telmisartan and hydrochlorothiazide. 62nd Indian Pharmaceutical Congress Abstract No. F-241 (2010). TLC of hydrochlorothiazide and telmisartan on silica gel with ethyl acetate - chloroform - methanol 6:3:1. The hR_F value was 38 for telmisartan and 55 for hydrochlorothiazide. Quantitative determination by absorbance measurement at 280 nm. The method was found to be linear in the range of 50-600 ng/band for both drugs. The sample was subjected to different stress conditions (acid, alkali, H_2O_2, thermal, photolytic) and was analyzed by the proposed method. The drugs were well separated from their degradation products. The method can be used for stability studies.

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

107 056 A. NAIK*, S. NAIK, M. PAI (*Goa College of Pharmacy, 18th June road, St. Inez, Panaji-Goa, 403001, India): Development and validation of a sensitive method for the quantitative analysis of atorvastatin calcium, ezetimibe and fenofibrate in a combined dosage form using HPTLC. 62nd Indian Pharmaceutical Congress Abstract No. F-246 (2010). TLC of atorvastatin calcium, ezetimibe and fenofibrate on silica gel with toluene - methanol - chloroform 6:3:4. Quantitative determination by absorbance measurement at 280 nm. The method was found to be linear in the range of 100-600 ng/band for atorvastatin calcium and ezetimibe and 50-300 ng/band for fenofibrate.

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

107 057 D. PANGAVHANE*, Smita LONDHE, Glory MAHAJAN, L. JAIN (*Dept. of Q. A. Techning, Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune, India): A stability-indicating HPTLC assay for the simultaneous determination of diclofenac sodium and omeprazole in commercial capsules. 62nd Indian Pharmaceutical Congress Abstract No. F-257 (2010). TLC of diclofenac sodium (DS) and omeprazole (OZ) in commercial capsules on silica gel with toluene - ethyl acetate 1:4. The hR_F values were 35 and 6 for DS and OZ, respectively. The linearity of the proposed method was in the range of 100-3000 ng/zone (r^2=0.9973) for OZ. The drugs were subjected to oxidation, acid and alkaline hydrolysis, photolysis, wet heat, dry heat and neutral degradation. Degradation products produced as a result of stress studies did not interfere with the detection of DS and OZ and the assay can thus be considered stability-indicating.

pharmaceutical research 11a

107 058 J. RAMESH*, R. VIJAYAMIRTHARAJ, B. JAYALAKSHMI, A. PRAKASAM, A. SURESH (*Dept. of Pharmaceutical Analysis, JKK Munirajah Medical Research Foundation College of Pharmacy, Komarapalayam 638183, Namakkal (DT), Tamilnadu, India): Development and validation of HPTLC method for the simultaneous estimation of atorvastatin and telmisartan in combined dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-234 (2010). HPTLC of atorvastatin and telmisartan on silica gel (pre-washed with methanol and dried at 60 °C for 5

min) with chloroform - benzene - methanol - glacial acetic acid 60:30:10:1. The hR_F values were 23 (telmisartan) and 56 (atorvastatin). Quantitative determination by absorbance measurement at 265 nm. The method was linear in the range of 40-200 ng/band for telmisartan and 10-50 ng/band for atorvastatin.

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

107 059 V. ROHIT*, H. KADIKAR, Vishranti TRIVEDI, V. SHAH (*Dept. of Q. A., Arihant School of Pharmacy and BRI, Adalaj, Gandhinagar, Gujarat, India): Development and validation of spectrophotometric and HPTLC methods for simultaneous estimation of ofloxacin and ornidazole in their combined dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-330 (2010). TLC of ofloxacin and ornidazole on silica gel with 1,4-dioxane - ethyl acetate - toluene - glacial acetic acid - water 5:5:3:2:2. The hR_F values were 16 and 89 for ofloxacin and ornidazole, respectively. Quantitative determination by absorbance measurement at 287 nm. The results by TLC were comparable to the results by a spectophotometric method.

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

107 060 E. SAJBEN*, L. MANCZINGER, A. NAGY, L. KREDICS, C. VAGVOLGYI (*Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary, sagben@gmail.com) : Characterization of pseudomonads isolated from decaying sporocarps of oyster mushroom. Microbiol. Res. 166, 255-267 (2011). TLC of lipopeptides (produced by Pseudomonas species in cultures of Pleurotus ostreatus; Pseudomonas reactans was used as a reference) on silica gel with chloroform - methanol - ammonia 80:25:4. Detection by spraying with 0.1 % bromothymol blue in ethanol, followed by heating.

food analysis, toxicology, qualitative identification 11d

107 061 I. SCHELLENBERG, Kathrin KABRODT* (*Anhalt University of Applied Sciences, Center of Life Sciences, Institute of Bioanalytical Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany, k.kabrodt@loel.hs-anhalt.de): Optimization of an AMD2 method for determination of stratum corneum lipids. CBS 105, 10-12 (2010). HPTLC of stratum corneum lipids (ceramides, cholesterol, phosphatidylcholine, squalene, sphingomyelin etc.) on silica gel by automated multiple development with a 8-step gradient from methanol to hexane in the AMD2 with pre-conditioning with 4M acetic acid before step 6. Detection by immersion in copper(II)sulfate reagent followed by heating at 170 °C for 8 min. Quantitative determination by absorbance measurement at 600 nm. Phosphatidylcholine and sphingomyelin remain at the start position, all other substances are separated.

pharmaceutical research, cosmetics, HPTLC, densitometry, qualitative identification, AMD, quantitative analysis 11

107 062 M. SONI*, A. MODH, H. BHATT, P. MEHTA (*Institute of Pharmacy, Nirma University, Ahmedabad 382481, Gujarat, India): Development, validation and comparison of HPTLC and UV methods for simultaneous estimation of ramipril and hydrochlorothiazide from its combined dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-332 (2010). TLC of ramipril and hydrochlorothiazide on silica gel with ethyl acetate - methanol - chloroform - glacial acetic acid 11:3:7:2. The hR_F values were 28 and 49 for ramipril and hydrochlorothiazide,respectively. Quantitative determination by absorbance measurement at 210 nm. The method was linear in the range of 500-1900 ng/band for both compounds. The recovery was 98-102 % for both drugs. The

results were comparable when the sample was analysed by a dual wave-length method. The proposed method can be used for analysis of formulation without any interference from excipients.

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

107 063 M. TOUFIK*, Kamini RAO, Janhavi RAO, Savita YADAV (*Dept. of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune 411038, India): Simultaneous HPTLC-densitometric analysis of metoprolol and ramipril in tablet. 62nd Indian Pharmaceutical Congress Abstract No. F-233 (2010). TLC of metoprolol and ramipril on silica gel with methanol - toluene - ethyl acetate - ammonia 25:30:50:7. Quantitative determination by absorbance measurement at 209 nm. The hR_F values of metoprolol and ramipril were 67 and 37, respectively. The reliability of the method was assessed by evaluation of linearity (2-12 µg/band for metoprolol and 0.2-1.2 µg/band for ramipril).

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

107 064 S. VARGHESE*, S. JOHNY, D. PAUL, T. RAVI (*College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641044 (TN), India): Development of validated HPLC and HPTLC method for the estimation of isotretinoin in capsule dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-384 (2010). TLC of isotretinoin on silica gel with toluene - ethyl acetate 4:1. The hR_F value was 54. Quantitative determination by absorbance measurement at 345 nm. The method was linear in the range of 20-100 ng/band. The sample was analysed by RP-HPLC and the result was comparable with the TLC method.

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

17. Amines, amides and related nitrogen compounds

107 065 S. AHMAD*, G.K. JAIN, MD. FAIYAZUDDIN, Z. IQBAL, S. TALEGAONKAR, Y. SULTANA, F.J. AHMAD (*Hamdard Univ. Dep. of Pharm., Faculty of Pharm., Hamdard Nagar, New Delhi 110062, India): Stability-indicating high-performance thin-layer chromatographic method for analysis of terbinafine in pharmaceutical formulations. Acta Chromatographica 21(4), 631-639 (2009). HPTLC on silica gel with toluene - ethyl acetate - formic acid 45:55:1. The hR_F value was 31. Quantification by densitometry at 284 nm. The limit of quantification was 35 ng/band, recovery was 97.6-101.6 %, and precision 2.19 *%RSD*. The method was applicable for routine analysis and accelerated stability testing of terbinafine in pharmaceutical drug-delivery systems. It can be used as a stability-indicating method because it separated the drug from its degradation products.

pharmaceutical research, quality control, HPTLC, quantitative analysis, qualitative identification, densitometry 17

107 066 H. DAVE*, Rajeshree MASHRU, A. PATEL (*Centre of Relevance & Excellence in Novel Drug Delivery Systems, Pharmacy Dept., G. H. Patel Bldg., Donor‘s Plaza. The M. S. Univ. of Baroda, Fatehganj, Vadodara 390002, Gujarat, Inida, rajshreemashru_msu@yagoo.com): TLC method for the determination of ternary mixture containing salbutamol sulphate, ambroxol hydrochloride and theophylline. Int. J. Pharma. Sci. 2(1), 390-394 (2010). TLC of salbutamol sulphate (SS), ambroxol hydrochloride (AH), and theophylline (THE) in a ternary fixed dose formulation on hand made silica gel plates with methanol - n-hexane 21:9. The hR_F values were 25 for SS, 72 for THE and 89 for AH. Detection under UV 254 nm and by exposure to iodine vapors. The bands of

the respective compounds were scraped off and quantified by spectrophotometry.

pharmaceutical research, quality control, qualitative identification 17a

107 067 H. DAVE*, Rajeshree MASHRU, A. PATEL (*Centre of Relevance and Excellence in Novel Drug Delivery Systems, Pharmacy Dept., G. H. Patel Bldg., Dono's Plaza, The M. S. University, Baroda, Fatehgung, Vadodara, Gujarat, India 390002, India, rajshreemashru_msu@yahoo.com): Thin-layer chromatographic method for the determination of ternary mixture containing salbutamol sulphate, bromhexine hydrochloride and etofylline. J. Pharm. Sci. & Res. 2(2), 143-148 (2010). TLC of salbutamol sulphate (SS), bromhexine hydrochloride (BH), and etofylline (ET) in fixed dose formulation on hand-made silica gel plates with methanol - *n*-hexane 2:1. All three compounds were well separated with hR_F values of 25 for SS, 71 for ET and 91 for BH. Detection at 254 nm as well as by exposure to iodine vapors. For spectophotometric quantification the bands of the selected drug in sample and standard mixture were scraped off, suspended in methanol, and the absorbance was measured at the maximum absorbance wavelength of each compound.

pharmaceutical research, quality control, quantitative analysis 17a

107 068 R. GHARGE*, G. WANKHEDE, R. KULKARNI, K. JAIN (*Dept. of Q. A. Techniques, Sinhgad College of Pharmacy, Vadgaon (BK) Pune 411041, M.S., India): Simultaneous estimation of lidocaine hydrochloride and clotrimazole by HPTLC with UV absorption densitometry. 62nd Indian Pharmaceutical Congress Abstract No. F-243 (2010). TLC of lidocaine hydrochloride and clotrimazole on silica gel with toluene - ethyl acetate - methanol - glacial acetic acid 45:30:20:1. Quantitative determination by absorbance measurement at 235 nm. The hR_F value was 28 for lidocaine HCl and 70 for clotrimazole. Linearity was in the range of 200-1200 ng/band for lidocaine HCl and 100-600 ng/band for clotrimazole. Recovery was found to be 99.6 % for lidocaine HCL and 99.0 % for clotrimazole.

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

107 069 S. HAVELE*, S. DHANESHWAR (*Research and Development Centre in Pharmaceutical Sciences and Applied chemistry, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune 411038, M.S., India): Estimation of metformin hydrochloride and glimepiride in multi-component formulation by HPTLC. 62nd Indian Pharmaceutical Congress Abstract No. F-235 (2010). TLC of metformin and glimepiride on silica gel with 0.5 % ammonium sulfate - water - methanol - ethyl acetate 2:2:1:1. Quantitative determination by absorbance measurement at 254 nm. The method was found to be linear in the range of 300-500 ng/band for glimepiride and 150-250 µg/band for metformin.

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

107 070 S. MULGUND*, A. BADANIKAI, A. BORKAR, M. PHOUJDAR (*Dept. of Pharmaceutical Chemistry, Sinhgad College of Pharmacy, Vadgaon (BK), Pune 411041, India): Stress degradation studies on fluvoxamine maleate using validated stability-indicating HPTLC method. 62nd Indian Pharmaceutical Congress Abstract No. F-244 (2010). TLC of fluvoxamine maleate on silica gel with benzene - methanol 5:4. The hR_F value was 52. Quantitative determination by absorbance measurement at 256 nm. The linearity was in the range of 500-3000 ng/band with r^2=0.998. The drug was subjected to acidic, alkaline and oxidative, dry heat, UV and photolytic stress. Since the method could effectively separate the drug from its degradation products, it can

be used for stability studies.

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

107 071 V. RENUKAPRIYA*, M. SHAIBA, V. RAMAKRISHNA, K. DEVI (*KVSR Siddhartha College of Pharmaceutical Science, Vijayawada 520010 (AP), India): High-performance thin-layer chromatographic estimation of ranolazine. 62nd Indian Pharmaceutical Congress Abstract No. F-290 (2010). TLC of ranolazine on silica gel with methanol - 10 mM ammonium acetate 3:2. The hR_F value was 54. Quantitative determination by absorbance measurement at 271 nm. The recovery was 99.9 %.

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

107 072 M. SINDHURA*, M. SHAIBA, G. RAO (*K.V.S.R. Siddhartha College of Pharmaceutical Sciences, Vijayawada 520010,AP, India): HPTLC estimation of tolterodine tartarate in formulation. 62nd Indian Pharmaceutical Congress Abstract No. F-237 (2010). TLC of tolterodine tartarate on silica gel with acetonitrile - water - formic acid 50:50:3. Quantitative determination by absorbance measurement at 281 nm. The method was found to be linear in the range of 1-30 µg/band.

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

107 073 A. SUGANTHI*, P. KUMAR, Nimisha MATHEW & T. RAVI (*College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641044, TN, India): Development of validated HPTLC method for the simultaneous estimation of ambroxol hydrochloride and doxophylline in tablet dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-253 (2010). TLC of ambroxol hydrochloride and doxophylline on silica gel with *n*-butanol - toluene - ethyl acetate - 25 % ammonia 50:30:20:1. The hR_F values were 36 and 45 for doxophylline and ambroxol, respectively. Quantitative determination by absorbance measurement at 258 nm. The method was linear in the range of 300-1100 ng/band for ambroxol and 100-1100 ng/band for doxophylline.

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

107 074 S. VARGHESE*, H. JOHN, M. JAGADEESHWARAN, T. RAVI (*Dept. of Pharmaceutical analysis, College of Pharmacy, Sri Ramakrishan Institute of Paramedical Sciences, Coimbatore 641044, (TN), India): Development of validated RP-HPLC and HPTLC method for the estimation of milnacipran from capsule dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-371 (2010). TLC of milnacipran on silica with n-butyl acetate - chloroform - glacial acetic acid 1:2:2. The hR_F value was 25. Quantitative determination by absorbance measurement at 220 nm. The results by an RP-HPLC method were comparable.

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

18. Amino acids and peptides, chemical structure of proteins

107 075 Susanne MINARIK, M. SCHULZ*, G. VAN BERKEL (*Merck KGaA, ABT. MM-LER-C, Frankfurter Str. 250, 64293 Darmstadt, Germany, michael.schulz@merckgroup.com): Use of planar chromatography for the analysis of peptides from tryptic protein digest. CBS 106, 5-6 (2011). HPTLC on 1) ProteoChrom silica gel with 2-butanol - pyridine - ammonia 25 % - water 39:34:10:26; on 2) ProteoChrom cellulose with 2-butanol - pyridine - acetic acid - water 15:10:3:12 by two-dimensional development and on 3) silica gel with the developing solvent

from 2). Detection by spraying with ninhydrin, fluorescamine, or triethylamine reagent. Evaluation under daylight and UV 366 nm. Detection by mass spectrometry by scanning the plate with a self modified desorption electrospray beam. In one-dimensional HPTLC up to 20 bands can be separated. By two-dimensional separation this number can be increased. Particularly suited are cellulose HPTLC plates.

pharmaceutical research, HPTLC, qualitative identification 18

107 076 C. ROULLIER, M. KRUGLER, E. WENSIG, A. MAILLARD, G. RECHBERGER, B. LEGOUIN, R. BAUER, J. BOUSTIE* (*Group of Natural Products, Synthesis and Medicinal Chemistry, Faculty of Pharmaceutical and Biological Sciences, University of Rennes, France, joel.boustie@univ-rennes1.fr): Characterization and identification of mycosporines-like compounds in cyanolichens. Isolation of mycosporine hydroxyglutamicol from *Nephroma laevigatum* Ach. Phytochemistry 72, 1348-1357 (2011). HPTLC of mycosporines and mycosporines-like amino acids on silica gel with chloroform - methanol - water 6:4:1. The plate was dried in a stream of nitrogen and protected from light. Detection by absorbance measurement at the respective maximum wavelength between 310 and 360 nm. The hR_F values of the compounds ranged between 20 and 80. HPTLC allowed a rapid and simultaneous comparison of 12-20 extracts for UV-absorbing compounds within 2 h.

pharmaceutical research, HPTLC, densitometry, qualitative identification 18a

21. Purines, pyrimidines, nucleic acids and their constituents

107 077 K. SHANKER*, Shalini GUPTA, Pooja SRIVASTAVA, S. SRIVASTAVA, S. SINGH, M. GUPTA (*Analytical Chemistry Dept., Central Institute of Medicinal & Atomatic Plant, (CSIR), Lucknow 226015, India, guptammg@rediffmail.com): Simultaneous determination of three steroidal glycoalkaloids in Solanum xanthocarpum by HPTLC. J. Pharm. Biomed. Anal. 54, 497-502 (2011). HPTLC of three bioactive steroidal glycoalkaloid markers, solasonine (SN), solamargine (SM) and khasianine (KN) in the plant *Solanum xanthocarpum*. The extraction effciency of targeted SGAs from plant matrix using methanol and acidified methanol were studied using percolation, ultrasonication and microwave techniques. HPTLC on silica gel with chloroform - methanol - water. The hR_F values were 31, 37, and 52 for SN, SM, and KN, respectively. Quantitative determination by absorbance measurement at 520 nm after derivatization using Dragendorffs reagent. The linearity range was 2-10 µg/band for SN and SM and 6-30 µg/band for KN. Method specificity was confirmed using hR_F values, correlation of UV spectra and comparison of ionization mass spectra (ESI-MS) of marker compounds in the sample track.

herbal, HPTLC, densitometry, quantitative analysis 21a

22. Alkaloids

107 078 M. WAKSMUNDZKA-HAJNOS*, D. MATOSIUK, A. PETRUCZYNIK, U. KIJKOWSKA-MURAK (*Medical University of Lublin, Department of Inorganic Chemistry, 20-081 Lublin, Poland): Determination of the lipophilicity of selected isoquinoline alkaloids by RP-TLC. Acta Chromatographica 20(4), 563-573 (2008). TLC of nine alkaloids on 1) RP-18 with aqueous acetone or aqueous dioxane using a variety of additives (ammonia, diethylamine, tetrabutylammonium chloride) to suppress ionization of the alkaloids and/or reduce ionic interactions with surface silanol groups, and on 2) ion-pair RP phase with aqueous acetone and additives such as pentane sulphonic acid, octane sulphonic acid, or di-(2-ethylhexyl)orthophosphoric acid. For the investigation of the relationship between RM and the modifier concentration a linear semilogarithmic equation was fitted to experimental data and used to obtain lipophilicity values RMW (RM for pure water), the slope, and f0, the intercept with the x-axis. The retention of standards with known lipophilicity logP was then determined using the chromatographic systems and RMW va-

lues were calculated. Equations relating logP and RMW from these experimental data were calculated for each system separately. These equations were used to estimate logP exp values for the alkaloids, and correlation of logPexp, slope, and f0 values obtained by different TLC systems.

pharmaceutical research 22

23. Other substances containing heterocyclic nitrogen

107 079 A. BHADIVADRA*, Y. KOLADIYA, H. BHATT, P. MEHTA (*Dept. of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, Ahmedabad 382481, Gujarat, India): Simultaneous estimation of amlodipine besylate and telmisartan in their combined dosage form by spectophotometric and HPTLC methods. 62nd Indian Pharmaceutical Congress Abstract No. F-389 (2010). TLC of amlodipine and telmisartan on silica gel with chloroform - methanol - toluene 10:7:3. The hR_F values were 23 and 75 for amlodipine besylate and telmisartan respectively. Quantitative determination by absorbance measurement at 238 nm. The sample was also analysed by spectophotometry and the results by both methods were comparable.

pharmaceutical research, quality control, densitometry, comparison of methods, quantitative analysis 23d

107 110 B. BIRADAR et al., see section 32

107 080 H. BODALWALA*, P. JAIN, R. KHATAL, K. AGRAWAK (*R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist. Dhule 425405 (MS), India): Stability-indicating HPTLC determination of brimonidine tartrate in bulk drug and pharmaceutical dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-301 (2010). TLC of brimonidine tartrate on silica gel with methanol - toluene - triethylamine 10:35:2. The hR_F value was 48. Quantitative determination by absorbance measurement at 247 nm. The method was linear in the range of 100-600 ng/band. The sample was subjected to different stress conditions (acid, base, oxidative, thermal and photolytic). With the proposed method all the degradation products were well resolved from the drug.

pharmaceutical research, quality control, quantitative analysis, densitometry 23e

107 081 K. DUTTA*, A. GARG, H. ASHIMA, G. ISHAN (*P.D.M. College of Pharmacy, Bahadurgarh, Haryana, India): Development of novel HPTLC method for the estimation of lamivudine, zidovudine and nevirapine either alone in bulk drug or combined in tablets. 62nd Indian Pharmaceutical Congress Abstract No. F-264 (2010). TLC of lamivudine, zidovudine and nevirapine on silica gel with chloroform - methanol 9:1. The hR_F values were 7, 46 and 77 for lamivudine, zidovudine and nevirapine, respectively. Quantitative determination by absorbance measurement at 265 nm. The method was linear in the range of 90-210 µg/band, 180-240 µg/band and 120-280 µg/band for lamivudine, zidovudine and nevirapine respectively.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 082 S. KATHIRVEL*, A. SUNEETHA, S. SUJANI (*Hindu College of Pharmacy, Amaravati Rd., Guntur-522002, India): Development and validation of TLC-densitometry method for the estimation of anti-psychotic drug in bulk and tablet formulation. 62nd Indian Pharmaceutical Congress Abstract No. F-13 (2010). HPTLC of risperidone in bulk and pharmaceutical dosage form on silica gel with dichloromethane - methanol - ethanol - triethylamine 120:120:60:1. Quantitative determination by absorbance measurement at 280 nm. The linearity was obtained in the range 4-8 µg/spot (r^2 =0.9989). The limit of detection and the limit of quantification for risperidone

were 98 ng/zone and 599 ng/zone, respectively. The recovery was 99.5 %.

pharmaceutical research, quality control, HPTLC, densitometry, quantitative analysis 23e

107 083 S. KELA*, P. DESAI, C. MODI, P. MEHTA (*Institute of Pharmacy, Nirma University, Ahmedabad 382481, Gujarat, India): Stability indicating HPTLC assay method for determination of irbesartan in pharmaceutical formulations 62nd Indian Pharmaceutical Congress Abstract No. F-255 (2010). TLC of irbesartan on silica gel with ethyl acetate - toluene - glacial acetic acid 35:15:1. Quantitative determination by absorbance measurement at 240 nm. The method was linear in the range of 200-800 ng/band. The sample was subjected to different stress conditions (acid, alkali, oxidation, thermal & photolytic). The compound was well separated from the different degradation products and could be estimated without any interference from the degradation product. The proposed stability indicating assay method was found suitable for routine quality control.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 084 G. KUMAR*, D. VASU, P. NARESH, P. SURESH (*Gitam Institute of Pharmacy, GITAM University, Rushikonda, Vizag 530045, India): Estimation of harmine from the stem bark of Symplocos racemosa Roxb. by HPTLC. 62nd Indian Pharmaceutical Congress Abstract No. F-248 (2010). TLC of harmine in stem bark of Symplocos racemosa Roxb. on silica gel with toluene - ethyl acetate - methanol 3:1:1. Quantitative determination by absorbance measurement at 324 nm. The linearity of the method was in the range of 100-500 ng/band.

traditional medicine, herbal, densitometry, quantitative analysis 23e

107 085 S. LAKSHMI*, P. CHAITHANYA, N. ANJANEYULU & M. MAHESHWARI (*Geethanjali College of Pharmacy, Cheeryal, Keesara, Hyderabad, India): HPTLC method development and validation for the simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in tablet dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-245 (2010). TLC of amlodipine besylate and nebivolol hydrochloride on silica gel with methylene chloride - methanol - 25 % ammonia 17:2:1. Both drugs were well resolved with hR_F values of 19 and 41 for amlodipine besylate and nebivolol hydrochloride respectively. Quantitative determination by absorbance measurement at 285 nm. The method was linear in the range of 200-600 ng/band for both drugs. Recovery was 99.9-102.1 %.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 086 Kranti MALPURI*, S. YAMUNA, R. VIJAYAGEETHA, Shantha ARCOT (*Final year B. Pharmacy, Faculty C. L. Baid Matha College of Pharmacy Chennai, India): Simultaneous estimation of risperidone and trihexyphenidyl hydrochloride in tablets by HPTLC. 62nd Indian Pharmaceutical Congress Abstract No. F-249 (2010). TLC of risperidone and trihexyphenidyl hydrochloride on silica gel with methanol - chloroform - glacial acetic acid 160:40:0.1. Quantitative determination by absorbance measurement at 254 nm. The linearity was in the range of 20-60 µg/band and 10-30 µg/band for risperidone and trihexyphenidyl hydrochloride, respectively. The recovery was 99.4-99.9 % for both drugs.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 087 K. MANIKANTA*, K. ALAGAWADI (*Dept. of Pharmaceutical Chemistry, KLE University, Belgaum, Karnataka 590010, India): Development and validation of HPTLC method for the si-

multaneous estimation of pioglitazone and metformin in pharmaceutical dosage forms. 62nd Indian Pharmaceutical Congress Abstract No. F-266 (2010). TLC of metformin and pioglitazone, extracted with methanol from bulk and pharmaceutical formulation, on prewashed silica gel with toluene - methanol - triethylamine 40:10:1. The hR_F values of metformin and pioglitazone were 25 and 50, respectively. Quantitative determination by absorbance measurement at 230 nm. The linearity was in the range of 100-1000 ng/band for metformin and 200-1200 ng/band for pioglitazone: the correlation coefficients (r) were 0.9958 and 0.9992, respectively.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

07 088 R. PATEL*, M. PATEL, J. PATEL, S. PATEL (*A. R. College of Pharmacy and G. H. Institute of Pharmacy, Vallabh Vidyanagar 388120 Gujarat, India): Desloratadine quantification using HPTLC method. 62nd Indian Pharmaceutical Congress Abstract No. F-263 (2010). TLC of desloratadine on silica gel with methanol - chloroform - toluene - ammonia 50:50:10:3. Quantitative determination by absorbance measurement at 254 nm. The hR_F value was 61. The linearity was in the range of 150-750 ng/zone with $r^2 = 0.9997$. The limit of detection was 21 ng/zone, whereas the limit of quantitation was 65 ng/zone.

quality control, HPTLC, densitometry, quantitative analysis 23e

107 089 R. PATEL*, M. PATEL, K. BHATT, B. PATEL (*A. R. College of Pharmacy & G. H. Patel Inst. of Pharmacy, Vallabh Vidyanagar 388120, Gujarat, India): New HPTLC method for quantification of risperidone in mucoadhesive microemulsion formulations and invitro diffusion study. 62nd Indian Pharmaceutical Congress Abstract No. F-250 (2010). TLC of risperidone on silica gel with methanol - ethyl acetate 4:1. The hR_F value was 34. Quantitative determination by absorbance measurement at 254 nm. The method was linear in the range of 100-600 ng/band. The proposed method was employed for estimation of solubility equilibrium, analysis of mucoadhesive microemulsion formulations and in vitro diffusion studies.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 090 Nilam PATEL*, P. CHAUDHARY, S. PANCHOLI (*Shree Krishna Institute of Pharmacy, Shankhalpur, Gujarat, India): Development and validation of stability indicating HPTLC method for simultaneous estimation of montelukast sodium and levocetirizine dihydrochloride in tablet dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-261 (2010). TLC of montelukast sodium and levocetirizine dihydrochloride on silica gel with ethyl acetate - methanol - ammonia 10:2:1. Quantitative determination by absorbance measurement at 231 nm. Montelukast and levocetirizine were subjected to acid, base, peroxide, and photodegradation. In stability tests the drugs were susceptible to acid and basic hydrolysis, oxidation and photolytic degradation. The stressed samples were analyzed by the proposed method and no intereference of the degradation products or the excipients with the drugs was found. The linearity ranges were 50-600 ng/zone for levocetirizine and 100-1200 ng/zone for montelukast. The recovery was 99.3 % for levocetirizine and 99.9 % for montelukast.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 091 S. PATEL*, P. PATEL, N. PATEL, B. PATEL (*Dept. of Pharmaceutical Q. A. Shree S. K. Patel College of Pharmaceutical Education and Research, Ganapat University, Kherva, Mehsana 382711, Gujarat, India): Development and validation of HPTLC method for simultaneous estimation of gatifloxacin and ornidazole in tablets. 62nd Indian Pharmaceutical Congress Abstract No. F-242 (2010). TLC of gatifloxacin and ornidazole on silica gel with n-butanol - ethanol - 8M

ammonia 10:1:3. Quantitative determination by absorbance measurement at 299 nm. The hR_F value of gatifloxacin was 27 and of ornidazole 83. The method was found to be linear between 20-100 ng/zone for gatifloxacin and 50-250 ng/zone for ornidazole ($r^2 > 0.99$). The limit of detection and quantitation were found to be 4.1 and 12.5 ng/zone, respectively for gatifloxacin and 10.3 and 31.2 ng/zone, respectively for ornidazole.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 092 M. PATEL*, R. PATEL, B. PATEL, D. SHAH (*Indukaka Ipcowala College of Pharmacy, Sardar Patel University, New Vallabh Vidyanagar 388121, Gujarat, India): A new eco friendly HPTLC method for quantification of carbamazepine in formulations and invitro diffusion study. 62nd Indian Pharmaceutical Congress Abstract No. F-262 (2010). TLC of carbamazepine on silica gel with ethyl acetate - toluene - methanol 5:4:1. Quantitative determination by absorbance measurement at 285 nm. The hR_F value was 47. The linearity was in the range of 100-600 ng/zone with $r^2 = 0.9995$. The limit of detection was found to be 7 ng/zone, whereas the limit of quantitation was found to be 4 ng/zone.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 093 M. PATIL*, A. TAMBOLI, V. BHALERAO, R. DESHMUKH (*Sahyadri College of Pharmacy, Methewade, Tal. Sangola, Dist. Solapur, MS, India): Simultaneous determination of amlodipine besylate and enalapril maleate by HPTLC method. 62nd Indian Pharmaceutical Congress Abstract No. F-256 (2010). TLC of amlodipine besylate and enalapril maleate on silica gel with toluene - isopropanol - glacial acetic acid - methanol 50:20:6:5. The hR_F values were 15 and 23 for amlodipine besylate and enalapril maleate, respectively. Quantitative determination by absorbance measurement at 223 nm.

pharmaceutical research, quality control, densitometry, quantitative analysis 23d

107 094 K. RAGHAVI*, M. SHAIBA, G. RAO (*KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada 520010, AP, India): Development and validation of HPTLC method for estimation of rupatadine fumarate in formulation. 62nd Indian Pharmaceutical Congress Abstract No. F-236 (2010). TLC of rupatadine formulation on silica gel with acetonitrile - water - formic acid 50:50:3. The hR_F value was 67. Quantitative determination by absorbance measurement at 263 nm. The method was linear in the range of 1-20 µg/band.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 095 N. RAJPUT*, S. SHUKLA, V. PATEL (*A.R. College of Pharmacy & G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat, India): Validated HPTLC method for quantification of bebeerine and oleanolic acid in roots of Cissampelos pareira Linn. var hirsuta. 62nd Indian Pharmaceutical Congress Abstract No. F-238 (2010). TLC of the two marker compounds bebeerine and oleanolic acid from the roots of Cissampelos pareira Linn. on silica gel with toluene - ethyl acetate - diethylamine 7:2:1 for bebeerine and toluene - ethyl acetate - formic acid 70:30:3 for oleanolic acid. The hR_F value was 20 (bebeerine) and 56 (oleanolic acid). Quantitative determination by absorbance measurement at 254 nm and under visible light after spraying with dragendorff‘s reagent (bebeerine) or anisaldehyde sulfuric acid reagent (oleanolic acid).

herbal, densitometry, quantitative analysis 23e

107 096 J. SHAH*, H. PATEL, S. PATEL, S. PANCHOLI (*Dept. of Pharmaceutical Analysis, Babaria Institute of Pharmacy, Vadodara-Mumbai NH#8, Varnama, Vadodara 391240, Gujarat, India): Development and validation of HPTLC and derivative spectroscopic method for simultaneous estimation of nebivolol and hydrochlorothiazide in combined dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-258 (2010). TLC of nebivolol HCl and hydrochlorothiazide on silica gel with methanol - chloroform - toluene - triethylamine 10:25:14:1. Quantitative determination by absorbance measurement at 284 nm. The hR_F value of nebivolol was 78 and of hydrochlorthiazide 41. The method was linear in the range of 5-100 ng/band and 20-140 ng/band for nebivolol HCl and hydrochlorothiazide, respectively. Recovery was in the range of 98.8-100.0 % for both drugs.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 097 S. SHUKLA*, Swarnlata SARAF, S. SARAF (*University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India): TLC densitometric fingerprint development and validation of berberine as markers in poly-herbal Unani formulations. Der Pharma Chemica 2(3), 8-18 (2010). HPTLC of berberine on silica gel with methanol - acetic acid - water 8:1:1. The band corresponding to berberine showed an hR_F value of 74. Quantitative determination by absorbance measurement at 350 nm. The method was linear in the range of 100-500 ng/band. Different samples analysed by the proposed method were found to contain 11.8-12.5 mg/g berberine. The recovery was between 98.0-100.3 %.

quality control, herbal, HPTLC, densitometry, quantitative analysis 23e

107 098 D. TAJANE*, K. INGALE, V. CHOUDHARI, B. KUCHEKAR (*Dept. of Pharmaceutical Analysis & Q. A., MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune 411038, India): Simultaneous estimation of drotaverine hydrochloride and etoricoxib by HPTLC. 62nd Indian Pharmaceutical Congress Abstract No. F-339 (2010). TLC of drotaverine hydrochloride (DRT) and etoricoxib (ETR) on silica gel with toluene - ethyl acetate - methanol 1:4:1. The hR_F values were 45 and 66 for DRT and ETR, respectively. Quantitative determination by absorbance measurement at 304 nm. The method was linear in the range of 200-700 ng/band for DRT and 225-787 ng/band for ETR. The recovery was in the range of 99.9-100.1 % for both drugs.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 099 A. THOMAS*, S. JAGDALE, S. BHOSALE, A. DESHPANDE (*DR. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411018, MS, India): Stability indicating HPTLC method for the simultaneous determination of amlodipine besylate and telmisartan from tablet dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-254 (2010). TLC of amlodipine besylate and telmisartan on silica gel with ethyl acetate - methanol - 25 % ammonia - glacial acetic acid 75:15:1:2. The hR_F value was 34 and 60 for amlodipine besylate and telmisartan, respectively. Quantitative determination by absorbance measurement at 226 nm. The linearity was in the range of 500-6000 ng/band for amlodipine and 1000-8000 ng/band for telmisartan. The sample was subjected to various stress conditions and all the degradation products were well resolved from the pure drugs. The method can be used for stability studies.

pharmaceutical research, quality control, densitometry, quantitative analysis 23d

107 100 M. TRYAMBAKE*, S. SHINDE, A. CHABUKSWAR, S. JAGDALE (*MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune 411038 (MS), India): Development and validation of HPTLC method for simultaneous estimation of hydrochlorothiazide and irbesartan in combined

dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-343 (2010). TLC of hydrochlorothiazide (HCTZ) and irbesartan (IRBE) on silica gel with toluene - acetic acid - methanol 70:2:50. Quantitative determination by absorbance measurement at 264 nm. The hR_F values were 15 for HCTZ and 45 for IRBE. The linearity was in the range of 90-540 ng/band and 180-900 ng/band with r^2= 0.9989 for HCTZ and IRBE. The recovery of HCTZ was 95.3-97.7 % and of IRBE 95.2-98.7 %.

pharmaceutical research, quality control, densitometry, quantitative analysis 23e

107 101 S. VARGHESE*, R. KUMAR, K. KRISHNAN, T. RAVI (*College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore 641044, (TN), India): Development of validated HPLC and HPTLC method for the estimation of citicoline sodium in tablet dosage form. 62nd Indian Pharmaceutical Congress Abstract No. F-381 (2010). TLC of citicoline sodium on silica gel with chloroform - methanol - water 3:7:3. The hR_F value was 53. Quantitative determination by absorbance measurement at 280 nm. The results of the method were comparable with the results of a RP-HPLC method.

pharmaceutical research, quality control, densitometry, comparison of methods, quantitative analysis 23e

24. Organic sulfur compounds

107 102 A. HAWRYL*, L. POPIOLEK, M. DOBOSZ, E. PIKULA, M. WAKSMUNDZKA-HAJNOS (*Med. Univ. of Lublin, Dep. of Inorg. Chem., Staszica 6, 20-081 Lublin Poland): RP-HPTLC determination of the lipophilicity of some new derivatives of thiosemicarbazide and 1,2,4-triazole of sulphanylacetic acid. Acta Chromatographica 22 (1), 37-55 (2010), DOI:10.1556/AChrom.22.2010.1.3. Separation of some new derivatives of thiosemicarbazide and the 1,2,4-triazole of sulphanylacetic acid by HPTLC on RP-18 with mobile phases containing water and an organic modifier (methanol, dioxane, acetone, 2-propanol, or tetrahydrofuran). Description of the relationships between solute retention and modifier concentration by Snyder‘s linear equation. RM0 and slope values were determined by extrapolation based on linear retention and mobile phase concentration; both values characterize the lipophilicity of the substances. Correlation of the calculated values of RM0 with log P values for the drugs investigated by use of the software HyperChem, and correlations between intercept (RM0) and slope from the linear equations.

HPTLC 24

27. Vitamins and various growth regulators

107 103 A. MOHAMMAD*, A. ZEHRA (*Aligarh Muslim University Analytical Research Laboratory, Department of Applied Chemistry, Faculty of Engineering and Technology, Aligarh, India): Specific separation of thiamine from hydrophilic vitamins with aqueous dioxane on precoated silica TLC plates. Acta Chromatographica 20(4), 637-642 (2008). Specific separation of thiamine hydrochloride from riboflavin, nicotinic acid, calcium D-pantothenate, pyridoxine hydrochloride, cyanocobalamin, and ascorbic acid by TLC on silica gel with dioxane - water 1:1. Detection under UV light. Examination of the effect of impurities (metal cations and inorganic anions) on the chromatography of thiamine hydrochloride. The detection limit for thiamine hydrochloride was 0.09 µg/zone and the relative standard deviation of the hR_F value of thiamine hydrochloride in five analyses was 14.9 %.

pharmaceutical research, quality control, quantitative analysis, qualitative identification 27

28. Antibiotics, Mycotoxins

107 104 Wioleta BAK, Irena CHOMA*, Edyta Grzelak, Barbara MAJER-DZIEDZIC, K. PILORZ (*Dpt.

of Chromatographic Methods, University of Maria Curie-Sklodowska, M. Sklodowska Sq. 3, 20-031 Lublin, Poland, irena.choma@poczta.umcs.lublin.pl): Determination of enrofloxacin and ciprofloxacin in milk by direct bioautography detection. CBS 106, 2-4 (2011). TLC of milk samples on silica gel with dichloromethane - methanol - isopropanol - 25 % aqueous ammonia 3:3:5:2 in the horizontal DS chamber. Bioautography detection with *Bacillus subtilis* using the Chrom Biodip Antibiotic Kit and by dipping in a broth of *Escherichia coli*. Detection by spraying with an aqueous tetrazolium salt (MTT) solution of 0.2% and evaluation under daylight. With the E. coli assay the limit of detection for ciprofloxacin was 25 µg/kg, which is lower than with the Chrom Biodip test, while for enrofloxacin it was slightly higher (75 µg/kg).

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

107 105 Juliane WELKE*, M. HOELTZ, H. DOTTORI, I. NOLL (*Institute of Food Science and Technology, Rio Grande do Sul Federal University, Porto Alegre, Brazil, juliwelke@yahoo.com.br) : Patulin accumulation in apples during storage by Penicillium expansum and Penicillium griseofulvum strains. Brazilian Journal of Microbiology 42, 172-180 (2011). TLC of patulin on silica gel with toluene - ethyl acetate - formic acid 5:4:1. Detection by spraying with 0.5 % aqueous methyl-benzothiazolinone hydrazone hydrochloride monohydrate, followed by heating at 130 °C for 15 min. Quantitative determination by absorbance measurement at 366 nm. Linearity was between 45 and 2100 µg/kg. The limits of detection and quantification were 0.005 µg/kg and 14 µg/kg. The relative standard deviation for repatibility was 6.2 %. Recovery (by standard addition) was 88 % for patulin.

food analysis, quality control, HPTLC, quantitative analysis, densitometry 28b

29. Pesticides and other agrochemicals

107 025 R. AKKAD et al., see section 3

30. Synthetic and natural dyes

107 106 J.D. VASTA*, J. SHERMA (*Lafayette College, Department of Chemistry, Easton PA 18042-1782, USA): Analysis of lycopene in nutritional supplements by silica gel high-performance thin-layer chromatography with visible-mode densitometry. Acta Chromatographica 20(4), 673-683 (2008). Presentation of a quantitative method for the analysis of lycopene in nutritional supplements consumed to reduce the risk of prostate cancer and other forms of cancer and cardiovascular disease. HPTLC on silica gel with petroleum ether - dichloromethane 9:1. Quantification by densitometry at 416 nm. Four products containing 300 µg, 3 mg, 5 mg, or 10 mg lycopene plus other ingredients were quantified using a lycopene standard: the measured amounts ranged from 77.7 to 98.1 % of the stated label values. The accuracy by spiked blank analysis was within 1.90 % of theoretical values for the 3 mg softgels and 1.10 % of theoretical values for the 10 mg softgels. The precision of replicate analyses showed a *RSD* of 1.44 % for the 10 mg softgels and 2.39 % *RSD* for the spiked blank for the 3 mg softgels. The results obtained for Lycopene standards available from two other companies showed 55.6, 57.6, and 20.0 % of the minimum amount expected from the stated label values.

quality control, food analysis, agricultural, quantitative analysis, qualitative identification, HPTLC, densitometry 30b

107 107 X. ZHANG* (Zhang Xiaomei), X. WEI (Wei Xining), Y. LEI (Lei Yong), X. CHENG (Cheng Xiaolin), Y. ZHOU (Zhou Yang) (*Sch. of Archaeol. & Museol., Peking Univ., Beijing 100871, China): (Micro and nondestructive analysis of blue dyes from silk fabrics and decorative painting

of ancient building) (Chinese). Spectroscopy and Spectral Anal. 30(12), 3254-3257 (2010). Dye analysis is important for the understanding of fabric color degradation and technical development of ancient printing and dyeing. TLC of blue dyes extracted from 6 silk fabrics of the Tang dynasty and decorative paintings of Jian Fu Gong (Forbidden City) on silica gel with benzene - nitrobenzene - acetone 8:1:1. Identification of indigo by comparison of the colors and the R_f value with the zone by the standard, and by Raman spectroscopy of raw samples. Raman spectroscopy is a nondestructive analysis whereas TLC requires small amounts of sample but may give more information. Both methods may be applicable for cultural heritages. The results obtained indicate that all these blue substances are indigo, which was not only used as dye in ancient fabrics, but also as pigment in decorative painting of historic buildings.

qualitative identification 30b

32. Pharmaceutical and biomedical applications

107 108 S.G. BHOPE*, V.V. KUBER, D.H. NAGORE (*MIDC Ranjangaon Tulip Lab Pvt Ltd, F-20/21 Pune 412220, India): Validated HPTLC method for simultaneous quantification of sennoside a, sennoside b, and kaempferol in Cassia fistula Linn. Acta Chromatographica 22 (3), 481-489 (2010). HPTLC on silica gel with toluene - ethyl acetate - methanol - formic acid 8:10:5:2. The hR_F values were 22, 19, and 81 for sennosides A and B and kaempferol, respectively. Quantification by densitometry at 270 nm. The recovery of sennosides A and B and kaempferol from *Cassia fistula* extract were 98.0, 98.7, and 99.1 %, respectively. The linearity was in the range of 100-400 ng/band. Instrument precision was in the range of 1.03-1.33 % and method precision in the range of 1.3-1.8 %.

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

107 109 V.K. BHUSARI*, M.V. MAHADIK, S.R. DHANESHWAR (*Bharati Vidyapeeth Univ., Poona Coll. of Pharm., Dep. of Pharm. Chem., Pune, Maharashtra, India 411038): Application of a stability-indicating HPTLC method for quantitative analysis of amtolmetin guacil in a pharmaceutical dosage form. Acta Chromatographica 21(2), 299-317 (2009). HPTLC of amtolmetin guacil on silica gel with toluene - ethyl acetate 2:3. Identification and quantification by densitometric analysis in absorbance mode at 320 nm. The samples were subjected to acidic and alkaline hydrolysis, oxidation, dry heat treatment, and photo-degradation. The method was suitable for stability studies and to study the kinetics of degradation of amtolmetin guacil.

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

107 110 B. BIRADAR*, T. RADHASI, D. GOHIL, NAGARAJ (*Dept. of Pharmaceutical Analysis, PES College of Pharmacy, Hanumanthanagar, Bangalore 560050, India): Validated HPTLC method for simultaneous quantitation of levocetirizine and phenylpropanolamine in bulk drug and formulation. 62nd Indian Pharmaceutical Congress Abstract No. F-251 (2010). TLC of levocetirizine and phenylpropanolamine on silica gel with methanol - ethyl acetate - toluene - ammonia 15:4:5:2. Quantitative determination by absorbance measurement at 210 nm. The hR_F value was 30 and 60 for levocetrizine and phenylpropanolamine, respectively. The linearity was in the range of 45-270 ng/band for both drugs.

pharmaceutical research, quality control, densitometry, quantitative analysis 32a, 23e

107 111 H. CHEN* (Chen Honghui), B. XU (Xu Baoli), G. PENG (Peng Guanghua) (*Bio-chem. Dep,

Wenshan Univ., Wenshan, Yunnan 663000, China): (Isolation and identification of chlorogenic acid in Yacon (Smallanthus sonchifolius) leaves by thin-layer chromatography) (Chinese). Chinese J. Food R & D, Test & Anal. 31 (10), 134-138 (2010). TLC of chlorogenic acid on silica gel with ethyl acetate - water - formic acid 17:2:2. Detection by spraying with 2 % FeCl - 1 % KFe(CN) 4:1.

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

107 112 L. CHENG* (Cheng Lijuan), F. WAN (Wan Fugui), Y. ZHOU (Zhou Yan) (*Yingshan County People's Hosp., Yingshan, Hubei 438700, China): (Preparation and quality control of Chuanqi Kuoguan capsules) (Chinese). Modern J. of Integrated Trad. Chinese & Western Med. 19(31), 3439-3441 (2010). TLC on silica gel with chloroform - methanol - water 13:7:2. Detection by spraying with 10 % slfuric acid in ethanol and heating at 105 °C until the zones are visualized, evaluation under UV 366 nm. Identification of the component drugs Radix Astragali and Rhizoma Chuanxiong P.E by comparison of the retention values and color of the zones by the active compounds astragaloside and ferulic acid in the individual drug.

pharmaceutical research, quality control, traditional medicine, qualitative identification, autoradiography, quantitative analysis, astragaloside, ferulic acid 32e

107 113 P. CHOTHE*, S. DESHMUKH, A. KAKADE, I. RAUT (*Dept. fo Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon 415404, Tal. Walwa Dist. Sangli, (MS), India): A new simple method for determination of partition coefficient by normal phase TLC. 62nd Indian Pharmaceutical Congress Abstract No. F-382 (2010). The partition coefficient (logP) of a drug in benzene - water is an important parameter to determine the absorbance of the drug in body, thus influencing its therapeutic response. A NP-TLC method for the determination and calculation of log P values is proposed. By this method differential values like Rfb/w, LogRfb/logRfw were calculated, which were very close to values reported in literature. LogP values of different drugs were 0.46 for paracetamol, 0.22 for atenolol, 5.11 for telmisertan, and 0.9 for nimesulide.

pharmaceutical research 32a

107 114 S. CORAN*, G. BARTOLUCCI, M. ALBERTI (*Dept. of Pharmaceutical Sciences, University of Firenze, Via Ugo Schiff 6, 1-50019, Sesto fiorentino, Italy, silvia.coran@unifi.it): Selective determination of aloin in different matrices by HPTLC densitometry in fluorescence mode. J. Pharm. Biomed. Anal. 54, 422-425 (2011). HPTLC of aloin in several aloe dried extracts and related commercial formulations on silica gel with ethyl formate - methanol - water 200:29:20. Evaluation under 254 nm. Detection by immersion in 10 % H_3BO_3 in methanol, followed by heating at 110 °C for 10 min. Quantitative determination by fluorescence measurement at 365/K540 nm.

herbal, HPTLC, densitometry, quantitative analysis 32e

107 115 M.C. DAMLE*, K.S. TOPAGI, K.G. BOTHARA (*AISSMS College of Pharmacy, Pharm. Chem. Dep., Kennedy Road, Near RTO Pune 411001, Maharashtra, India): Development and validation of a stability-indicating HPTLC method for analysis of nebivolol hydrochloride and hydrochlorothiazide in the bulk material and in pharmaceutical dosage forms. Acta Chromatographica 22 (3), 433-443 (2010). HPTLC on silica gel with ethyl acetate - methanol - acetic acid 13:2:1. The hR_F values were 46 and 78 for nebivolol hydrochloride and hydrochlorothiazide, respectively. Detection and quantification by densitometry at 280 and 270 nm for nebivolol hy-

drochloride and hydrochlorothiazide, respectively. The drugs were subjected to hydrolysis under acidic, basic, and neutral conditions, oxidation, heat, and photolysis as stress conditions. The drug showed degradation when subjected to oxidative stress and acidic conditions, which also affected the tablet sample substantially. However there was no interference of the drug peak by any of the degradation products. The method was therefore applied for stability testing of these drugs during stability studies.

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

107 116 P.V. DEORE*, A.A. SHIRKHEDKAR, S.J. SURANA (*R.C. Patel College of Pharmacy, Department of Pharmaceutical Chemistry Shirpur Dist. Dhule (M.S.), India, 425 405): Simultaneous TLC-densitometric analysis of atenolol and lercanidipine hydrochloride in tablets. Acta Chromatographica 20(3), 463-473 (2008). TLC on silica gel with toluene - methanol - triethylamine 35:15:1. The hR_F of atenolol and lercanidipine hydrochloride was 24 and 68, respectively. Detection and quantitative determination by absorbance measurement at 275 nm. The linearity was in the range of 2-12 µg/band for atenolol and 400-2400 ng/band for lercanidipine hydrochloride. The recovery was 98.9 % for atenolol and 99.7 % for lercanidipine hydrochloride.

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

107 117 R.R. DURÓN, L.C. ALMAGUER, A. DE J. GARZA-JUÁREZ, MA. LUZ, SALAZAR CAVAZOS, N. WAKSMAN-DE-TORRES (Universidad Autónoma de Nuevo León, Departamento de Química Analítica, Facultad de Medicina P.O. Box 2316 Sucursal Tecnológico, 64841 Monterrey Nuevo León, México): Development and validation of thin-layer chromatographic methods for quality control of herbal products. Acta Chromatographica 21(2), 203-215 (2009). HPTLC of commercial products containing *Heterotheca inuloides, Citrus aurantium, Peumus boldus, Equisetum arvense, Eucalyptus globulus, Ginkgo biloba, Mentha piperita, Aloe vera, Salvia officinalis*, and *Cassia senna* on silica gel with different mobile phases. The mobile phase for aloin, boldine, chlorogenic acid, rutin, kaempferol, caffeic acid, and quercetin was ethyl acetate - methanol - water 100:17:13; for menthol, cineole, menthone, alpha- and beta-thujone, geraniol, linalyl acetate and linalool it was toluene - ethyl acetate 93:7; for ginkolide B toluene - ethyl acetate - acetone - methanol 50:25:25:3; and for sennoside B ethyl acetate - formic acid - acetic acid - water 100:11:11:27. Detection with natural products reagent, anisaldehyde reagent or Liebermann-Burchard reagent. We found that in only 20 % of the 40 commercial products analysed the chromatographic characteristics of the respective plants matched those of the specific respective marker compounds. This highlights a problem arising from the lack of regulation of these products, and emphasizes the need to develop simple and reliable analytical methods like TLC methods that can be performed in any laboratory for the purpose of quality control of dietary supplements or commercial herbal products sold in Mexico.

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

107 118 J. FU* (Fu Jingjuan), ZH. LIU (Liu Zhihui), F. QIAN (Qian Fang), X. CHANG (Chang Xingjie) (*Affiliated Hosp., Nanjing Univ. Trad. Chinese Med. & Pharm., Jiangsu, Nanjing 210029, China): (Study on the quality specification of Baibanding tincture) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. (1), 55-57 (2011). TLC of components of Baibanding tincture: 1) for Gardenia jasminoides, on silica gel with ethyl acetate - acetone - methanol - water 5:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 110 °C until the zones were visualized;

2) for *Cuscuta chinensis*, on polyamide phase with methanol - glacial acetic acid - water 4:1:5, detection by spraying with $AlCl_3$ solution and 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 % NaOH in methanol and evaluation under UV 366 nm. Identification by fingerprint comparison with the individual component drug of the preparation.

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

107 119 G.P. GANU*, S.S. JADHAV, A.D. DESHPANDE (*Pad. Dr D.Y. Patil Inst. of Pharm. Sci. & Res., Dep. of Pharmacy, Pimpri, Pune, India): Development and validation of a method for densitometric analysis of lupeol from Mimosoups elengi. Acta Chromatographica 22 (3), 491-497 (2010). HPTLC of lupeol (methanolic Soxhlet extract from the bark of Mimosoups elengi) on silica gel with toluene - ethyl acetate - formic acid 12:2:1. The hR_F value of lupeol was 64. Evaluation by densitometry at 220 nm. The linearity was in the range of 1-4 µg/band. The precision was 1.06 and 1.03 *%RSD*, respectively. Recovery was 97.3 %.

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

107 120 A. GOEL*, R. GOEL, G.K. JAIN, R.M. SINGH, F.J. AHMAD, G.N. SINGH (*Government of India, Central Indian Pharmacopoeia Laboratory, Ministry of Health and Family Welfare, Ghaziabad Uttar Pradesh, India): Development and validation of a stability-indicating HPTLC method for analysis of 3-acetyl-11-keto-beta-boswellic acid in a herbal extract and a nanoparticles formulation. Acta Chromatographica 20(3), 497-511 (2008). HPTLC of 3-acetyl-11-keto-beta-boswellic acid (AKBA) on silica gel with toluene - ethyl acetate 7:3 at room temperature (25 ± 2 °C) in a twin-trough chamber with chamber saturation. Quantification of AKBA (hR_F 52) by densitometry in absorbance mode at 250 nm. The linearity was in the range of 200-1200 ng/band (r=0.9989), recovery was 99.4-100.2 %, and the limits of detection and quantification were 3 and 9 ng/band, respectively. AKBA was subjected to various stress conditions: acid and alkali hydrolysis, oxidation, photodegradation, and dry and wet heat treatment. The degradation products were separated from the pure drug with significantly different hR_F values.

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

107 121 C.L. GOPU*, S.S. GILDA, A.R. PARADKAR, K.R. MAHADIK (*Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune 411038, Maharashtra, India): Development and validation of a densitometric TLC method for analysis of trigonelline and 4-hydroxyisoleucine in Fenugreek seeds. Acta Chromatographica 20(4), 709-719 (2008). HPTLC of trigonelline and 4-hydroxyisoleucine from Fenugreek seeds (*Trigonella foenum-graceum*) on silica gel with *n*-butanol - methanol - acetic acid - water 8:3:2:2. Detection by spraying with ninhydrin reagent. Quantification by densitometry at 266 nm for trigonelline, and at 395 nm for 4-hydroxyisoleucine. The linearity was in the range of 100-1000 ng/band for trigonelline and 50-500 ng/band for 4-hydroxyisoleucine, respectively, with r=0.9992 and 0.9986 respectively. The average recovery at three different levels was 99.4 % for trigonelline and 99.1 % for 4-hydroxyisoleucine.

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

107 122 F. HASAN*, R. KHAR, F. AHMAD, M. ALI, M. REZA (*Dept. of Pharmaceutical, Faculty of

Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India): Validated HPTLC method for estimation of biomarkers in sesame oil. 62nd Indian Pharmaceutical Congress Abstract No. F-265 (2010). TLC of cholesterol on silica gel with carbon tetra chloride - methanol - formic acid 270:30:11. The hR_F value was 55. Quantitative determination by absorbance measurement at 366 nm. The method was linear in the range of 100-600 ng/band.

herbal, densitometry, quantitative analysis 32g

107 123 Maha HEGAZY*, Fadia H. METWALY, M. ABDELKAWY, Nada S. ABDELWAHAB (*Anal. Chem. Dep., Faculty of Pharm., Cairo Univ., Kasr El-Aini St., 11562 Cairo, Egypt): Validated chromatographic methods for determination of hydrochlorothiazide and spironolactone in pharmaceutical formulation in presence of impurities and degradants. J. of Chromatogr. Sci. 49, 129-135 (2011). TLC on silica gel with ethyl acetate - chloroform - formic acid - triethyl amine 70:30:1:1. Detection and quantification by densitometry. Good correlation between the integrated peak area of the studied drugs and their corresponding concentrations was found in different ranges.

pharmaceutical research, quality control, quantitative analysis, qualitative identification, comparison of methods, densitometry 32c

107 124 M. KACHROO*, S. AGRAWAL (*Dept. of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Hosur Rd., Bangalore 560027, India): HPTLC method for estimation of isolated derivative in fractions of seeds of *Ensete superbum*. J. Chem. Pharm. Res. 2(1), 155-161 (2010). A chroman derivative ($C1_6O_4H_{22}$) was isolated from the ethanolic extract of dried seeds of *Ensete superbum*. HPTLC on silica gel with toluene - ethyl acetate - formic acid 5:4:1. Quantitative determination by absorbance measurement at 254 nm. The linear range was 300-900 ng/band. The amount of the chroman in different fractions of the extract was 1.83 % (ethanol fraction), 1.74 % (ethyl acetate fraction) and 0.74 % (methanol fraction).

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

107 125 I. KHAN, P. SANGWAN, S. ABDULLAH, B. GUPTA, J. DHAR, R. MANICKAVASAGAR, S. KOUL* (*Bioorganic Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Jammu and Kashmir 180001, India, skoul@iiim.res.in): Ten marker compounds-based comparative study of green tea and guava leaf by HPTLC densitometry methods: antioxidant activity profiling. J. Sep. Sci. 34, 749-760 (2011). HPTLC of (-)-epicatechin (1), (-)-epicatechin gallate (2), (-)-epigallocatechin gallate (3), caffeine (4), rutin (5), quercetin (6), gallic acid (7), ellagic acid (8), caffeic acid (9), and ferulic acid (10) in the leaves of green tea (*Camellia sinensis*) and guava (*Psidium guajava*) on silica gel with toluene - acetone - formic acid 5:4:1 for compounds (1) - (6) and toluene - ethyl acetate - formic acid - methanol 15:15:4:1 for compounds (7) - (10). Quantitative determination by absorbance measurement at 282 nm for compounds (1) - (6) and 285 nm for compounds (7) - (10). The hR_F values of compounds (1) - (10) were 49, 37, 26, 60, 8, 66, 49, 34,62 and 70, respectively. Linearity was between 100-350 ng/band for compounds (1) - (5), 66.6-233.2 ng/band for compound (6) and between 50-300 ng/band for compounds (7) - (10). The limits of detection were found to be 60 ng/band for compounds (1) - (3), 30 ng for compounds (4), (5) and (8), 40 ng/band for compound (6), 20 ng/band for compound (7) and 10 ng/band for compounds (9) and (10). The limits of quantification were 100 ng/band for compounds (1) - (3), 60 ng/band for compounds (4) - (7), 30 ng/band for compounds (9) - (10), and 75 ng/band for compound (8). Inter- and intraday precisions were below 1.50 % and 2.84 %, respectively. Recoveries were found in the range of 95-100 %.

herbal, HPTLC, quantitative analysis, densitometry 32e

107 126 L. KOMSTA*, R. SKIBINSKI, Anna BERECKA, Anna GUMIENICZEK, B. RADKIEWICZ, M. RADON (*Dept. of Medicinal Chemistry, Medical University of LUblin, Jaczewskiego 4, 20-090 Lublin, Poland): Revisiting thin-layer chromatography as a lipophilicity determination tool - a comparative study on several techniques with a model solute set. J. Pharm. Biomed. Anal. 53, 911-918 (2010). Comparative study on several approaches of TLC lipophilicity determination with the goal of standardization: single TLC runs, extrapolation of retention, principal component analysis of a retention matrix, PARAFAC on a three-way array and a PLS regression. All techniques were applied to 35 simple model solutes (e.g. benzoic acid, caffeine, benzocaine, isoniazide) using nine concentrations of six modifiers (acetonitrile, acetone, dioxane, propan-2-ol, methanol and tetrahydrofurane). Methanol and dioxane were most suitable as modifiers, while acetonitrile provided no suitable correlation of retention with lipophilicity. The approach of single TLC runs provided surprisingly good results. The chemometric processing methods (PCA, PARAFAC and PLS) did not show any advantage compared to classical methods. There is a need to use robust regression and correlation measures due to presence of significant outliers.

pharmaceutical research 32a

107 127 X. LI (Li Xia) (Pharm. Preparation Section, The Second People's Hosp. of Xiangtan, Hunan Province, Xiangtan 411100, China): (Establishment of a method for determining rhaponitin in Sihuang Xiehuo tablets and Maren pills) (Chinese). J. Chinese Modern Med. & Pharm. 17 (34), 52-53 (2010). TLC on silica gel with chloroform - ethyl acetate - methanol - formic acid 2000:25:50:1. Detection under UV 366 nm. Identification of rhaponitin in both medicines by comparison with the standard.

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

107 128 L. LIU* (Liu Liangyu), H. ZHU (Zhu Hong), J. LAI (Lai Juanhua) (*Jiangxi Inst. Pharm., Nanchang 330029, China): (Study of the identification of Shujinhuoxue pills by thin-layer chromatography) (Chinese). J.of Jiangxi Univ. of TCM 22 (5), 55-57 (2010). TLC of Shujinhuoxue pills: 1) for Angelica sinensis, on silica gel with cyclohexane - ethyl acetate 12:1, detection under UV 365 nm; 2) for Rheum officinale, on silica gel with petroleum ether (30-60 ºC) - ethyl formate - formic acid 15:5:1, detection by exposure to ammonia vapors; 3) for Radix *Rehmanniae praeparata*, on silica gel with petroleum ether (60-90 ºC) - ethyl acetate 1:1, detection under UV 254 nm; 4) for *Gardenia jasminoides*, on silica gel with ethyl acetate - acetone - formic acid - water 5:5:1:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 110 ºC until the zones were visualized; 5) for Lignum Sappan, on polyamide phase with 36 % acetic acid, detection by spraying with 5 % $AlCl_3$ in ethanol and heating mildly until the spots were visualized.

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

107 129 S.V. LONDHE*, S.V. MULGUND, R.S. DESHMUKH, K. S. JAIN (*Sinhgad College of Pharmacy, Dep. of Pharm. Chem., Vadgaon, Pune 411041, India): Simultaneous HPTLC analysis of aspirin, atorvastatin calcium and clopidogrel bisulphate in the bulk drug and in capsules. Acta Chromatographica 22 (2), 297-305 (2010). Description of a simple, precise, and accurate method for simultaneous quantification of aspirin, atorvastatin calcium and clopidogrel bisulphate by HPTLC on silica gel with toluene - methanol - formic acid 65:35:1. The hR_F values were 26, 47, and 78 for aspirin, atorvastatin calcium, and clopidogrel bisulphate, respectively. Quantification by densitometry at 254 nm. The precision intra-day and inter-day was in the ranges of 0.2-0.7 *%RSD* and 0.5-1.0 *%RSD* for aspirin, 0.4-0.9 *%RSD* and 0.4-0.6 *%RSD* for atorvastatin calcium,

and 0.3-0.7 *%RSD* and 0.4-0.9 *%RSD* for clopidogrel bisulphate.

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

107 130 J. LONG* (Long Jinyuan), X. LU (Lu Xiaoling) (*Lianyuan People's Hosp., Lianyuan, Hunan 417100, China): (Study of the identification of Biyanling tablets by thin-layer chromatography) (Chinese). Chinese J. Mod. Drug Appl. 4(15), 16-17 (2010). TLC on silica gel 1) with *n*-butanol - glacial acetic acid - water 4:1:5 for *Xanthium sibiricum Patr.*, detection by exposure to iodine vapors; 2) with trichloromethane - diethyl ether 5:1 for Flos magnoliae, detection by spraying with 10 % sulfuric acid in ethanol and heating at 90 °C until the zones were visualized; 3) with *n*-butanol - ethyl acetate 17:3 for herba Menthae, detection by spraying with vanillin reagent and heating at 105 °C until the zones were visible; 4) with petroleum ether (30-90 °C) - ethyl acetate 17:3 for *Angelica dahurica* (Fisch. ex Hoffm.) Benth. et Hook. f. ex Franch. et Sav., detection under UV 366 nm.

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

107 131 V.K. MAHAJAN*, S.B. BARI, A.A. SHIRKHEDKAR, S.J. SURANA (*R.C. Patel College of Pharmacy, Shirpur, Dist. Dhule (M.S.), 425 405 India): Simultaneous densitometric TLC analysis of aceclofenac, paracetamol, and chlorzoxazone in tablets. Acta Chromatographica 20(4), 625-636 (2008). TLC of of aceclofenac, paracetamol, and chlorzoxazone on silica gel (prewashed with methanol) with toluene - 2-propanol - ammonia 10:10:1. Detection and quantification by densitometry at 274 nm. The hR_F values of aceclofenac, paracetamol, and chlorzoxazone were 28, 72 , and 51, respectively. The linearity was in the range of 400-1400 ng/band for aceclofenac, 2-7 µg/band for paracetamol, and 1-3.5 µg/band for chlorzoxazone, with r=0.9995, 0.9993, and 0.9996, respectively. The recovery of aceclofenac was 99.5-100.4 %, for paracetamol 100.0-100.5 %, and for chlorzoxazone 99.4-99.8 %.

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

107 039 M. MEHTA et al., see section 8

107 132 X. MIAO* (Miao Xiaolou), Y. LI (Li Yun), H. PAN (Pan Hu), Y. YANG (Yang Yaoguang), P. SU (Su Peng), Y. WANG (Wang Yu), Z. JIAO (Jiao Zenghua) (*Key Lab. Animal Med. Proj., Lanzhou Inst. Animal & Veterinary Pharm. Sci., Chinese Acad. Agr. Sci., Lanzhou, Gansu 730050, China): (Determination of stachydrine in Gongkang perfusion by thin-layer chromatography) (Chinese). J. Trad. Chinese Veterinary Med. (5), 53-55 (2010). TLC on silica gel with acetone - ethanol - hydrochloric acid 10:6:1. Detection by spraying with bismuth potassium iodide - 1 % $FeCl_3$ in ethanol 5:1 and heating at 100 ºC. Quantitative determination of stachydrine by absorbance measurement at 510 nm. The precision was 3.7 *%RSD* within plate (n=8), and the stability of the measurement within 120 minutes was 4.5 *%RSD* (n=5). The linearity range was 3.2-38.3 µg/zone (r=0.997, n=6) and standard addition recovery was 96.6 % (*RSD*=2.0 %, n=6).

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

107 133 D.H. NAGORE*, V.K. GHOSH, M.J. PATIL, A.M. WAHILE (*Tulip Lab Pvt. Ltd. F-20/21 MIDC Ranjangaon, Tal-Shirur, Pune 412220, India): Validated HPTLC method for quantification of epicatechin in extracts of leaves of *Cassia fistula Linn.* Acta Chromatographica 22 (2),

259-265 (2010), DOI:10.1556/AChrom.22.2010.2.8. Description of a new, simple, precise, and accurate method for quantification of (-)-epicatechin in the leaves of *Cassia fistula* by HPTLC on silica gel with toluene - ethyl acetate - formic acid - methanol 205:3:1:1. Quantification by densitometry at 280 nm. The linearity was in the range of 200-800 ng/band. Method precision was 1.4 *%RSD* and instrumental precision 1.1 *%RSD*. Recovery was 98.1 % and specificity regarding matrix was given.

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

107 134 Ciara O'SULLIVAN, J. SHERMA* (*Department of Biology, Lafayette College, Easton PA 18042-1782, USA): Transfer of thin-layer chromatography pharmaceutical product screening methods designed for use in developing countries to quantitative high-performance TLC densitometry methods. Abstracts, 42nd Middle Atlantic Regional Meeting of the American Chemical Society, College Park MD, USA, May 21-24 (2011). The four TLC methods for acetaminophen, acetylsalicylic acid, ibuprofen, and chlorpheniramine maleate contained in the Compendium of methods developed by A.S. Kenyon and T.P. Layloff at the US FDA for use in countries with limited resources were transferred to quantitative HPTLC. The used sample preparation methods provide suitable calibration curves covering the range of 70-130 % of the label value of the products. Quantitative determination by absorbance measurement at 254 nm.

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

107 135 Y. PAN* (Pan Yanrong), X. WEI (Wei Xiaorui) (*Xuchang Inst. for Drug Contr. of Henan Prov., Henan, Xuchang 461000,China): (Study on the analysis of Ziyinzhike capsules by thin-layer chromatography) (Chinese). J. Chinese Modern Med. & Pharm. 18 (1), 40-42 (2011). TLC on silica gel with petroleum ether (60-90 ºC) - ethyl acetate 1:1. Detection under UV 254 nm. Identification by comparison of the fingerprint of the main component, *Rehmanniae Radix*.

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

107 136 H.J. PANCHAL*, B.N. SUHAGIA (*Shree S.K. Patel College of Pharm. Educ. & Research, Ganpat Vidyanagar, Kherva, Mehsana 382711 Gujarat, India): Simultaneous analysis of atorvastatin calcium and losartan potassium in tablet dosage forms by RP-HPLC and HPTLC. Acta Chromatographica 22 (2), 173-187 (2010), DOI:10.1556/AChrom.22.2010.2.2. HPTLC on silica gel with methanol - carbon tetrachloride - ethyl acetate - glacial acetic acid 80:636:280:4. The hR_F values were 45 and 30 for atorvastatin calcium and losartan potassium, respectively. Quantification by densitometry at 238 nm. Linearity was in the range of 50-500 ng/band for each substance. The recoveries were 100.6 % and 100.5 % for atorvastatin calcium and losartan potassium, respectively. No interference from excipients was observed. The results were compared statistically using a paired t-test with results by an RP-HPLC method. Both methods provided comparable results.

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

107 137 D.B. PATEL*, N.J. PATEL (*Ganpat Univ., Dep. of Pharm. Chem., S.K. Patel College of Pharm. Educ. & Res., Kherva, Mehsana 382711 Gujarat, India): Validated reversed-phase high-performance liquid chromatographic and high-performance thin-layer chromatographic methods for si-

multaneous analysis of tamsulosin hydrochloride and dutasteride in pharmaceutical dosage forms Acta Chromatographica 22 (3), 419-431 (2010), DOI:10.1556/AChrom.22.2010.3.6. Simultaneous analysis of tamsulosin hydrochloride and dutasteride in tablet formulations by HPTLC on silica gel with toluene - methanol - triethylamine 18:3:2. Quantification by densitometry at 280 nm over the concentration range 200-2000 ng/band for both drugs. The recovery was 99.7 % and 100.1 % for tamsulosin hydrochloride and dutasteride, respectively.

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

107 138 K.K. ROUT*, S.K. MISHRA, J. SHERMA (*Utkal Univ. Pharm. & Phytochem. Div., Univ. Dep. of Pharm. Sci., Bhubaneswar 751004 Orissa, India): Development and validation of an HPTLC method for analysis of zerumbone, the anticancer marker from *Zingiber zerumbet*. Acta Chromatographica 21(3), 443-452 (2009). HPTLC on silica gel with ethyl acetate - hexane 3:17. Detection and quantification by densitometry at the maximum absorbance wavelength of 250 nm. The linearity was in the range of 60-260 ng/zone with r=0.9997. The limits of detection and quantification were 20 and 60 ng/zone, respectively. The precision and repeatability of the method were found to be 0.8 and 1.1 %, respectively. Recovery ranged from 97.9-100.1 %. The maximum zerumbone content in the rhizome was 1.81 %.

pharmaceutical research, clinical chemistry research, quantitative analysis, qualitative identification, HPTLC, densitometry 32e

107 139 A. RUIKAR, R. JADHAV, A. TAMBE, A. MISAR, A. MUJUMDAR, V. PURANIK, N. DESHPANDE (Dr. T. R. Ingle Research Lab., Dept. of Chemistry, Sir Parashurambhau College, Pune 411030, India, anjaliruikar07@yahoo.com): Quantification of santonin from Artemisia pallens Wall by HPTLC. International Journal of Pharma and Bio Sciences 1(1), 1-3 (2010). Shade dried aerial parts of the plant were extracted with acetone (A) and methanol (B) and the solvent was removed to get the crude extract. Extract A was further fractioned over silica gel (60-120) by eluting with *n*-hexane (C) and *n*-hexane - acetone 9:1(D) . TLC of all fractions on silica gel with *n*-hexane - ethyl acetate. Quantitative determination by absorbance measurement at 258 nm. The linearity was in the range of 1-5 µg/band. The amount of santonin found in different fractions of the acetone extract was 31.3 mg/g (A), 40.7 mg/g (B), 1.9 mg/g (C), and 20.9 mg/g (D).

herbal, densitometry, quantitative analysis 32e

107 140 M.R. SENGAR*, S.V. GANDHI, U.P. PATIL, V.S. RAJMANE, K.G. BOTHARA (*A.I.S.S.M.S. College of Pharm. Dep. of Pharm. Anal., Kennedy Road, Pune 411001, India): A validated densitometric TLC method for analysis of cefuroxime axetil and potassium clavulanate in combined tablet dosage forms. Acta Chromatographica 22 (1), 91-97 (2010), DOI:10.1556/AChrom.22.2010.1.7. TLC on silica gel with chloroform - methanol - toluene 4:3:3. The hR_F value was 77 and 29 for cefuroxime axetil and potassium clavulanate, respectively. Quantification by densitometry at 225 nm. The linearity was in the range of 0.5-2.5 and 2-10 µg/band, respectively. Application of the method for analysis of the drugs in a pharmaceutical formulation with a recovery of 100.1 % for cefuroxime axetil and 99.9 % for potassium clavulanate.

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

107 141 R. SHARMA*, R. GUPTA, I. SINGH (*Dept. of Natural Products, National Institute of Pharmaceutical Education & Research (NIPER), Mohali 160062, India, ramji_np2007@yahoo.com): Densitometric determination of anthocyanins in *Eugenia jambolana*. 2nd International Con-

ference on New Development in Drug Discovery from Natural Product & Traditional Medicine PP82, 82 (2010). *Eugenia jambolana* pulp was dried in vacuum and enriched by chromatography on XAD 7HP ion-exchange resin, followed by Sephadex LH 20. HPTLC of both enriched extracts on silica gel with ethyl acetate - formic acid - acetic acid - water 100:11:11:26. Quantitative determination by absorbance measurement at 520 nm. The vaccum dried pulp and the enriched extracts 1 and 2 were found to contain 0.08 %, 17 % and 10 % of anthocyanins, respectively. Malvidin-3-laminariobioside was used as marker compound for quantitative analysis.

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

107 142 J. SHI* (Shi Junhan), X. NIW (Niw Xiaojing) (*The First Affil. Hosp. of Henan Univ. of TCM, Zhengzhou 450000, China): (An improved method for identification of Weizhangshu compound oral liquid by thin-layer chromatography) (Chinese). J. of Qilu Med. & Pharm. 29(11), 658-659 (2010). TLC on silica gel with 1) benzene - methanol 27:1; 2) toluene - methanol 17:1 ; 3) cyclohexane - propanone 10:3:4) petroleum ether (60-90 ºC) - ethyl acetate - formic acid 85:15:2, or 80:20:1. Detection by spraying with 1 % vanillin in sulfuric acid and heating at 100 °C until the zones were visualized. Identification by comparison of the fingerprint with the characteristic reference standards magnolol and honokiol. System 4) provided the best separation.

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

107 143 A.A. SHIRKHEDKAR*, R.R. THORVE, R.A. FURSULE, S.J. SURANA (*R.C. Patel College of Pharmacy Shirpur 425 405, M.S., India): Development and validation of a stability-indicating HPTLC method for analysis of rupatadine fumarate in the bulk drug and tablet dosage form. Acta Chromatographica 20(3), 423-437 (2008). HPTLC of rupatadine fumarate on silica gel with toluene - methanol - triethylamine 20:5:1. The hR_F value was 61. Quantitative determination by absorbance measurement at 264 nm. The linearity was in the range of 400-1400 ng/band (r=0.9992). The limits of detection and quantitation were 67 and 202 ng/band, respectively. Moreover, rupatadine fumarate was subjected to acid and alkaline hydrolysis, oxidation, and photochemical and thermal degradation and underwent degradation under all these conditions. The method proved to be repeatable, selective, and accurate for the analysis of the drug by statistical analysis, and is able to separate the degradation products from the drug.

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

107 144 C. SINDHU (Noida Institute of Engineering & Technology, Greater Noida, Uttar Pradesh 201306, India, phdgs77@indiatimes.com): Phytochemical screening of *Calendula officinalis Linn* leaf extract by TLC. International J. Research in Ayurveda & Pharmacy 1(1), 131-134 (2010). Dried leaves of *Calendula officinalis* were extracted with petroleum ether, chloroform, methanol and water, the solvents were removed and the extracts were subjected to phytochemical analysis for amino acids, essential oils, triterpens, alkaloids, saponins, sterols, and fatty acids. TLC on silica gel with *n*-hexan - acetic acid - water 12:3:5, detection by spraying with ninhydrin solution, followed by heating at 105 °C revealed violet bands which indicated the presence of amino acids. For essential oils TLC on silica gel with dichloromethane - chloroform - ethyl acetate - *n*-propanol 94:90:4:5, followed by spraying with vanillin-sulfuric acid reagent and heating at 105 °C for 2 min. Pink brown coloured zones indicated the presence of essential oils. For triterpenoids TLC on silica gel with n-butanol - 2M ammonia 1:1 , detection by spraying with antimony trichloride solution. Purple coloured zones indicated the presence of triterpenoids. For alkaloids TLC on silica gel with chloroform - methanol 1:1, detection by alkaloids-reagent. For saponins TLC on

silica gel with chloroform - methanol 12:1, detection by spraying with vanillin-sulfuric acid reagent. For sterols TLC on silica gel with chloroform - methanol 3:4, detection by anisaldehyde reagent. For fatty acids TLC on silica gel with *n*-hexane - ethyl acetate 19:1, detection by $KMnO_4$ reagent.

herbal, qualitative identification 32e

107 145 ZH. SU* (Su Zhijian), CH. JIANG (Jiang Changming), Y. XIAO (Xiao Yuqin) (*Xiamen Munic. Hosp., Xiamen, Fujian 361009, China): (Study on the quality standard of Yinhu granules) (Chinese). J. Strait Pharm. 22(11), 90-92 (2010). TLC of components of Yinhu granules: 1) for *Artemisia capillaris Thunb.* on polyamide phase with acetic acid; 2) for Radix Notoginseng on silica gel with chloroform - methanol - water 13:7:2. Detection 1) under UV 366 nm; 2) by spraying with 10 % sulfuric acid in ethanol and heating at 105 ºC until the zones were visualized.

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

107 014 CH. TISTAERT et al., see section 1

107 146 R. VELHO-PEREIRA, C. BARHATE, S. KULKARNI, A. JAGTAP* (*Department of Pharmacology, Bombay College of Pharmacy, Mumbay 400098, India, jagtaparti@gmail.com): Validated high-performance thin-layer chromatography method for the quantification of thymoquinone in *Nigella sativa* extracts and formulations. Phytochem. Anal. 22, 367-373 (2011). HPTLC of thymoquinone in the seeds of *Nigella sativa* on silica gel with toluene - cyclohexane 4:1. Quantitative determination by absorbance measurement at 254 nm. The hR_F of thymoquinone was 28. The linearity range was 100-1400 ng/zone. The limit of detection and limit of quantification was 50 and 150 ng/spot, respectively. Inter- and intraday precisions were 1.6 and 2.4 % (n=6), respectively. Recovery (by standard addition) was 100.1 %. The method is reproducible and selective for the analysis of thymoquinone with added advantages of low cost of reagents, speed and minimal sample preparation, satisfactory precision and accuracy.

herbal, HPTLC, quantitative analysis, densitometry 32e

107 147 J. WANG, J. SHIN, M. CHOI, H. KIM, C. SON* (*Liver and Immunology Research Center, Daejeon Oriental Hospital of Daejeon University, 22-5 Daeheung-dong Jung-gu, Republic of Korea, ckson@dju.ac.kr): An herbal fruit, *Amomum xanthoides*, ameliorates thioacetamide-induced hepatic fibrosis in rat via antioxidative system. J. Ethnopharmacol. 135, 344-350 (2011). HPTLC of *Amomum xanthoides* fruit on silica gel with hexane - acetone 1:1. Detection by spraying with 4 % vanillin sulfuric acid and evaluation under white light. The sample showed a similar hR_F value as borneol.

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

107 148 R. WANG* (Wang Rui), Q. JIA (Jia Qi), L. GU (Gu Lihua), Z. ZHANG (Zhang Zijia), ZH. WANG (Wang Zhengtao), Y. LI (Li Yiming) (*School of Pharm., Shanghai University of TCM, Shanghai 201203, China): (Application of thin-layer chromatography/bioautography in chemical education laboratory for the analysis of traditional Chinese medicine) (Chinese). J. of Guangzhou Chem. Engin. 39(1), 144-145 (2011). A course on the technology of TLC/bioautography applied for the analysis of TCM was set-up to enhance students‘ understanding of theoretical knowledge and to train and improve the interest and skill of students in chemical experiments. Demonstrati-

on of the TLC analysis of rutin and quercetin in *Flos Sophorae* on silica gel with ethyl acetate - formic acid - water 8:1:1. Detection under UV 254 nm and 366 nm, and by immersing into a solution of 1,1-diphenyl-2-picrylhydrazyl in ethanol (DPPH radical reagent). The result of this practice was satisfactory, and the course proved to be a good example to utilize the modern technology in the experimental teaching.

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

107 149 X. WANG* (Wang Xiaofei), L. YU (Yu Ling), H. DU (Du Huashuang), J. WANG (Wang Jie) (*Inst. for Drug Contr. of People's Armed Police Forces, Beijing 102613, China): (Optimization of the procedure for identification of quercetin in Herba Saururi Chinensis by thin-layer chromatography) (Chinese). Chinese J. of Ethnomed. & Ethnopharm. (23), 61-64 (2010). Optimization of the sample preparation procedure for Herba *Saururi chinensis* 1) by ultrasonication with methanol for 60 min; 2) by ultrasonication with methanol for 20 min and filtration through neutral alumina column with methanol; 3) by reflux extraction with 80 % methanol for 60 min and extraction with diethyl ether; 4) by ultrasonication with ethanol for 60 min; 5) by ultrasonication with methanol - 25 % hydrochloric acid 4:1 for 60 min and extraction with ethyl acetate. Procedure 5) was best suited. TLC on silica gel 1) with petroleum ether (60-90 °C) - acetone 5:2, and detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 ºC until the zones appear; 2) with toluene - ethyl acetate - formic acid 5:2:1, and detection by spraying with 1 % $AlCl_3$ in ethanol and evaluation under UV 366 nm; 3) with *n*-hexane - ethyl acetate - formic acid 70:50:8, and detection by spraying with 1 % $AlCl_3$ in ethanol, heating at 105 ºC and evaluation under daylight or under UV 366 nm; 4) with toluene - ethyl acetate - formic acid 5:4:1 with chamber saturation with hydrochloric acid vapor, detection under daylight or UV 366 nm.

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

107 150 X. WEN* (Wen Xianmin), M. YANG (Yang Miannan) (*Res. Cent. of Natural Drugs, Yunnan Mingyang Pharm. Co., Kunming 650200, China): (Study of the quality standard for Cishushi suppository) (Chinese). Yunan J. of Chinese Trad. Med. & Pharm. 31(10), 58-60 (2010). TLC of extracts of Cishushi suppository: 1) for *Panax notoginseng*, on silica gel with chloroform - methanol - water 14:6:1, detection by spraying with 10 % sulfuric acid in ethanol and heating at 105 ºC; 2) for *Fructus Cnidii*, on silica gel with petroleum ether (60-90 °C) - ethyl acetate 7:3, detection under UV 366 nm; 3) for Borneolum Syntheticum, on silica gel with toluene - ethyl acetate 19:1, detection by spraying with 5 % vanillin-sulfuric acid reagent and heating at 105 °C; 4) for *Fructus Sophorae*, on silica gel with chloroform - methanol - water - formic acid 700:300:50:1, detection by spraying with 1 % $AlCl_3$ in ethanol, heating and evaluation under UV 366 nm.

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

107 151 D. YADAV, N. TIWARI, M. GUPTA* (*Analytical Chemistry Department, Central Institute of Medicinal and Aromatic Plants, Uttar Pradesh 226015, India, guptammg@rediffmail.com) : Simultaneous quantification of diterpenoids in *Premna integrifolia* using a validated HPTLC method. J. Sep. Sci. 34, 286-291 (2011). HPTLC of 1beta,3alpha,8beta-trihydroxy-pimara-15-ene (1), 6alpha,11,12,16-tertahydroxy-7-oxo-abieta-8,11,13-triene (2) and 2alpha,19-dihydroxy-primara-7,15-diene (3) in the root bark of Premna integrifolia on silica gel with hexane - acetone - ethyl acetate 3:1:1. Detection by dipping into vanillin-sulfuric acid reagent (2 g vanillin in 190 mL ethanol with 10 mL sulfuric acid) followed by air drying and heating for 3 min at 110 °C.

Quantitative determination by absorbance measurement at 475 nm. The hR_F values of (1), (2) and (3) were 58, 44, and 32, respectively and selectivity regarding matrix was given. Linearity was between 1-10 µg/spot for (1), (2) and (3), respectively. The limits of detection were found to be 230, 106 and 336 ng/band for compounds (1), (2) and (3), respectively, whereas the limits of quantification were 769, 354 and 1122 ng/band, respectively. Inter- and intraday precisions were 0.9-1.3 % and 1.2-1.24 %, respectively. The average recoveries for compounds (1) to (3) were found to be 100.6, 103.9 and 97.6 %, respectively, within the acceptable *%RSD*.

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

107 152 X. YANG* (Yang Xuming), J. ZHANG (Zhang Jiali), J. LI (Li Jianghua) , J. FANG (Fang Jun) (*School of Med. & Pharm., Jiangnan Univ., Wuxi, Jiangsu 214122, China): (Determination of gentamicin in fermentation broth by thin-layer chromatography) (Chinese). J. of Food Sci. & Biotechnol. 27(5), 129-133 (2008). TLC of gentamicin on silica gel with the lower phase of chloroform - methanol - 25 % ammonia 5:4:3 and after chamber saturation with the upper phase of the developing solvent. Detection by exposure to iodine vapor. Identification by comparison of the hR_F values with the standards of the main components of gentamicin (Cl, C1 and C2). The results were compared with results obtained by HPLC and good agreement between both methods was found.

pharmaceutical research, quality control, quantitative analysis, qualitative identification, comparison of methods 32c

35. Other technical products and complex mixtures

107 153 Elisabeth DYTKIEWITZ, W. SCHWACK* (*University of Hohenheim, institute of Food Chemistry, Garbenstrasse 28, 70599 Stuttgart, Germany, wolfgang.schwack@uni-hohenheim.de): Determination of additives in plastic foils. CBS 105, 13-15 (2010). HPTLC of PVC foil samples on silica gel with isooctane - toluene - diethyl ether - ethyl acetate 8:7:4:1 after chamber saturation for 10 min, up to a migration distance of 65 mm. Detection of the biological activity of any compound migrated from the plastic foils in migration studies by dipping in *Vibrio fischeri* bacteria suspension and documentation with the Bioluminizer. Also direct extraction of additives from plastic foils by the TLC-MS Interface coupled to an Agilent LC-MS system and recording of the eluted additives in the positive ESI mode. Exact masses of unknowns were calculated with MassWorks software allowing their improved specification and thus their confirmation.

food analysis, quality control, HPTLC, quantitative analysis, qualitative identification, bioassay 35

107 154 S.N. FEDOSOV*, J. BRASK, X. XU (*Dept. Molecular Biology, Aarhus Univ., Science Park, Gustav Wieds Vej 10C, 8000 Aarhus C, Denmark): Analysis of biodiesel conversion using thin-layer chromatography and nonlinear calibration curves. J. Chromatogr. A 1218 (19), 2785-2792 (2011). Examination of the applicability of TLC for the analysis of biodiesel conversion. Biodiesel is a complex mixture which complicates the analytical separation and requires a large set of data for understanding reaction kinetics. A flame ionization detector (FID) and a modified TLC staining procedure were evaluated in comparison with the well-established but time-consuming and expensive GC and HPLC methods. The TLC staining method is suited for quantitative analysis due to no background. Demonstration by using several experimental samples produced by enzymatic conversion of rapeseed oil to biodiesel. It was found that the first reaction step (6 h) resulted in 85-95 % conversion and the second step (after removal of glycerol and water) increased the yield to 97-98 %. All components of the mixtures were separated and quantified. Relation of the biodiesel contents measured by TLC and GC gave the values of 1.03 ± 0.07 (TLC-staining)

and 0.95 ± 0.04 (TLC-FID), which indicated the applicability of the TLC methods.

quantitative analysis, comparison of methods 35

107 009 C. NEUMANN et al., see section 1

37. Environmental analysis

107 155 Gertrud MORLOCK*, L. SCHUELE, S. GRASHORN (*Univ. of Hohenheim, Inst. of Food Chem., Garbenstrasse 28, 70599 Stuttgart, Germany): Development of a quantitative high-performance thin-layer chromatographic method for sucralose in sewage effluent, surface water, and drinking water. J. Chromatogr. A 1218 (19), 2745-2753 (2011). HPTLC of sucralose in waste water on silica gel with isopropyl acetate - methanol - water 15:3:1. The developing time was 15 min. Detection with *p*-aminobenzoic acid reagent. Quantification by absorbance measurement at 400 nm. The limit of quantification was 100 ng/L at a recovery rate of 80 % and the extraction of a 0.5 L water sample. An interlaboratory trial in 2008 showed good agreement of the sucralose content determined in four water samples by HPTLC and other methods (HPLC-MS/MS or HPLC-TOF-MS). The good accuracy and high sample throughput capacity proved HPTLC as a well suited method for quantification of sucralose in various aqueous matrices.

environmental, HPTLC, quantitative analysis, qualitative identification, postchromatographic derivatization, comparison of methods, densitometry, sweetener, mass spectrometry 37c

38. Chiral separation

107 156 M. DEL BUBBA*, A. CINCINELLI, L. CHECCHINI, L. LEPRI (*Dep. of Chem., Univ. of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy): Chiral separations and quantitative analysis of optical isomers on cellulose tribenzoate plates. J. Chromatogr. A 1218 (19), 2737-2744 (2011). Investigation of new cellulose tribenzoate/gypsum layers in the ratio up to 8:1 (w/w) for the chiral resolution of closely related aromatic ketones (e.g. tetralones and indanones), alcohols (e.g. benzhydrols) and racemates or enantiomers of other compound classes (e.g. dinitrophenyl amino acids). 16 racemates were baseline or partially resolved by eluting with methanol or 2-propanol/water mixtures on 4:1 (w/w) layers among 22 investigated compounds. The study provided better understanding of the retention and resolution mechanisms on this chiral stationary phase, however, some results were unexpected and confirmed the complexity of enantioseparation mechanisms. Evidence from experimental tests is necessary. Quantification of the investigated compounds by densitometry in the visible region of cellulose tribenzoate/gypsum plates after their exposure to iodine vapours.

densitometry, qualitative identification, quantitative analysis 38

107 157 M. SAJEWICZ*, E. JOHN, D. KRONENBACH, M. GONTARSKA, M. WRÓBEL, T. KOWALSKA (*Silesian University, Inst. of Chem., 9 Szkolna Street, 40-006 Katowice, Poland): How to suppress the spontaneous oscillatory in-vitro chiral conversion of a-substituted propionic acids? A thin-layer chromatographic, polarimetric, and circular dichroism study of complexation of the Cu(II) cation with L-lactic acid. Acta Chromatographica 21(1), (2009) . This study focused on the attempt to suppress the spontaneous oscillatory in-vitro chiral conversion of a-substituted propionic acids using, as example, L-lactic acid dissolved in water in the presence of copper(II) cations to check whether the coordinate covalent bonds between copper(II) and L-lactic acid ligands prevented the latter species from oscillatory chiral conversion. Aqueous solutions of copper(II) acetate and lactic acid in the molar ratios 1:1, 1:2, and 1:3 were stored and the possible chiral conversion of L-lactic acid was monitored by TLC, polarimetry, and circular dichroism spectroscopy. It was found that chelating of copper(II)cations with L-lactic acid did not result in

suppression of the spontaneous oscillatory in-vitro chiral conversion of the acid from the TLC data. Different molar proportions of copper(II) cation and L-lactic acid had somewhat different effects on the dynamics of conversion, in contrast, when L-lactic acid is dissolved in water in the presence of copper(II)cations almost no chiral conversion is observed from polarimetric and circular dichroism studies. It was therefore concluded that chelating of copper(II) cations with L-lactic acid stabilizes the chiral structure of the acid in solution. The structure-stabilizing effect of copper(II) cations is weakened by the TLC system due to the interaction of the copper(II)-L-lactic acid complex with the silica gel.

qualitative identification, quantitative analysis, densitometry 38

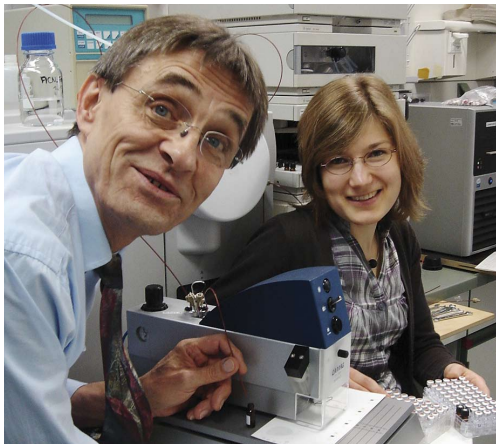
107 158 M. SAJEWICZ*, E. JOHN, D. KRONENBACH, M. GONTARSKA, T. KOWALSKA (*Silesian University, Institute of Chemistry, 9 Szkolna Street, 40-006 Katowice, Poland): TLC study of the separation of the enantiomers of lactic acid. Acta Chromatographica 20(3), 367-382 (2008). Investigation of the separation of the enantiomers of D,L-lactic acid with transition metal cations (i.e., Co^{2+}, Ni^{2+}, and Mn^{2+}, rather than Cu^{2+} as stated in the literature) used to impregnate the silica gel. The goal was first to achieve a resolution that might enable the quantification of the two lactic acid enantiomers and second to gain deeper insight into the mechanism of separation. For comparison D,L-lactic acid was chromatographed on non-impregnated silica gel, and then efficient separation conditions with the Ni^{2+} and Co^{2+} cations were established, which outperformed the previously reported procedure with Cu^{2+}. The Mn^{2+} cation proved unsuitable for the purpose. The enantiomers of D,L-lactic acid were also separated on non-impregnated silica gel, which seems yet more proof of the microcrystalline chirality of silica gel used as stationary phase and of its substantial contribution to the enantiomer separation investigated.

pharmaceutical research 38

107 159 P. SITADEVI, P. RAO (Analytical Chemistry Div., Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, India, sitadevi@iict.res.in): Development and validation of a method for the enantioseparation of oxybutynin chloride by HPTLC. Analytical Chemistry, An Indian Journal 9(3) (2010). HPTLC of a racemic mixture of oxybutynin chloride on chiral phase with toluene - acetone - methanol 8:1:1. Both enantiomers were well separated with hR_F values of 47 and 63. The identity of the isomers was established by on-line UV, NMR and MS data. The method was validated using NP-TLC and the same mobile phase. The method was linear in the range of 50-350 µg/band with a recovery of 98.2-101.7 %.

pharmaceutical research, quality control, HPTLC, quantitative analysis 38

Planare Festphasenextraktion – ein neues Clean-up Konzept in der Rückstandsanalytik von Pestiziden



10

Prof. Dr. W. Schwack und Claudia Oellig

Ein Forschungsschwerpunkt von Professor Schwack, Universität Hohenheim, liegt in der Methodenentwicklung zur Rückstandsanalytik von Pflanzenschutzmitteln in Obst und Gemüse. Neben der intensiven Beschäftigung mit der speziellen Klasse der Dithiocarbamat-Fungizide geht es dabei um Automatisierung der Extraktion und um Methoden zum Clean-up von Extrakten.

Einleitung

In der EU sind für über 500 Pestizide Rückstandshöchstgehalte in Lebens- und Futtermitteln festgelegt. Zur Überprüfung dieser Grenzwerte für den Verbraucherschutz sind sichere, robuste, schnelle und sensitive analytische Methoden notwendig. In Obst und Gemüse ist eine Vielzahl an störenden Matrixkomponenten enthalten, welche die Signale in der GC-MS und LC-MS stark beeinflussen, meistens Signalsuppressionen bewirken. Der sicherste Weg zur Vermeidung derartiger Matrixeffekte in der Rückstandsanalytik von Pflanzenschutzmitteln (PSM) in Obst und Gemüse ist eine sorgfältige Reinigung der Extrakte. Ziel dieser Arbeit war es daher, unter Einsatz der Planar-Chromatographie eine effiziente, einfache und schnelle Clean-up Methode zur nachfolgenden Bestimmung mittels LC-MS zu entwickeln.

Im Vergleich zu aktuellen Clean-up Methoden wie dispersive oder Kartuschen-SPE in der Rückstandsanalytik von PSM in Lebensmitteln stellt die neue high-throughput planar solid phase extraction (HTpSPE) eine kostengünstige, zuverlässige und schnelle Alternative dar. Sie bietet eine sehr effiziente Reinigung von Extrakten bei geringen laufenden Kosten und einem Lösungsmittelverbrauch von nur 1 mL/Probe. Ein Clean-up von 20 Proben ist parallel in einem Lauf möglich. [1]

Herstellung der Probenextrakte

Bio Obst- und Gemüse-Proben (10 g) wurden nach der QuEChERS-Methode [2] extrahiert.

Pestizid-Standardlösungen

Sieben repräsentative Pestizide kamen zum Einsatz, mit denen die Proben-Rohextrakte auf 0,1 und 0,5 mg/kg dotiert wurden: Acetamiprid, Azoxystrobin, Chlorpyriofos, Fenarimol, Mepanipyrim, Penconazol, Pirimicarb. Als interne Standards wurden Tris(1,3-dichlorisopropyl)phosphat TDCPP (zur Quantifizierung) und Sudan II (zur Sichtbarmachung der Pestizidzonen) verwendet.

Schicht

DC-Aluminiumfolien Kieselgel 60 NH_2 F_{254s} (Merck), 20 x 20 cm, vorgewaschen mit Acetonitril (15 cm); verwendet werden die unteren 20 x 10 cm.

Probenauftragung

Flächenförmig mit DC Probenautomat 4 (ATS 4), Länge 3,0 mm, Höhe 4,0 mm, Bahnabstand 8,5 mm, seitlicher Randabstand 16,5 mm, unterer Randabstand 13 mm, Auftragevolumen 50 µL

Planares Festphasen Clean-up

In der Automatischen Entwicklungskammer ADC 2 mit 10 mL Acetonitril, Laufstrecke 75 mm, Laufzeit 10 min, Trocknungszeit 5 min; nach 180° Drehung der Folie 2. Entwicklung mit Aceton, Laufstrecke 45 mm, Laufzeit 3 min, Trocknungszeit 5 min

Dokumentation des Clean-up

Mit TLC Visualizer bei UV 254 nm, UV 366 nm und Weisslicht-Beleuchtung sowie unter UV 366 nm nach Tauchen in Primulin-Lösung

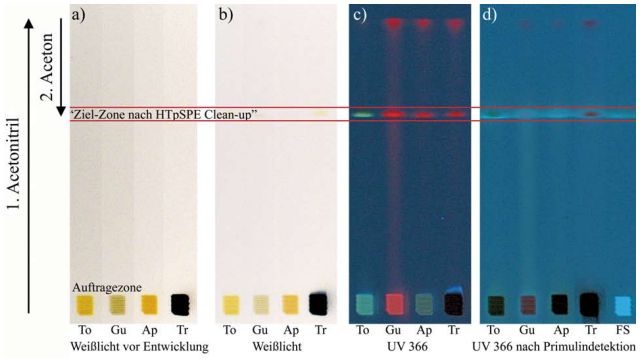
TLC-LC-(ESI)-MS-Kopplung

Extraktion der Zielzone mittels TLC-MS Interface in Autosampler Vials mit Acetonitril/10 mM Ammoniumformiatpuffer (1:1, v/v), Flussrate 0,2 mL/min,

Extraktionszeit 60 Sekunden; Trennung der Pestizide an einer Chromolith Performance RP-18e (100 mm x 3,0 mm, Merck) mit Gradientenelution.

Ergebnisse und Diskussion

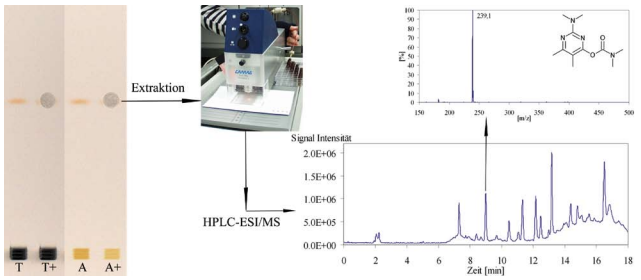
Die Planar-Chromatographie wurde eingesetzt, um PSM-Wirkstoffe und mitextrahierte Matrixkomponenten quantitativ voneinander zu trennen und dabei alle Wirkstoffe in einer Zone zu fokussieren. Durch Variation von Sorbens und Fliessmittelsystem konnte die angestrebte Trennung leicht optimiert werden. Das beste planar-chromatographische Clean-up von QuEChERS-Extrakten verschiedener Obst- und Gemüseproben wurde mit einer Zweifachentwicklung an Aminophasen erzielt. Mit den vielfältigen Möglichkeiten der planar-chromatographischen Detektion kann der Erfolg sichtbar gemacht werden.



11

Trennung von Matrix und Pestiziden auf DC Kieselgel 60 NH_2 F_{254s} Aluminiumfolien (Tomate (To), Gurke (Gu), Apfel (Ap) und Traube (Tr); zum Vergleich Ölsäure (FS)): vor der Entwicklung (a), nach der Entwicklung unter Weisslicht (b), UV 366 (c), UV 366 nach Tauchen in Primulin-Lösung (d).

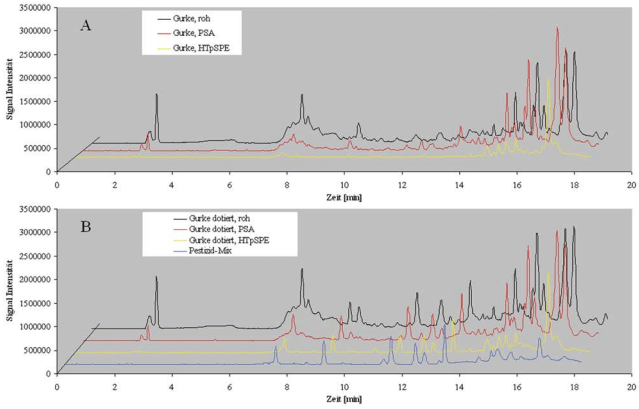
Nach diesem HTpSPE Clean-up wird die Zielzone (Pestizide) mittels TLC-MS Interface in Autosampler-Vials extrahiert oder direkt online auf das LC-MS System übergeben.



12

Extraktion einer Zielzone nach HTpSPE Clean-up mittels TLC-MS Interface mit nachfolgender LC-MS; Total-Ionenstrom-Chromatogramm eines Apfelextraktes dotiert mit einem Pestizid-Mix aus verschiedenen Substanzklassen (A+) und Massenspektrum des Peaks bei 9 min (Pirimicarb, m/z 239,1, $[M+H]^+$).

Auf einer 20 cm-Platte lassen sich leicht 20 Probenextrakte simultan bei einer Entwicklungszeit von 20 min aufreinigen; inklusive Probenauftragung benötigt das gesamte Clean-up 70 min. Mit 3,5 min/Probe sowie dem sehr geringen Lösungsmittelverbrauch von nur 1 mL/Probe liefert HTpSPE eine sehr effiziente und schnelle Alternative zu derzeit gängigen Clean-up Techniken. Der neue Ansatz wurde mit Wirkstoffen aus verschiedenen Substanzklassen in diversen pflanzlichen Matrices erfolgreich geprüft. Im Vergleich zu bisherigen dispersiven SPE-Verfahren sind die Probenextrakte deutlich sauberer und Matrixeffekte nahezu vollständig eliminiert.



13

LC-MS Totalionenstrom-Chromatogramme von Gurkenextrakten nach HTpSPE Clean-up im Vergleich zum Rohextrakt und nach dispersiver SPE (PSA); (A) undotiert, (B) dotiert mit einem Pestizid-Mix (0,5 mg/kg) (Reprinted from [1] with permission from Elsevier).

Wiederfindungen der eingesetzten Wirkstoffe wurden auf zwei Dotierniveaus in verschiedenen Obst- und Gemüsematrices bestimmt. Mittelwerte über alle Wirkstoffe und Matrices von 90–104 % mit exzellenten relativen Standardabweichungen von 0,3–4,1 % ($n = 5$) zeigen, dass das neue HTpSPE Clean-up reproduzierbar und verlustfrei arbeitet.

[1] Oellig, C., Schwack, W. J Chromatogr A (2011), 1218, 6540–6547

[2] www.quechers.com

Weitere Informationen sind von den Autoren auf Anfrage erhältlich.

Kontakt: Claudia Oellig, Institut für Lebensmittelchemie, Universität Hohenheim, 70599 Stuttgart, Claudia.Oellig@uni-hohenheim.de

Quantifizierung und Nebenkomponentenanalyse des kosmetischen Wirkstoffes Tiliroside mittels der Planar-Chromatographie


14


15

Michael Schulz, Susanne Minarik, Michaela Oberle, Sylvia Eisenberg*

Sowohl in der Analytik-Abteilung als auch in der Forschung verwendet Merck die Planar-Chromatographie für verschiedene Aufgaben. Einen hohen Stellenwert hat die HPTLC in der Kosmetikforschung.

Einleitung

Die Planar-Chromatographie bietet speziell für die Analyse natürlicher Kosmetikwirkstoffe viele Vorteile und ein weitreichendes Anwendungsgebiet. Ein Beispiel ist der Kosmetikwirkstoff RonaCare® Tiliroside von Merck, der aus einer Pflanze der Familie Sterculiaceae gewonnen wird. Hier wird mit der HPTLC ein breites Anwendungsspektrum abgedeckt, angefangen bei der Quantifizierung des Wirkstoffs in komplexer Pflanzenmatrix zur Qualitätsbestimmung des Rohstoffs, über die »In-Prozess-Kontrolle« und das Monitoring des Verunreinigungsprofils während des Herstellungsprozesses bis hin zur Quantifizierung des Wirkstoffs im Endprodukt. Die Methode hat sich auch bei Stabilitätsprüfungen bewährt. In dieser Arbeit wird eine Quantifizierung und eine Nebenkomponentenanalyse mit visueller Auswertung am Beispiel des kosmetischen Wirkstoffs Tiliroside vorgestellt.

Die Vorteile eines hohen Probendurchsatzes und einer einfachen Probenvorbereitung machen die Planar-Chromatographie zu einer schnellen und leistungsfähigen Analysenmethode in der Kosmetik. Aufgrund der einmaligen Verwendung der HPTLC-Platte ist das bei der HPLC bestehende Risiko der Kontamination der stationären Phase durch Pflanzenmatrix und Öle des Kosmetikums ausgeschlossen.

Standardlösungen

1. Quantifizierung aus einem Pflanzenextrakt
Tiliroside-Standard in Methanol, 0,5 mg/mL

2. Nebenkomponentenanalyse des Pflanzenextraktes
Tiliroside-Standard in Methanol, 2,6 mg/mL. Tiliroside-Proben 1 und 2, Kaempferol-3-Glucosid, Kaempferol-3-Rutinosid, Kaempferol, Cumarinsäure, jeweils 1 mg/mL in Methanol, Glucose 1 mg/mL in Wasser.

Probenvorbereitung

1. Quantifizierung aus einem Pflanzenextrakt
25 mg Tiliroside wurden in 50 mL Methanol gelöst. Jeweils ca. 5,0 g Pflanzenmaterial wurden 4 mal mit 80 mL Methanol unter Rückfluss extrahiert, die Extrakte über ein Faltenfilter filtriert und nach dem Abkühlen auf Raumtemperatur auf 500 mL aufgefüllt. Die Lösungen wurden nochmals über ein 0,45 µm Membranfilter gegeben.

2. Nebenkomponentenanalyse des Pflanzenextraktes
Die jeweiligen Standards wurden eingewogen und im Ultraschallbad gelöst. Die klaren gelben bzw. farblosen Lösungen wurden über ein 0,45 µm Membranfilter gegeben.

Schicht

HPTLC-Platten Kieselgel 60 F_{254s} Merck, 20 x 10 cm

Probenauftragung

Bandförmig mit DC-Probenautomat 4 (ATS 4), Bandlänge 5 mm, Bahnabstand 10 mm, Abstand vom unteren Rand 10 mm, Auftragevolumen 2 µL

Chromatographie

In der Doppeltrogkammer 20 x 10 cm mit Ethylacetat – Ameisensäure – Eisessig – Wasser (100: 11:11:27) + 1 % Heptan

Postchromatographische Derivatisierung

Mit dem DC-Sprühgerät werden folgende Derivatisierungen durchgeführt:

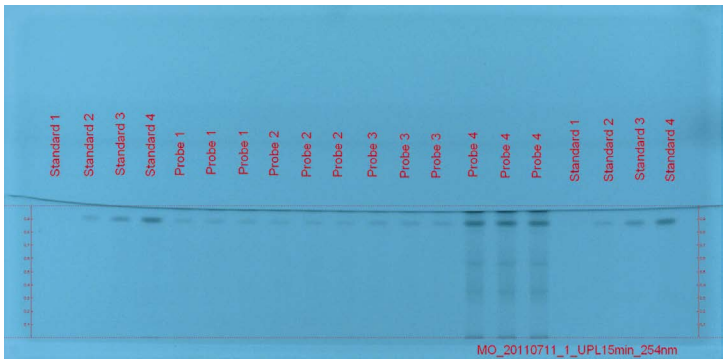
- Naturstoffreagenz nach Neu (NSR): 1 % Diphenylborsäure-2-aminoethylester in Methanol → UV 366 nm
- Anisaldehyd-Schwefelsäure-Reagenz (ASR): 0,5 mL Anisaldehyd in 85 mL Methanol, 10 mL Eisessig und 8 mL konz. Schwefelsäure (zugesetzt unter Eiskühlung) → Platte bei 90 °C für max. 15 min erhitzen → Weisslicht

Densitometrie

CAMAG TLC Scanner 3, Absorptionsmessung bei 315 nm, Spaltgrösse 4 x 0,3 mm, Messgeschwindigkeit 20 mm/s

Ergebnisse und Diskussion

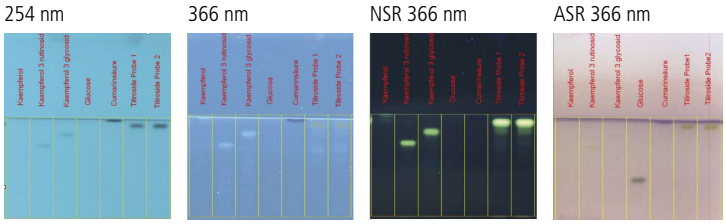
1. Quantifizierung



16

Aufnahme unter UV 254 nm

Die Platte wurde im Adsorptionsmaximum von Tiliroside bei 315 nm gescannt. Die Quantifizierung über die ermittelten Peakflächen ergab nach einer Dreifachbestimmung der Extraktproben und einer 4 Punkte-Kalibrierung einen Gehalt in Extraktprobe 1 von 1,09 µg (*RSD* = 0,4 %), Extraktprobe 2 von 0,93 µg (*RSD* = 0,8 %), Extraktprobe 3 von 1,19 µg (*RSD* = 1,2 %) und Extraktprobe 4 von 3,32 µg (*RSD* = 0,3 %).



17

Bahn 1: Kaempferol, Bahn 2: Kaempferol-3-Rutinosid, Bahn 3: Kaempferol-3-Glucosid, Bahn 4: Glucose, Bahn 5: Cumarinsäure, Bahn 6: Tiliroside Probe 1, Bahn 7: Tiliroside Probe 2

2. Nebenkomponentenanalyse

Cumarinsäure zeigt eine Bande im UV 254 nm bei R_F = 1, die in den Tiliroside-Proben nicht vorkommt. Kaempferol (bei R_F = 1) wird mit dem Naturstoffreagenz nachgewiesen und wurde in den Tiliroside-Proben nicht detektiert. Die Anwesenheit von Cumarinsäure und Kaempferol kann dadurch eindeutig ausgeschlossen werden, ebenso die Abwesenheit von Glucose aufgrund der Anfärbung mit ASR. Kaempferol-3-Rutinosid und Kaempferol-3-Glucosid zeigen nach Anfärbung mit dem Naturstoffreagenz zwei stark fluoreszierende Banden deutlich unterhalb des kosmetischen Wirkstoffs Tiliroside. Ein Vergleich mit den Tiliroside-Proben zeigt die typischen, sehr geringen Mengen an Kaempferol-3-Rutinosid und Kaempferol-3-Glucosid im Pflanzenextrakt.

Hinweis: Im Rahmen dieser Arbeit wurden keine Methodenoptimierungen durchgeführt. Der R_F-Wert von Tiliroside ist relativ hoch, und die Vergleichssubstanzen Cumarinsäure und Kaempferol laufen in der Front. Die gezeigten Ergebnisse sind direkt aus der Praxis. Dabei werden mit der HPTLC schnell aussagekräftige Ergebnisse erzielt und anschliessend mit der HPLC verifiziert. In diesem Fall wurde eine gute Übereinstimmung festgestellt.

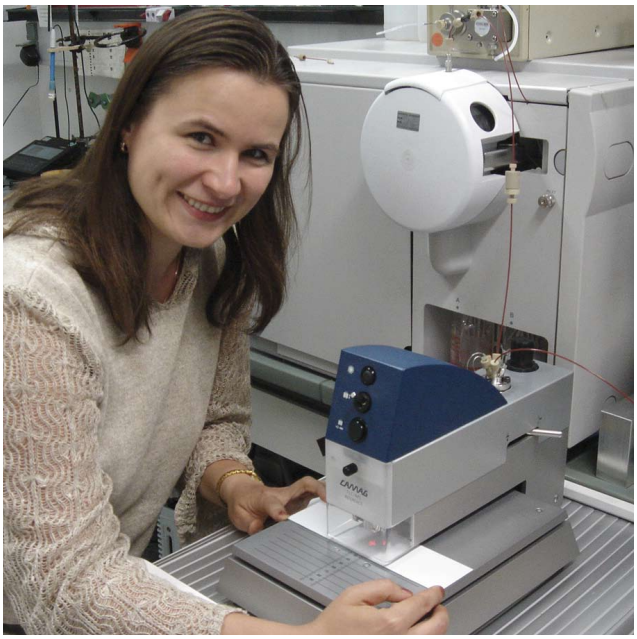
Fazit

Die Methode eignet sich sehr gut zum gezielten Ausschluss relevanter Nebenkomponenten. Durch die Verwendung geeigneter Derivatisierungsmittel konnten auch UV-inaktive Substanzen auf der gleichen HPTLC-Platte nachgewiesen und somit das gesamte Nebenkomponentenprofil dargestellt werden. Hoher Probendurchsatz und präzise Quantifizierung weisen die HPTLC als eine sehr leistungsfähige Methode zur Analytik kosmetischer Wirkstoffe aus.

Weitere Informationen sind von den Autoren auf Anfrage erhältlich.

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

Schnelle Quantifizierung von 5-Hydroxymethyl-furfural in Honig



18

Dr. Elena Chernetsova

In der Arbeitsgruppe von Prof. Dr. Gertrud Morlock am Institut für Lebensmittelchemie der Universität Hohenheim in Stuttgart forscht Frau Dr. Elena Chernetsova, Gastwissenschaftlerin aus Russland, zur Kopplung der Planar-Chromatographie mit der Massenspektrometrie.

Einleitung

Von jeder analytischen Methode, die in der Qualitätskontrolle eingesetzt werden soll, wird erwartet, dass sie einen hohen Probendurchsatz ermöglicht sowie verlässlich und kostengünstig ist. 5-Hydroxymethylfurfural (HMF) bildet sich aus Fruktose oder Glucose in Honig nach längerer Lagerung oder Erwärmung. Sein Gehalt ist somit ein Indikator für dessen Frische und Naturbelassenheit. Die Quantifizierung von HMF in Honig erfolgt üblicherweise entweder mittels HPLC oder spektrophotometrisch nach White oder Winkler [1]. Letztere haben Nachteile. Die Methode nach Winkler ist wenig genau, die nach White benutzt karzinogene Reagenzien und ist ausserdem unsicher. Daher wird für die Quantifizierung von HMF überwiegend HPLC eingesetzt, was jedoch recht aufwändig ist. Die Proben werden in Wasser gelöst, mit Carrez-Reagenz behandelt, um die Zersetzung von HMF zu unterdrükken, filtriert und dann einzeln chromatographiert, was 10–15 Minuten pro Einzelprobe in Anspruch nimmt.

Planar-Chromatographie erwies sich als effiziente, schnelle und kostengünstige Alternative [2]. Nach minimaler Probenvorbereitung werden 24 Honig-Proben gleichzeitig unter identischen Bedingungen innerhalb 5 Minuten und mit geringem Fliessmittelverbrauch getrennt. Erfolgt die Entwicklung in der Horizontal-Entwicklungskammer von beiden Seiten zur Mitte hin, können sogar 48 Proben gleichzeitig getrennt werden. Die Zuverlässigkeit der neuen Methode wurde durch TLC/MS-Kopplung, selektive Derivatisierung und Methodenvergleich belegt.

Schicht

HPTLC-Platten Kieselgel 60 bzw. Kieselgel 60 F_{254}, vorgewaschen mit Methanol – Wasser 6:1, Trocknung 20 min bei 110 °C

Standardlösungen

Wässrige Lösungen von HMF zu 0.1, 1.0, 2.5, 5 und 10 µg/mL

Probenvorbereitung

Honigproben homogenisiert und je ca. 1 g eingewogen in 10 mL Messkolben, aufgefüllt mit Wasser

Probenauftragung

Bandförmig mit DC-Probenautomat 4 (ATS4), Bandlänge 6.5 mm, Bahnabstand 7.5 mm, Abstand vom unteren Rand 8 mm, Auftragevolumen 1–12 µg, 24 Bahnen

Chromatographie

Automatische Entwicklungskammer (ADC 2) mit 10 mL Ethylacetat, Laufstrecke 50 mm, Trocknungszeit 5 min

Densitometrie

TLC Scanner 3 mit winCATS Software, Spektrenaufnahme von 200–800 nm, Quantifizierung bei

290 nm, Spaltgrösse 5 x 0.45 mm, Messgeschwindigkeit 20 mm/s; Auswertung je nach Konzentrationsbereich durch polynome oder Michaelis Menten 2 Regression.

HPTLC-MS (optional)

Die Positionen der HMF-Zonen wurden mit einem weichen Bleistift markiert. Die Elution erfolgte mit dem TLC-MS Interface mit rundem Elutionskopf mit Methanol 0.2 mL/min. In der Auslasskapillare wurde ein Inline-Filter mit einer Frittengrösse von 0.5 µm (Upchurch) integriert. Aufnahme der Massenspektren mit Elektrospray-Ionisierung-MS (ESI-MS) und der LC/MSD Chemstation (Agilent).

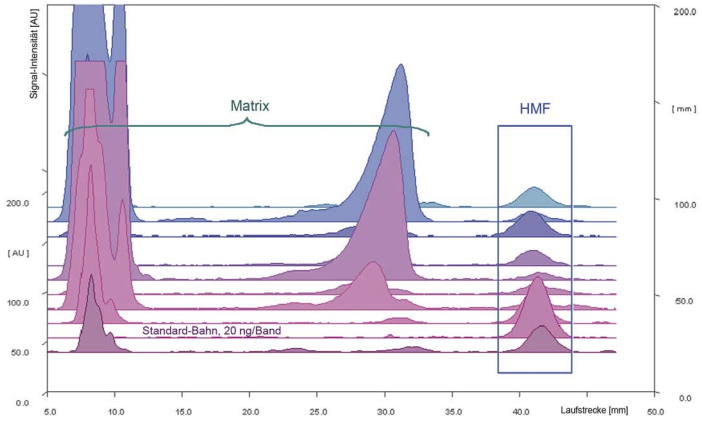
Postchromatographische Derivatisierung (optional)

Mittels Chromatogramm-Tauchvorrichtung (Eintauchgeschwindigkeit 5.0 mm/s, Eintauchzeit 0 s) in *p*-Aminobenzoesäure-Reagenz (1 g gelöst in 36 mL Essigsäure, dann Zugabe von 40 mL Wasser, 2 mL Phosphorsäure 86 % und 120 mL Aceton); anschliessend Erhitzen auf 110 °C, 5–10 min mit DC-Plattenheizer.

Ergebnisse und Diskussion

Im Densitogramm zeigten sich die HMF-Zonen (hR_F 80) deutlich getrennt von allen Matrix-Komponenten der Honig-Proben. Die Identität von HMF konnte durch Vergleich der UV-Spektren der Proben mit denen der Standards abgesichert werden. Die Spektrenmessung lieferte gleichzeitig die zur Quantifizierung optimale Wellenlänge von 290 nm.

Die Nachweisgrenze (LOD, S/N 3, Peakhöhe) von HMF in der Honig-Matrix war vergleichbar mit der ohne Matrix und entsprach 0.75 mg/kg bei einem Auftragevolumen von 12 µL. Die Bestimmungsgrenze (LOQ, S/N 10, Peakhöhe) betrug 2.4 mg/kg. Somit ist die neue HPTLC-Methode geeignet, die weltweit strengsten Grenzwerte für den HMF-Gehalt in Honig von 15 mg/kg zu kontrollieren. Die Bestimmungsgrenze kann bei Bedarf weiter herabgesetzt werden, indem man das Auftragevolumen erhöht.



19

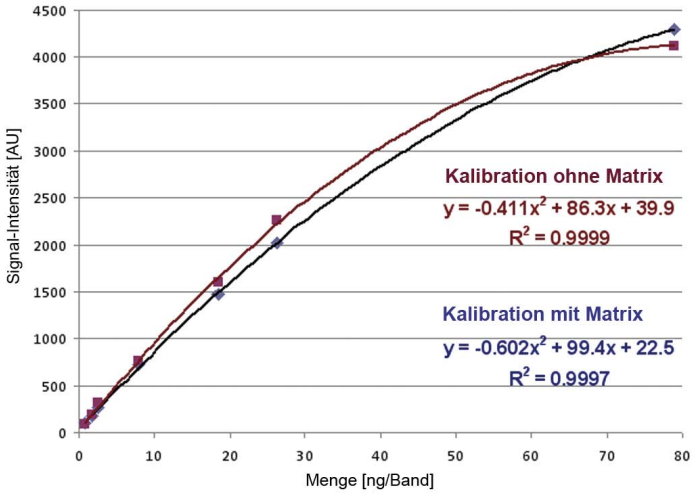
Densitogramme von Honig-Proben und HMF Standard (Bahn 2), erhalten durch Absorptionsmessung bei 290 nm

Im Arbeitsbereich 1:100 entsprach die Kalibrationskurve einem Polynom, während sich für höhere HMF-Mengen auf der Platte die Michelis Menten 2-Regression anbot.

Regression	Kalibrationsbereich	Korrelationskoeffizient r	Relative Standardabweichung sdv
Polynom	1:100 (0.8–80 ng/Band)	≥ 0.9998 (F) ≥ 0.9999 (H)	≤ 2.5 % (F) ≤ 1.4 % (H)
Michelis Menten 2	1:1000 (11–1100 ng/Band)	n.b. n.b.	≤ 1.5 % (F) ≤ 2.3 % (H)

F: Peakfläche; H: Peakhöhe; n.b. nicht bestimmbar

Die Kalibration erfolgte über externe Standards, da keine Störung durch die Honigmatrix ersichtlich war.



20

Kalibrierkurven von HMF mit und ohne Honig-Matrix

Für 10 Honig-Proben, die von der Landesanstalt für Bienenkunde, Stuttgart, und vom Institut für Bienenkunde in Celle zur Verfügung gestellt wurden, sind nachstehend die Ergebnisse der Proben, die um bzw. über dem streng-

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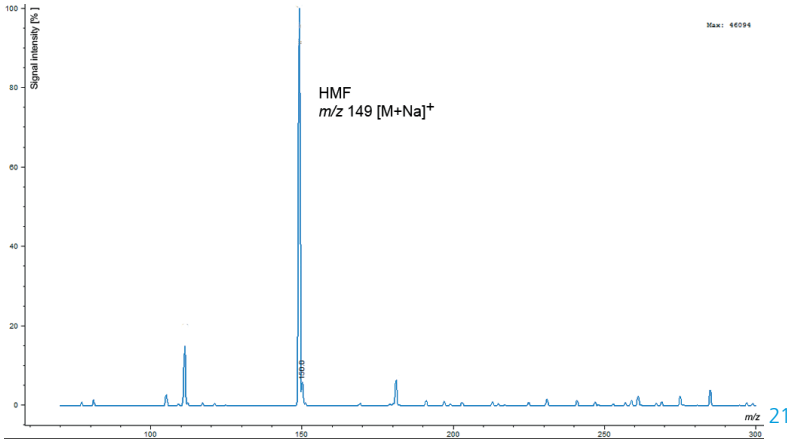
Honigprobe #	Winkler Methode	HPLC-UV		HPTLC-UV		
	HMF in Honig, mg/kg	**HMF in Honig, mg/kg**	Abweichung zur Winkler Methode, %	**HMF in Honig, mg/kg**	Abweichung zur Winkler Methode, %	Abweichung zur HPLC, % (mg/kg)
1	95.3	–	–	75.2	22	–
2	41.8	–	–	30.8	30	–
3	46.1	38.5	16	39.3	16	2.1 % (0.8)
5	17.6	13.5	23	13.7	20	1.4 % (0.2)
7	21.6	18.1	16	18.8	13	3.9 % (0.7)
8	40.2	30.4	24	28.7	26	5.6 % (1.7)
10	23.9	–	–	25.1	5	–
Mittelwert			**20**		**19**	**3.3 % (0.9)**

Die Wiederholbarkeit in Matrix (*%RSD*, *n* = 6, Peakhöhe) betrug 2.9 % für 10 ng/Band HMF und 0.6 % für 100 ng/Band HMF. Die mittlere Reproduzierbarkeit (*%RSD*, *n* = 2, Peakhöhe) über das gesamte Verfahren lag für 4 Honigproben bei 3.0 % (zwischen 1.9 und 4.4 %).

Die HPTLC-MS-Kopplung kann zur zusätzlichen Absicherung der HMF-Ergebnisse dienen. Nach der UV-Absorptionsmessung können gefundene HMF-Zonen von der HPTLC-Platte eluiert werden. HPTLC-ESI-MS erlaubt die Bestätigung positiver, über dem Grenzwert liegender Funde (full scan-Modus) oder eine zweite quantitative Auswertung (selected ion monitoring, SIM-Modus). Die mittlere Abweichung der HMF-Ergebnisse in Honig zwischen HPTLC-UV und HPTLC-MS war 11 % (5.1 mg/kg).



Full scan-Massenspektrum einer als HMF zugeordneten Zone in einer Honig-Probe (80 ng/Band)

Neben der UV- oder MS-Auswertung ist eine weitere Möglichkeit zur Absicherung der HMF-Ergebnisse die Derivatisierung von HMF mit p-Aminobenzoesäure zu einer blau fluoreszierenden Zone (Fluoreszenzmessung bei 366/>400 nm).

Fazit

Es konnte gezeigt werden, dass die Quantifizierung von HMF in Honig mittels der vorgestellten HPTLC-Methode zu den gleichen Ergebnissen führt wie die herkömmliche HPLC-Methode und wie diese den spektrophotometrischen Methoden überlegen ist. Der hohe Probendurchsatz kombiniert mit einer verlässlichen und zugleich kostengünstigen Methode sind für die Qualitätskontrolle ideal.

Weitere Informationen sind von den Autoren erhältlich.

[1] S. Bogdanov *et al.* Apidologie 35 (2004) S4

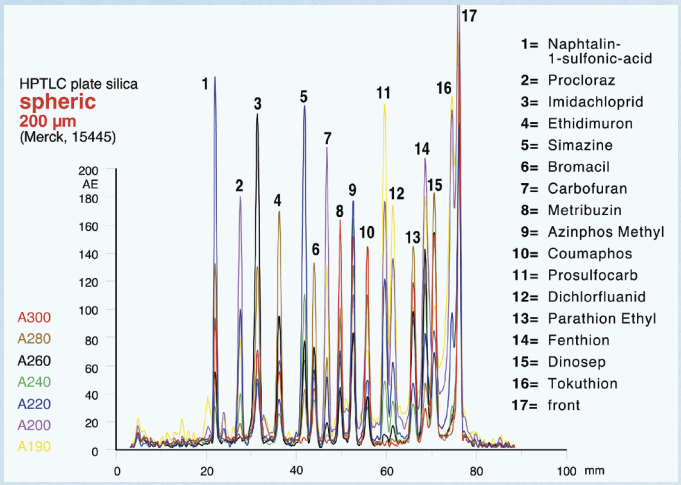
[2] E. Chernetsova, I.Revelsky, G. Morlock Anal Bioanal Chem, 401 (2011) 325-332

Kontakt: apl. Prof. Dr. Gertrud Morlock und Dr. Elena Chernetsova, Institut für Lebensmittelchemie, Universität Hohenheim, Garbenstrasse 28, 70599 Stuttgart, Deutschland, gerda.morlock@uni-hohenheim.de

CAMAG AMD 2 System

Automatisierte Mehrfachentwicklung von Dünnschicht-Chromatogrammen

22



23

Densitogramm (Mehrwellenlängen-Scan) eines AMD-Chromatogramms von Pestiziden, getrennt auf HPTLC-Platte LiChrospher Kieselgel 60 F_{254} Merck.

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.

Die gegenwärtig häufigsten Einsatzgebiete der AMD-Methode

Umweltschutz-Analytik, u. a. Verunreinigungen in Trinkwasser, Boden-Altlasten

CBS 105, S. 7–9, W. Weber et al: 1H-Benzotriazol und Tolyltriazol in der aquatischen Umwelt (AMD – MS)
Der Einsatz von AMD bei der Untersuchung von Roh- und Brauchwässern bietet eine Reihe von Vorteilen: U. a. können beim so genannten Non-Target-Screening unbekannte Substanzen durch Kopplung mit MS oder durch bioeffektive Detektion identifiziert und somit beurteilt werden.

Lipide, Phospholipide

CBS 105, S. 10–12, I. Schellenberg, K. Kabrodt: Optimierung einer AMD 2-Methode zur Bestimmung von Stratum corneum-Lipiden (AMD – Biolumineszenz)
AMD ist der Schlüssel zum Einsatz der Planar-Chromatographie in der Lipid-Analytiik. Die Gradientenelution bietet eine genügend hohe Trennzahl, während das planare Medium eine post-chromatographische Derivatisierung leicht macht.

Inhaltsstoffe von Pflanzen und anderen Naturprodukten

CBS 102, S. 4–7, G. Morlock *et al.*: Screening nach aktiven Naturstoffen aus Schwämmen (AMD-UV/Vis/FLD-bioluminescence-MS)
Die bei HPLC-MS erforderlichen zeitaufwändigen Isolier- und Reinigungsprozesse entfallen komplett. Da alle Lösungsmittel nach der Chromatographie entfernt werden, können diese nicht störend auf die effektorientierte Detektion bioaktiver Metabolite wirken.

CAMAG **Weltweit führend in der Planar-Chromatographie**

CBS 107 D 996.1310-10